AmpliTaq and AmpliTaq Gold DNA Polymerase

The Most Referenced Brand of DNA Polymerase in the World

Date: 2005-05

Notes:

1) **Segmented by Journal which predominantly publish articles on Microbial, Plant and Non-human animals.**

2) **Journals are listed alphabetically from A to Z**

Acta Tropica (6)

Bromidge, T., W. Gibson, et al. (1993). "Identification of Trypanosoma brucei gambiense by PCR amplification of variant surface glycoprotein genes." Acta Tropica **53**(2): 107.

http://www.sciencedirect.com/science/article/B6T1R-476YG0D-2M/2/4f22d3380220b2c2ddbff121a6580ad6

We have developed a sensitive and specific method to identify Trypanosoma brucei gambiense using the polymerase chain reaction (PCR) to amplify the gene encoding variant surface glycoprotein (VSG) Antat 11.17. The test was capable of distinguishing T. b. gambiense from T. b. brucei in most foci of gambian sleeping sickness and gave positive results with previously well-characterised Type I T. b. gambiense stocks from Ivory Coast, Nigeria, Cameroon, Congo, Zaire and Sudan. The test gave negative results with T. b. rhodesiense from Zambia, Kenya and Uganda, virulent or Type II T. b. gambiense from Ivory Coast and T. b. brucei stocks from East and West Africa. The test was modified for colorimetric detection in dot blot format by using nested biotinylated primers in a two-step reaction.Comparison of DNA sequences of VSG genes from T. b. gambiense and other T. brucei ssp. stocks showed a high level of homology, suggesting recent gene flow.

Doedens, A., A. Loukas, et al. (2001). "A cDNA encoding Tc-MUC-5, a mucin from Toxocara canis larvae identified by expression screening." Acta Tropica **79**(3): 211.

http://www.sciencedirect.com/science/article/B6T1R-4384P6V-2/2/dc4720074b590ef3c92972f1135a16bb

Toxocara canis is an ascarid nematode parasite of canids. Larvae infect a wide range of accidental hosts including humans, in whom they are the aetiologic agent of visceral and ocular Larva migrans. The labile surface coat of T. canis larvae consists of a family of mucin glycoproteins termed TES-120, for which the cDNAs have recently been cloned. In this paper, we describe the identification of a novel cDNA (Tc-muc-5) encoding an apomucin by expression screening of a cDNA library with antiserum raised to T. canis excretory/secretory products, and

compare the predicted Tc-MUC-5 protein with those of other T. canis mucins (Tc-MUC-1-Tc-MUC-4) that include the TES-120 surface coat glycoproteins. Tc-MUC-5 has both a larger open reading frame and a more divergent sequence than the other T. canis mucins. It contains a putative signal peptide followed by two six-cysteine (SXC) domains, an extended threonine-rich central mucin core domain and two C-terminal SXC domains. Amino acid composition analysis of secreted TES-120 glycoproteins revealed a distinct lack of lysine residues; while this finding is in agreement with the primary sequences of Tc-MUC-1-Tc-MUC-4, Tc-MUC-5 is conspicuous by its relative abundance of lysines (6.7%), suggesting that this protein is not part of the TES-120 family of surface coat proteins.

Magesa, S. M., K. Y. Mdira, et al. (2002). "Diversity of Plasmodium falciparum clones infecting children living in a holoendemic area in north-eastern Tanzania." Acta Tropica **84**(2): 83.

http://www.sciencedirect.com/science/article/B6T1R-46WWBK3-1/2/779256dfc9f8a53fd5a1b34b9f9e45f4

The diversity of Plasmodium falciparum clones and their role in progression from asymptomatic to symptomatic condition in children have been investigated. Attempts to identify whether particular parasite genotypes were associated with the development of clinical symptoms have been made. A cohort of 34 initially asymptomatic parasitaemic children aged 1-5 years were followed daily for 31 days. Clinical examinations were made each day for signs and symptoms of clinical malaria, followed by parasitological investigation. Nineteen children developed symptoms suggestive of clinical malaria during this period. Daily blood parasite samples from 13 children who developed clinical malaria symptoms and 7 who remained asymptomatic were genotyped by PCR-amplification of the polymorphic regions of the merozoite surface proteins 1 and 2 (MSP1 and MSP2) and the glutamate rich protein (GLURP) genes. Infections were found to be highly complex in both groups of children. Every isolate examined from both groups had a mixture of parasite clones. Daily changes were observed in both parasite density and genotypic pattern. The mean number of genotypes per individual was estimated at 4.9 and 2.7 for asymptomatic and symptomatic groups of children, respectively. Analysis of allele frequency distributions showed that these differed significantly for the MSP1 locus only.

Palsson, K., J. Pinto, et al. (1998). "The palpal ratio method compared with PCR to distinguish between Anopheles gambiae s.s. and A. melas from Guinea Bissau, West Africa." Acta Tropica **70**(1): 101.

http://www.sciencedirect.com/science/article/B6T1R-3SY8DCX-B/2/a963542d2f190bf961794fce0b27dd13

We compared the palpal ratio method with the polymerase chain reaction (PCR) to distinguish between Anopheles gambiae s.s. and A. melas. At the end of the rainy season of 1995, female mosquitoes were collected indoors in the Antula area of Bissau, Guinea Bissau. A subsample of 354 mosquitoes were identified first with PCR and then with the palpal ratio method (study A). Subsequently, another 195 mosquitoes were identified first with the palpal ratio method and then with PCR (study B). The highest percentage (100%, n=16) of correctly identified A. melas was obtained at the palpal ratio cut-off point of 0.83. However, at this point 4.0% (14/347) and 11.3% (21/186) of the A. gambiae were erroneously identified as A. melas in study A and B, respectively. This suggests that the palpal ratio method is not sufficiently reliable to distinguish between A. gambiae and A. melas from the Bissau area.

Stothard, J. R., S. Hughes, et al. (1996). "Variation within the Internal Transcribed Spacer (ITS) of ribosomal DNA genes of intermediate snail hosts within the genus Bulinus (Gastropoda: Planorbidae)." Acta Tropica **61**(1): 19.

http://www.sciencedirect.com/science/article/B6T1R-44BFJ67-F/2/b66c307751560a0d99ef800224e40243

Species within the genus Bulinus are responsible for transmission of schistosomes within the Schistosoma haematobium group. In order to provide a molecular insight into the species relationships within the genus, genetic variation between species representing the four species groups was assayed by Polymerase Chain Reaction (PCR) amplification of the ribosomal Internal Transcribed Spacer (ITS) region followed by Restriction Fragment Length Polymorphism (RFLP) analysis of this product with six restriction enzymes. This PCR-RFLP methodology detected considerable variation within the ITS region indicating that restriction profiles will be useful as genetic markers for identification purposes. The complete ITS1 spacer was sequenced for B. globosus, B. cernicus and B. truncatus. There were numerous nucleotide differences between taxa mainly insertions and deletions. Nucleotide divergence was calculated between species from the restriction profiles: the B. truncatus/tropicus complex and B. reticulatus group were most similar which were in turn more closely related to the B. africanus group than to the B. forskalii group. The nucleotide divergence between the species groups is substantial and questions the placement of these groups within the same genus.

Zindrou, S., E. Orozco, et al. (2001). "Specific detection of Entamoeba histolytica DNA by hemolysin gene targeted PCR." Acta Tropica **78**(2): 117.

http://www.sciencedirect.com/science/article/B6T1R-42DX1WB-4/2/323ab83a0dd194f26936db76c459c354

Diagnostic differentiation of pathogenic Entamoeba histolytica from non-pathogenic Entamoeba dispar is of great clinical importance. We have developed and evaluated a new polymerase chain reaction (PCR) assay (haemo-PCR) based on the novel E. histolytica hemolysin gene HLY6. The specificity of this assay was confirmed by analyzing different Entamoeba species, faeces samples, human and bacterial DNA, and digestion of amplification products with appropriate restriction enzymes. The sensitivity was confirmed by serial dilutions of E. histolytica HM-1:IMSS DNA in the excess of human DNA. Totally, 45 clinical samples were analyzed by the haemo-PCR assay including amoebic liver abscess (ALA) fluids from 23 patients suspected for amoebiasis, four faeces samples containing E. histolytica and E. dispar, and positive and negative controls. The results were compared with those obtained with PCRs for cystein-rich surface protein (P30) and small subunit ribosomal RNA (ssu rRNA) genes. The haemo-PCR gave a positive result in 18 (89%) ALA fluids compared with 14 (77%) and five (28%) by PCR for p30, and ssu rRNA, respectively. PCR products were obtained only from specimens containing E. histolytica DNA. The haemo-PCR assay was therefore found to be a valuable diagnostic tool for identification of E. histolytica infections both in faeces and ALA samples.

Advances in Space Research (2)

Chen, D. J., K. Tsuboi, et al. (1994). "Charged-particle mutagenesis II. Mutagenic effects of high energy

charged particles in normal human fibroblasts." Advances in Space Research **14**(10): 347.

http://www.sciencedirect.com/science/article/B6V3S-47STFGW-1K/2/f1ee51de313eecdf8f9d57fa1c296589

The biological effects of high LET charged particles are a subject of great concern with regard to the prediction of radiation risk in space. In this report, mutagenic effects of high LET charged particles are quantitatively measured using primary cultures of human skin fibroblasts, and the spectrum of induced mutations are analyzed. The LET of the charged particles ranged from 25 KeV/[mu]m to 975 KeV/gmm with particle energy (on the cells) between 94 - 603 MeV/u. The X-chromosome linked hypoxanthine guanine phosphoribosyl transferase (hprt) locus was used as the target gene. Exposure to these high LET charged particles resulted in exponential survival curves; whereas, mutation induction was fitted by a linear model. The Relative Biological Effect (RBE) for cell-killing ranged from 3.73 to 1.25, while that for mutant induction ranged from 5.74 to 0.48. Maximum RBE values were obtained at the LET of 150 keV/[mu]m. The inactivation cross-section ([alpha]i) and the action-section for mutant induction ([alpha]m) ranged from 2.2 to 92.0 [mu]m2 and 0.09 to 5.56 x 10-3 [mu]m2, respectively. The maximum values were obtained by 56Fe with an LET of 200 keV/[mu]m. The mutagenicity ([alpha]m/[alpha]i) ranged from 2.05 to 7.99 x 10-5 with the maximum value at 150 keV/[mu]m. Furthermore, molecular analysis of mutants induced by charged particles indicates that higher LET beams are more likely to cause larger deletions in the hprt locus.

Roux, S. J., A. Chatterjee, et al. (2003). "Early development of fern gametophytes in microgravity." Advances in Space Research **31**(1): 215.

http://www.sciencedirect.com/science/article/B6V3S-47RB619-14/2/3513037097ef06418b690a0d87753742

Dormant spores of the fern Ceratopteris richardii were flown on Shuttle mission STS-93 to evaluate the effects of micro-g on their development and on their pattern of gene expression. Prior to flight the spores were sterilized and sown into one of two environments: (1) Microscope slides in a video-microscopy module; and (2) Petri dishes. All spores were then stored in darkness until use. Spore germination was initiated on orbit after exposure to light. For the spores on microscope slides, cell level changes were recorded through the clear spore coat of the spores by video microscopy. After their exposure to light, spores in petri dishes were frozen in orbit at four different time points during which on earth gravity fixes the polarity of their development. Spores were then stored frozen in Biological Research in Canister units until recovery on earth. The RNAs from these cells and from 1-g control cells were extracted and analyzed on earth after flight to assay changes in gene expression. Video microscopy results revealed that the germinated spores developed normally in microgravity, although the polarity of their development, which is guided by gravity on earth, was random in space. Differential Display-PCR analyses of RNA extracted from space-flown cells showed that there was about a 5% change in the pattern of gene expression between cells developing in micro-g compared to those developing on earth.

Armstrong, P. M. and R. Rico-Hesse (2003). "EFFICIENCY OF DENGUE SEROTYPE 2 VIRUS STRAINS TO INFECT AND DISSEMINATE IN AEDES AEGYPTI." Am J Trop Med Hyg **68**(5): 539-544.

http://www.ajtmh.org/cgi/content/abstract/68/5/539

Dengue serotype 2 (DEN-2) viruses with the potential to cause dengue hemorrhagic fever have been shown to belong to the Southeast (SE) Asian genotype. These viruses appear to be rapidly displacing the American genotype of DEN-2 in the Western Hemisphere. To determine whether distinct genotypes of DEN-2 virus are better adapted to mosquito transmission, we classified 15 viral strains of DEN-2 phylogenetically and compared their ability to infect and disseminate in different populations of Aedes aegypti mosquitoes. Envelope gene nucleotide sequence analysis confirmed that six strains belonged to the American genotype and nine strains were of the SE Asian genotype. The overall rate of disseminated infection in mosquitoes from Texas was 27% for the SE Asian genotype versus 9% for the American genotype. This pattern of infection was similar in another population of mosquitoes sampled from southern Mexico (30% versus 13%). Together, these findings suggest that Ae. aegypti tends to be more susceptible to infection by DEN-2 viruses of the SE Asian genotype than to those of the American genotype, and this may have epidemiologic implications.

Elias, F. E., C. A. Vigliano, et al. (2003). "ANALYSIS OF THE PRESENCE OF TRYPANOSOMA CRUZI IN THE HEART TISSUE OF THREE PATIENTS WITH CHRONIC CHAGAS' HEART DISEASE." Am J Trop Med Hyg **68**(2): 242-247.

http://www.ajtmh.org/cgi/content/abstract/68/2/242

It is still unclear to what extent myocarditis-associated, chronic Chagas' heart disease is due to persisting Trypanosoma cruzi. In the present study, we have analyzed tissue samples from the hearts of three patients with this disease. In situ hybridization provided little evidence for the presence of intact T. cruzi, even at sites of strong inflammation. Nevertheless, micromanipulation techniques detected remnants of both T. cruzi kinetoplast DNA and nuclear DNA. Trypanosoma cruzi DNA was also detected in single macrophages dissected directly from frozen heart tissue sections. Thus, this analysis demonstrates that T. cruzi kinetoplast DNA and nuclear DNA are widely dispersed in the heart tissue, although in low amounts. Since we rarely detected intact T. cruzi parasites during the chronic phase of Chagas' heart disease, we can exclude heart tissue as a major parasite reservoir.

Fitness, J., S. Floyd, et al. (2004). "LARGE-SCALE CANDIDATE GENE STUDY OF LEPROSY SUSCEPTIBILITY IN THE KARONGA DISTRICT OF NORTHERN MALAWI." Am J Trop Med Hyg **71**(3): 330-340.

http://www.ajtmh.org/cgi/content/abstract/71/3/330

We present a large case-control candidate gene study of leprosy susceptibility. Thirty-eight polymorphic sites from 13 genes were investigated for their role in susceptibility to leprosy by comparing 270 cases with 452 controls in Karonga district, northern Malawi. Homozygotes for a silent T[-&gt]C change in codon 352 of the vitamin D receptor gene appeared to be at high risk (odds ratio [OR] = 4.3, 95% confidence interval [CI] = 1.6-11.4, P = 0.004), while homozygotes for the McCoy b blood group defining variant K1590E in exon 29 of the complement receptor 1 (formerly CD35) gene appeared to be protected (OR = 0.3, 95% CI = 0.1-0.8, P = 0.02). Borderline evidence for association with leprosy susceptibility was found for seven polymorphic

sites in an additional six genes. Some of these apparent associations may be false-positive results from multiple comparisons, and several associations suggested by studies in other populations were not replicated here. These data provide evidence of inter-population heterogeneity in leprosy susceptibility.

Gwinn, W., W. Sun, et al. (2003). "SEROTYPE-SPECIFIC TH1 RESPONSES IN RECIPIENTS OF TWO DOSES OF CANDIDATE LIVE-ATTENUATED DENGUE VIRUS VACCINES." Am J Trop Med Hyg **69**(90060): 39-47.

http://www.ajtmh.org/cgi/content/abstract/69/6_suppl/39

As part of a larger vaccine study, peripheral blood mononuclear cells (PBMC) were collected from volunteers for analysis of vaccine- induced T cell responses. The PBMC were re-stimulated in vitro with live dengue virus and assayed for Th1 or Th2 memory cell responses. Re-stimulated PBMC from the volunteers predominantly secreted interferon-{gamma}. Little interleukin-4 (IL-4) or IL-10 secretion was detected, indicating a Th1 type of T cell response. The interferon-{gamma} response was primarily serotype-specific with some serotype cross-reactivity. T cell depletion studies showed that the interferon-{gamma} was being secreted by CD4+ T lymphocytes and/or by cells other than CD8+ T lymphocytes that were being stimulated by the CD4+ T lymphocytes. CD3+ or CD8+ T cell depletion showed that granzyme B mRNA expression correlated with the presence of CD4+ T lymphocytes. However, depletion of CD4+ T cells after four days of stimulation indicated that the granzyme B mRNA was produced by cells in culture other than lymphocytes. In summary, an antigen-specific Th1 type T cell response was seen as a response to vaccination using live attenuated dengue virus.

Huang, D. B., Z.-D. Jiang, et al. (2003). "ASSOCIATION OF VIRULENCE FACTOR-POSITIVE AND -NEGATIVE ENTEROAGGREGATIVE ESCHERICHIA COLI AND OCCURRENCE OF CLINICAL ILLNESS IN TRAVELERS FROM THE UNITED STATES TO MEXICO." Am J Trop Med Hyg **69**(5): 506-508.

http://www.ajtmh.org/cgi/content/abstract/69/5/506

The objective of the study was to determine if the presence or absence of virulence factor-positive and -negative enteroaggregative Escherichia coli (EAEC) determined the occurrence of illness or sub-clinical EAEC infection in travelers from the United States to Mexico. Sixty-five newly arrived college students from the United States submitted weekly stool samples for a four-week period of time. Among EAEC-infected subjects, diarrhea occurred in those with a defined virulence factor with the following frequency: aggA, 5 of 15 (33%); aggR, 3 of 11 (27%); aafA, 3 of 8 (38%); and aspU, 1 of 6 (17%). Twenty-two of 31 students (71%) had two or more EAEC infections. After the initial EAEC infection, only 4 (11%) of 31 students had a subsequent symptomatic EAEC infection. Our study suggests that clinical illness by EAEC is not explained by presence of a defined EAEC virulence factors, and we provide suggestive evidence that EAEC infection protects against future symptomatic infection.

Jiang, J. U., T.-C. Chan, et al. (2004). "DEVELOPMENT OF A QUANTITATIVE REAL-TIME POLYMERASE CHAIN REACTION ASSAY SPECIFIC FOR ORIENTIA TSUTSUGAMUSHI." Am J Trop Med Hyg **70**(4): 351-356.

http://www.ajtmh.org/cgi/content/abstract/70/4/351

Two specific and sensitive polymerase chain reaction (PCR) assays were developed to detect and quantitate Orientia tsutsugamushi, the agent of scrub typhus, using a portion of the 47-kD outer membrane protein antigen/ high temperature requirement A gene as the target. A selected 47-kD protein gene primer pair amplified a 118-basepair fragment from all 26 strains of O. tsutsugamushi evaluated, but it did not produce amplicons when 17 Rickettsia and 18 less-related bacterial nucleic acid extracts were tested. Similar agent specificity for the real-time PCR assay, which used the same primers and a 31-basepair fluorescent probe, was demonstrated. This sensitive and quantitative assay determination of the content of O. tsutsugamushi nucleic acid used a plasmid containing the entire 47-kD gene from the Kato strain as a standard. Enumeration of the copies of O. tsutsugamushi DNA extracted from infected tissues from mice and monkeys following experimental infection with Orientia showed 27-5,552 copies/{micro}L of mouse blood, 14,448-86,012 copies/{micro}L of mouse liver/spleen homogenate, and 3-21 copies/{micro}L of monkey blood.

Mita, T., A. Kaneko, et al. (2003). "RECOVERY OF CHLOROQUINE SENSITIVITY AND LOW PREVALENCE OF THE PLASMODIUM FALCIPARUM CHLOROQUINE RESISTANCE TRANSPORTER GENE MUTATION K76T FOLLOWING THE DISCONTINUANCE OF CHLOROQUINE USE IN MALAWI." Am J Trop Med Hyg **68**(4): 413-415.

http://www.ajtmh.org/cgi/content/abstract/68/4/413

In 1993, Malawi stopped treating patients with chloroquine for Plasmodium falciparum malaria because of a high treatment failure rate (58%). In 1998, the in vitro resistance rate to chloroquine was 3% in the Salima District of Malawi; in 2000, the in vivo resistance rate was 9%. We assayed two genetic mutations implicated in chloroquine resistance (N86Y in the P. falciparum multiple drug resistance gene 1 and K76T in the P. falciparum chloroquine resistance transporter gene) in 82 P. falciparum isolates collected during studies in 1998 and 2000. The prevalence of N86Y remained similar to that in neighboring African countries that continued to use chloroquine. In contrast, the prevalence of K76T was substantially lower than in neighboring countries, decreasing significantly from 17% in 1998 to 2% in 2000 ($P < 0.02$). However, neither mutation was significantly associated with in vivo or in vitro resistance ($P > 0.29$). Withdrawal of the use of chloroquine appears to have resulted in the recovery of chloroquine efficacy and a reduction in the prevalence of K76T. However, other polymorphisms are also expected to contribute to resistance.

Ogg, M. M., R. J. Carrion, et al. (2003). "SHORT REPORT: QUANTIFICATION OF LEISHMANIAVIRUS RNA IN CLINICAL SAMPLES AND ITS POSSIBLE ROLE IN PATHOGENESIS." Am J Trop Med Hyg **69**(3): 309-313.

http://www.ajtmh.org/cgi/content/abstract/69/3/309

Leishmaniavirus (LRV) is a double-stranded RNA virus that infects the protozoa Leishmania and has been identified in numerous strains of Leishmania braziliensis and L. braziliensis guyanensis. In general, the species of Leishmania dictates disease manifestation except in the case of L. braziliensis, which is capable of causing either cutaneous or mucocutaneous leishmaniasis. We wanted to determine 1) the quantity of LRV RNA present in a clinical sample and 2) if infection with LRV was associated with a specific disease manifestation. A real-time reverse transcriptase-polymerase chain reaction assay was used to assay clinical samples for the presence of LRV. Of 47 samples tested, 12 positive samples were obtained from patients with cutaneous lesions, lesions in the process of scarring, and cutaneous scars. This is the first study to examine the prevalence of LRV RNA within a small cohort from Brazil.

Santolamazza, F., A. Della Torre, et al. (2004). "SHORT REPORT: A NEW POLYMERASE CHAIN REACTION-RESTRICTION FRAGMENT LENGTH POLYMORPHISM METHOD TO IDENTIFY ANOPHELES ARABIENSIS FROM AN. GAMBIAE AND ITS TWO MOLECULAR FORMS FROM DEGRADED DNA TEMPLATES OR MUSEUM SAMPLES." Am J Trop Med Hyg **70**(6): 604-606.

http://www.ajtmh.org/cgi/content/abstract/70/6/604

We present a polymerase chain reaction-restriction fragment length polymorphism method to simultaneously distinguish the two Anopheles gambiae M and S molecular forms and Anopheles arabiensis. This method uses different diagnostic sites than previously published methods, and it is based on the amplification of a smaller ribosomal DNA fragment. We have tested this protocol in a variety of samples from different geographic regions and various ages of preservation to ascertain the robustness of this protocol over a wide geographic window and on DNA templates of poor quality. This procedure is as efficient as previous ones in discriminating An. arabiensis from the two taxa in An gambiae s.s. However, it performs better than others on poor quality templates such as the ones from museum collections, and poorly stored field collected material. However, it must be noted that it does not allow the simultaneous discrimination of all the species in the An. gambiae complex.

Singer, L. M., R. D. Newman, et al. (2004). "EVALUATION OF A MALARIA RAPID DIAGNOSTIC TEST FOR ASSESSING THE BURDEN OF MALARIA DURING PREGNANCY." Am J Trop Med Hyg **70**(5): 481-485.

http://www.ajtmh.org/cgi/content/abstract/70/5/481

Plasmodium falciparum infection during pregnancy may cause placental malaria and subsequently low birth weight, primarily through the placental sequestration of infected red blood cells. Measuring the burden of malaria during pregnancy usually involves determining the prevalence of placental malaria infection through microscopic examination of placental blood films, a difficult and error-prone process. A number of rapid diagnostic tests (RDTs) for malaria have been developed, most of them immunochromatographic dipstick assays. However, none have been tested for the direct determination of malaria antigen in placental blood. We undertook an evaluation of the Malaria Rapid Test (MAKROmed(R)) in determining placental malaria infection. The prevalence of placental parasitemia was 22.6% by microscopy, 51.0% by a polymerase chain reaction (PCR), and 43.1% by RDT. When the PCR was used as the gold standard, RDTs had a sensitivity of 89% and a specificity of 76%. The MAKROmed RDT was highly sensitive in the detection of placental malaria, but had lower than expected specificity.

Singh, O. P., D. Chandra, et al. (2004). "DIFFERENTIATION OF MEMBERS OF THE ANOPHELES FLUVIATILIS SPECIES COMPLEX BY AN ALLELE-SPECIFIC POLYMERASE CHAIN REACTION BASED ON 28S RIBOSOMAL DNA SEQUENCES." Am J Trop Med Hyg **70**(1): 27-32.

http://www.ajtmh.org/cgi/content/abstract/70/1/27

Anopheles fluviatilis, one of the major vectors of malaria in India, is a complex of at least three cryptic species provisionally designated as species S, T, and U. Identification of the cryptic species of An. fluviatilis complex is of paramount importance in disease control program due to contrasting differences in their vectorial efficiency, preference for feeding on humans, and resting

behavior. Species S, T, and U are morphologically indistinguishable at any stage of their life cycle and can be identified only by the examination of species-specific fixed inversions in the polytene chromosomes. We report an allele-specific polymerase chain reaction assay for the differentiation of members of An. fluviatilis complex, which is based on differences in nucleotide sequences in D3 domain of 28S ribosomal DNA. The assay was evaluated against chromosomally examined individuals from different localities with different sympatric associations and was found to differentiate unambiguously all the members of the complex.

Vinoles, J., M. Serra, et al. (2005). "SEROINCIDENCE AND PHYLOGENY OF HUMAN IMMUNODEFICIENCY VIRUS INFECTIONS IN A COHORT OF COMMERCIAL SEX WORKERS IN MONTEVIDEO, URUGUAY." Am J Trop Med Hyg **72**(4): 495-500.

http://www.ajtmh.org/cgi/content/abstract/72/4/495

A cohort study involving 60 human immunodeficiency virus (HIV)-negative male transvestite commercial sex workers (CSWs) was conducted in Montevideo, Uruguay in 1999-2001. Serum samples were tested for HIV by an enzyme-linked immunosorbent assay screening with immunoblot confirmation. Six participants seroconverted for an incidence-density rate of 6.03 (95% confidence interval = 2.21-13.12) per 100 person-years. Inconsistent condom use during client sex (adjusted hazard ratio [AHR] = 6.7), during oral sex (AHR = 5.6), and at the last sexual encounter (AHR = 7.8), and use of marihuana (AHR = 5.4) were marginally associated with HIV seroconversion. Five samples were genotyped in the protease and reverse transcriptase regions; three were subtypes B and two were BF recombinants. Full genome analysis of four samples confirmed all three subtype B samples and one of the two BF recombinants. Male transvestite CSWs sustained a high rate of HIV infection. Larger prospective studies are required to better define subtypes and associated sexual and drug-related risk factors.

Am. J. Botany (17)

Athanasiou, A., D. Khosravi, et al. (2003). "Characterization and localization of short-specific polygalacturonase in distylous Turnera subulata (Turneraceae)." Am. J. Botany **90**(5): 675-682.

http://www.amjbot.org/cgi/content/abstract/90/5/675

We describe for distylous Turnera subulata a polygalacturonase specific to short-styled plants that is localized to the style transmitting tissue (the tissue through which pollen tubes grow). The polygalacturonase gene is linked to and may be upregulated by the S allele of the distyly locus. Because of its tissue-specific location, the polygalacturonase may be involved in the self-incompatibility response, acting in a complementary or antagonistic manner, or possibly in signalling downstream events. A pollen-specific polygalacturonase was also identified and may be a member of a small multigene family of pollen polygalacturonases. The role, if any, played by the pollen polygalacturonase in distyly, is presently unknown.

Bortiri, E., S.-H. Oh, et al. (2002). "The phylogenetic utility of nucleotide sequences of sorbitol 6-phosphate dehydrogenase in Prunus (Rosaceae)." Am. J. Botany **89**(10): 1697-1708.

http://www.amjbot.org/cgi/content/abstract/89/10/1697

Sequences from s6pdh, a gene that encodes sorbitol-6-phosphate dehydrogenase in the Rosaceae, are used to reconstruct the phylogeny of 22 species of Prunus. The s6pdh sequences alone and in combination with previously published sequences of the internal transcribed spacer (ITS) and the cpDNA trnL-trnF spacer are analyzed using parsimony and maximum likelihood methods. Both methods reconstructed the same phylogeny when s6pdh sequences are used alone and in combination with ITS and trnL-trnF, and the topology is in agreement with previous studies that used a larger sample size. The s6pdh sequences have about twice as many informative sites as ITS. A molecular clock is rejected for s6pdh, most likely due to greater rates of evolution in subgenera Padus and Laurocerasus than in the rest of the genus. Phylogenetic reconstruction of Prunus as determined by analysis of the combined data set suggests an early split into two clades. One is composed of subgenera Cerasus, Laurocerasus, and Padus. The second includes subgenera Amygdalus, Emplectocladus, and Prunus. Species of section Microcerasus (formerly in subgenus Cerasus) are nested within subgenus Prunus. The order of branching and relationships among early diverging lineages is weakly supported, as a result of very short branches that may indicate rapid radiation.

Gobert, V., S. Moja, et al. (2002). "Hybridization in the section Mentha (Lamiaceae) inferred from AFLP markers." Am. J. Botany **89**(12): 2017-2023.

http://www.amjbot.org/cgi/content/abstract/89/12/2017

The amplified fragment length polymorphism (AFLP) method was used to evaluate genetic diversity and to assess genetic relationships within the section Mentha in order to clarify the taxonomy of several interspecific mint hybrids with molecular markers. To this end, genetic diversity of 62 Mentha accessions from different geographic origins, representing five species and three hybrids, was assessed. Three EcoRI/MseI AFLP primer combinations generated an average of 40 AFLP markers per primer combination, ranging in size from 50 to 500 base pairs (bp). The percentage of markers polymorphic ranged from 50% to 60% across all accessions studied. According to phenetic and cladistic analysis, the 62 mint accessions were grouped into two major clusters. Principal coordinates analysis separated species into well-defined groups, and clear relationships between species and hybrids could be described. Our AFLP analysis supports taxonomic classification established among Mentha species by conventional (morphological, cytological, and chemical) methods. It allows the assessment of phenetic relationships between species and the hybrids M. spicata and M. x piperita, largely cultivated all over the world for their menthol source, and provides new insights into the subdivision of M. spicata, based for the first time on molecular markers.

Jorgensen, J. L., I. Stehlik, et al. (2003). "Implications of ITS sequences and RAPD markers for the taxonomy and biogeography of the Oxytropis campestris and O. arctica (Fabaceae) complexes in Alaska." Am. J. Botany **90**(10): 1470-1480.

http://www.amjbot.org/cgi/content/abstract/90/10/1470

Taxonomic consensus is lacking on the Oxytropis arctica and O. campestris species complexes, two polyploid complexes found in the interior and arctic areas of Alaska. One classification has emphasized flower size, whereas flower color is considered a key diagnostic character in another classification. Our analyses of internal transcribed spacer (ITS) sequences and random amplified polymorphic DNA (RAPD) markers provided no support for either classification system. The trees generated from ITS sequences and the phenogram derived from RAPD markers suggest that

most recognized taxa in the two complexes are probably polyphyletic, including O. arctica var. barnebyana, which is listed as threatened in Alaska. The only consistent pattern detected by both types of molecular markers was a geographic split dividing the northeastern arctic populations from most other populations (48.60-55.03% in AMOVA analyses). This genetic subdivision probably reflects a Pleistocene barrier formed by the northern coastal ice shield. Our molecular data, in conjunction with the previously reported variation of ploidy levels in these groups, suggest a scenario of recent and multiple origins of polyploidy. It is possible that most Alaskan populations of these two complexes are best referred to as a single taxonomic species despite morphological differentiation within the complexes.

Lindqvist, C. and V. A. Albert (2002). "Origin of the Hawaiian endemic mints within North American Stachys (Lamiaceae)." Am. J. Botany **89**(10): 1709-1724.

http://www.amjbot.org/cgi/content/abstract/89/10/1709

The Hawaiian endemic mints constitute a major island radiation, displaying a remarkable diversity of floral, fruit, and vegetative features. Haplostachys and Phyllostegia have flowers associated with insect pollination, whereas Stenogyne has flowers typical of bird pollination. The three genera had been thought to be closely related to East Asian members of Lamioideae tribe Prasieae because of the fleshy nutlets borne by Phyllostegia and Stenogyne. We evaluated the origins of the Hawaiian mints using phylogenetic analyses of DNA sequence data from the plastid rbcL and trnL intron loci and the nuclear ribosomal 5S nontranscribed spacer. The Hawaiian genera were found to be monophyletic but deeply nested inside another lamioid genus, Stachys. In particular, they were found to be most closely related to a group of temperate North American Stachys from the Pacific coast, suggesting that the Hawaiian mints derived from a single colonization event from western North America to the Hawaiian Islands. Furthermore, Stachys, which contains amphiatlantic and transberingian clades, was found to be polyphyletic, with some species more closely related to Gomphostemma, Phlomidoschema, Prasium, and Sideritis than to other species of Stachys. Based on chromosomal evidence and our phylogenetic analyses, we hypothesize that the Hawaiian mints may be polyploid hybrids whose reticulate genomes predate the Hawaiian dispersal event and are derived from Stachys lineages with flowers exhibiting insect- vs. bird-pollination characteristics. Thus, the Hawaiian endemic mints may provide yet another insular system for the combined study of polyploidy, hybrid cladogenesis, and adaptive radiation.

Little, D. P. and D. S. Barrington (2003). "Major evolutionary events in the origin and diversification of the fern genus Polystichum (Dryopteridaceae)." Am. J. Botany **90**(3): 508-514.

http://www.amjbot.org/cgi/content/abstract/90/3/508

Recent advances in molecular systematics of the ferns make it possible to address long-standing questions about classification of the major fern genera, such as the worldwide genus Polystichum (Dryopteridaceae), comprising at least 200 species. In this study we examined rbcL sequences and morphological characters from 55 fern taxa: 34 were from Polystichum and 21 were from other genera in the Dryopteridaceae. We found that Phanerophlebia, possibly including Polystichopsis, is the sister group to Polystichum sensu lato (s.l.), including Cyrtomium. Polystichum as commonly recognized is paraphyletic. Our results lead us to suggest recognizing the clade of earliest diverging Polystichum species as a distinct genus (Cyrtomidictyum) and to continue to recognize Cyrtomium as a separate genus, leaving a monophyletic Polystichum sensu stricto (s.s.). We resolved a tropical American clade and an African clade within Polystichum s.s. However, the resemblance between the once-pinnate, bulb-bearing calciphilic species found in Asia and the West Indies appears to be the result of convergent evolution.

Optimizing our morphological character transformations onto the combined phylogeny suggests that the common ancestor of Polystichum s.l. and Phanerophlebia had evolved the common features of the alliance, including ciliate petiole-base scales, once-pinnate fronds, ultimate segments with scarious tips, peltate indusia, and microscales.

Mansion, G. and L. Zeltner (2004). "Phylogenetic relationships within the New World endemic Zeltnera (Gentianaceae-Chironiinae) inferred from molecular and karyological data." Am. J. Botany **91**(12): 2069-2086.

http://www.amjbot.org/cgi/content/abstract/91/12/2069

The New World endemic genus Zeltnera consists of 25 species mainly distributed in the western part of the United States and Mexico. Chromosome counts performed on 113 populations (24 species) reveal extensive congruence between chromosomal groups and the assemblages obtained from analyses of nuclear ribosomal DNA (ITS) and chloroplast DNA (trnL intron and trnL-F intergenic spacer) sequences. Karyological and molecular data sets support three main biogeographic groups for Zeltnera. A first and mainly unresolved cluster (n = 17 and n = 20) occurs in California, whereas two other clades are centered in the Texas region (n = 20 and n = 21) and in Mexico (n = 21 and n = 22). Under the assumption of a molecular clock, and using both dispersal and vicariance explanations for the current distribution of the respective species, the genus is thought to have a North American origin with considerable diversification in the early Pliocene (ca. 5 million years ago). Geological events, such as desert formation and mountain orogenies, have created insuperable barriers that today separate the three major and likely vicariant groups.

Morton, C. M., M. Grant, et al. (2003). "Phylogenetic relationships of the Aurantioideae inferred from chloroplast DNA sequence data." Am. J. Botany **90**(10): 1463-1469.

http://www.amjbot.org/cgi/content/abstract/90/10/1463

The tribes and subtribes of Aurantioideae, an economically important subfamily of the Rutaceae, have a controversial taxonomic history because of the lack of a phylogenetic framework. The rps16 and trnL-trnF sequences of the chloroplast were analyzed phylogenetically to construct an evolutionary history and evaluate the most recent classification system of Swingle and Reece (The Citrus Industry, volume 1 [1967]). Taxa representing tribes Citreae and Clauseneae and five of the six subtribes were sampled. Conflicts in the positions of some taxa between the rps16 and trnL-trnF trees are poorly supported. In all analyses, the Aurantioideae are monophyletic. The strict consensus tree of the combined analysis indicates that the two tribes along with the subtribes sampled are not monophyletic. The combined topology is not congruent with the widely used classification of Aurantioideae by Swingle and Reece. The tribes and subtribes are in need of revision.

Nickrent, D. L., M. A. Garcia, et al. (2004). "A phylogeny of all species of Arceuthobium (Viscaceae) using nuclear and chloroplast DNA sequences." Am. J. Botany **91**(1): 125-138.

http://www.amjbot.org/cgi/content/abstract/91/1/125

The genus Arceuthobium (dwarf mistletoes, Viscaceae) comprises 42 species that parasitize hosts in Pinaceae and Cupressaceae in the Old and New Worlds. Maximum parsimony analyses

were conducted on two data partitions (separately and combined): nuclear ribosomal internal transcribed spacer (ITS) sequences for all 42 currently recognized species and chloroplast trnT-L-F sequences for 34 New World species. The Old and New World species were phylogenetically distinct using ITS, thus making subgenus Arceuthobium paraphyletic. Arceuthobium pendens and A. guatemalense comprise the basalmost clade of subgenus Vaginata, characterized by the presence of flabellate secondary branching. The trnT-L-F sequences, which vary widely in length depending upon taxon, contain three times less phylogenetic signal than ITS, although homoplasy for this partition is lower. Several of the clades obtained from analysis of nuclear ITS sequences are also recovered using trnT-L-F sequences such as A. guatemalense and A. pendens, the A. rubrum group, the A. vaginatum group, and the A. campylopodum group. The ITS + trnT-L-F tree is well resolved except for four internal nodes. A revised classification of the genus is discussed that recognizes only monophyletic species that are well differentiated by molecular data.

Oh, S.-H. and D. Potter (2005). "Molecular phylogenetic systematics and biogeography of tribe Neillieae (Rosaceae) using DNA sequences of cpDNA, rDNA, and LEAFY." Am. J. Botany **92**(1): 179-192.

http://www.amjbot.org/cgi/content/abstract/92/1/179

A phylogeny of the tribe Neillieae (Rosaceae), which comprises Neillia, Stephanandra, and Physocarpus, was reconstructed based on nucleotide sequences of several regions of cpDNA, the ITS and ETS regions of rDNA, and the second intron of LEAFY, to elucidate relationships among genera and species in Neillieae and to assess the historical biogeography of the tribe. Phylogenetic analyses indicated that Physocarpus and Neillia-Stephanandra were strongly supported as monophyletic and suggested that Stephanandra may have originated by hybridization between two lineages of Neillia. Dispersal-vicariance analyses suggested that the most recent common ancestor of Neillieae may have occupied eastern Asia and western North America and that Physocarpus and Neillia-Stephanandra may have been split by an intercontinental vicariance event in the early Miocene. The biogeographic analyses also suggested that species of Neillia and Stephanandra diversified in eastern Asia, whereas in Physocarpus one dispersal event from western North America to eastern Asia occurred. Two divergent types of LEAFY sequences were found in the eastern North American species, P. opulifolius, but only one type was present in each plant. The two types of sequences may represent homeologous genes that originated by hybridization between P. capitatus and P. monogynus, both western North American species.

Perret, M., A. Chautems, et al. (2003). "Systematics and evolution of tribe Sinningieae (Gesneriaceae): evidence from phylogenetic analyses of six plastid DNA regions and nuclear ncpGS." Am. J. Botany **90**(3): 445-460.

http://www.amjbot.org/cgi/content/abstract/90/3/445

For nearly all species in the three genera of tribe Sinningieae (Gesneriaceae), Sinningia, Paliavana, and Vanhouttea (mostly in southeastern Brazil) plus 10 outgroups, we have sequenced six non-coding DNA regions (i.e., plastid intergenic spacers trnT-trnL, trnL-trnF, trnS-trnG, atpB-rbcL, and introns in the trnL and rpl16 genes) and four introns in nuclear plastid-expressed glutamine synthetase gene (ncpGS). Separate and combined analyses of these data sets using maximum parsimony supported the monophyly of Sinningieae, but the genera Paliavana and Vanhouttea were found embedded within Sinningia; therefore a new infrageneric classification is here proposed. Mapping of pollination syndromes on the DNA-based trees supported multiple origins of hummingbird and bee syndromes and derivation of moth and bat syndromes from hummingbird flowers. Perennial tubers were derived from perennial stems in

non-tuberous plants.

Rauscher, J. T. (2002). "Molecular phylogenetics of the Espeletia complex (Asteraceae): evidence from nrDNA ITS sequences on the closest relatives of an Andean adaptive radiation." Am. J. Botany **89**(7): 1074-1084.

http://www.amjbot.org/cgi/content/abstract/89/7/1074

The subtribe Espeletiinae (Asteraceae, Heliantheae) comprises morphologically and ecologically diverse plants endemic to the tropical montane paramos of the Andes of Venezuela, Colombia, and Ecuador. Though the ecophysiology and ecology of this adaptive radiation have been well studied, relationships among taxa in the subtribe and between the subtribe and other taxa in the Heliantheae are poorly known. In this study, sequences from the internal transcribed spacer (ITS) region of nuclear ribosomal DNA are used to test previous hypotheses about the phylogenetic position of the Espeletiinae within the Heliantheae and to determine which taxa are the subtribe's closest relatives. Gene phylogenies based on maximum parsimony analyses reveal that the Espeletiinae clade is nested well within the subtribe Melampodiinae and thus should be considered a monophyletic complex of species, not a separate subtribe. The most parsimonious gene trees suggest that the genus Ichthyothere may be the sister taxon to the Espeletia complex and that the genus Smallanthus and a species of Rumfordia are likely among the complex's other closest living relatives. These data offer preliminary insights into the origins of this adaptive radiation and the broader phylogenetic context in which it occurred.

Rova, J. H. E., P. G. Delprete, et al. (2002). "A trnL-F cpDNA sequence study of the Condamineeae-Rondeletieae-Sipaneeae complex with implications on the phylogeny of the Rubiaceae." Am. J. Botany **89**(1): 145-159.

http://www.amjbot.org/cgi/content/abstract/89/1/145

DNA sequences from the chloroplast trnL-F region of 154 Rubiaceae and 11 outgroup taxa were analyzed cladistically. An emphasis was placed on the tribes Rondeletieae, Sipaneeae, and Condamineeae. Sipaneeae are not close to Rondeletieae and belong in the Ixoroideae. There is no support for a widely distributed Rondeletieae in a broad sense. Instead, Rondeletieae sensu stricto form an almost entirely Antillean clade. Support was found for the separation of Arachnothryx, Rogiera, Roigella, and Suberanthus from Rondeletia. The Guettardeae as well as Gonzalagunia are found close to a complex formed by Arachnothryx, Javorkaea, and Rogiera. Condamineeae, in a strict sense, belongs in the Ixoroideae. A number of Rondeletieae genera should be transferred to Condamineeae or other parts of Ixoroideae. Support is found for an emended tribe Naucleeae, comprising several genera with spherical pseudanthia. For the first time, tribal or subfamilial affiliation based on molecular sequence data is suggested for Allenanthus, Blepharidium, Chione, Coutaportla, Dolichodelphys, Mazaea, Neobertiera, Neoblakea, Phialanthus, Phyllacanthus, Phyllomelia, Schmidtottia, and Suberanthus.

Salazar, G. A., M. W. Chase, et al. (2003). "Phylogenetics of Cranichideae with emphasis on Spiranthinae (Orchidaceae, Orchidoideae): evidence from plastid and nuclear DNA sequences." Am. J. Botany **90**(5): 777-795.

http://www.amjbot.org/cgi/content/abstract/90/5/777

DNA sequences from plastid rbcL and matK genes and the trnL-F region, as well as the nuclear ribosomal ITS region, were used to evaluate monophyly and subtribal delimitation of Cranichideae and generic relationships in Spiranthinae. Cranichideae are moderately supported as monophyletic, with Chloraeinae and Pterostylis-Megastylis indicated as their collective sisters. Within Cranichideae, Pachyplectroninae and Goodyerinae form a well-supported monophyletic group sister to a "core spiranthid" clade that includes, according to their branching order, Galeottiellinae, Manniellinae, and a Prescottiinae-Cranichidinae-Spiranthinae subclade. Inclusion of Galeottiella in Spiranthinae, as in previous classifications, renders the latter paraphyletic to all other spiranthid subtribes. Cranichidinae and Spiranthinae (minus Galeottiella) are monophyletic and strongly supported, but Prescottiinae form a grade that includes a strongly supported prescottioid Andean clade and a weakly supported Prescottia-Cranichidinae clade sister to Spiranthinae. Well-supported major clades in Spiranthinae identified in this study do not correspond to previous alliances or the narrowly defined subtribes in which they have been divided recently. Morphological characters, especially those that have been used for taxonomic delimitation in Cranichideae, are discussed against the framework of the molecular trees, emphasizing putative synapomorphies and problems derived from lack of information or inadequate interpretation of the characters.

Sanchez-Baracaldo, P. (2004). "Phylogenetics and biogeography of the neotropical fern genera Jamesonia and Eriosorus (Pteridaceae)." Am. J. Botany **91**(2): 274-284.

http://www.amjbot.org/cgi/content/abstract/91/2/274

Jamesonia and Eriosorus are two traditionally recognized fern genera in the Neotropics that together form a monophyletic group. Molecular phylogenetic analyses for this study suggest, however, that neither genus is itself monophyletic and that several independent lineages with the jamesonia morphotype have each undergone a fairly recent radiation in paramo ecosystems. A robust phylogeny was generated based on sequence data of the nuclear external transcribed spacer (ETS) of 18S-26S rDNA, the plastid gene rps4 and the intergenic spacer rps4-trnS. Several conclusions can be made concerning the evolutionary history and biogeographic patterns of the Jamesonia-Eriosorus complex: (1) "jamesonia" is polyphyletic, making "eriosorus" paraphyletic; (2) all analyses recover three major clades in the Andes; (3) two well-supported clades can be recognized, corresponding to the northern vs. central Andes; and (4) the sister taxon of the Andean radiation is the Brazilian taxon Eriosorus myriophyllus. Jamesonia is a potential example of a recent adaptive radiation because the group is characterized as being morphologically and ecologically diverse and its habitat is of recent origin.

Scheen, A.-C., C. Brochmann, et al. (2004). "Northern hemisphere biogeography of Cerastium (Caryophyllaceae): insights from phylogenetic analysis of noncoding plastidnucleotide sequences." Am. J. Botany **91**(6): 943-952.

http://www.amjbot.org/cgi/content/abstract/91/6/943

Phylogenetic relationships and biogeography of the genus Cerastium were studied using sequences of three noncoding plastid DNA regions (trnL intron, trnL-trnF spacer, and psbA-trnH spacer). A total of 57 Cerastium taxa was analyzed using two species of the putative sister genus Stellaria as outgroups. Maximum parsimony analyses identified four clades that largely corresponded to previously recognized infrageneric groups. The results suggest an Old World origin and at least two migration events into North America from the Old World. The first event possibly took place across the Bering land bridge during the Miocene. Subsequent colonization of South America occurred after the North and South American continents joined during the Pliocene. A more recent migration event into North America probably across the northern Atlantic

took place during the Quaternary, resulting in the current circumpolar distribution of the Arctic species. Molecular clock dating of major biogeographic events was internally consistent on the phylogenetic trees. The arctic high-polyploid species form a polytomy together with some boreal and temperate species of the C. tomentosum group and the C. arvense group. Lack of genetic variation among the arctic species probably indicates a recent origin. The annual life form is shown to be of polyphyletic origin.

Walter, R. and B. K. Epperson (2005). "Geographic pattern of genetic diversity in Pinus resinosa: contact zone between descendants of glacial refugia." Am. J. Botany **92**(1): 92-100.

http://www.amjbot.org/cgi/content/abstract/92/1/92

Although red pine (Pinus resinosa) generally has low or completely lacks variation for molecular markers, some variation is observed for chloroplast microsatellites (cpSSRs). We sampled and examined 10 cpSSRs for 19 populations. Analysis of these populations plus 10 previously studied populations shows that the geographic distribution of genetic diversity over the range of P. resinosa is markedly nonuniform. Although the pattern exhibits little isolation by distance, there is a region centered in northeastern New England where populations contain much greater chloroplast haplotype diversity than elsewhere. This area is band-shaped, with the longer axis nearly parallel with latitude, and very sharply delineated. The area of high diversity was buried by the Laurentide ice sheet. The geographic pattern indicates that P. resinosa is not at equilibrium, and the species has had a more complex postglacial history than typically purported for forest trees in eastern North America. The results suggest that the area of high diversity is a stable transition zone between descendants of two distinct refugia, one in the southern Appalachians and another near the North Atlantic coastline of the Wisconsinian glacial period. Plausible explanations are given that selection between two lineages, along latitudinal zones, may have maintained the transition zone.

Anaerobe (3)

Bradshaw, M., S. S. Dineen, et al. (2004). "Regulation of neurotoxin complex expression in Clostridium botulinum strains 62A, Hall A-hyper, and NCTC 2916." Anaerobe **10**(6): 321.

http://www.sciencedirect.com/science/article/B6W9T-4DD8F07-1/2/d94f8df08d315a30ddedd7f459020662

The kinetics of botulinum toxin gene expression have been investigated in Clostridium botulinum type A strains 62A, Hall A-hyper, and type A(B) strain NCTC 2916 during the growth cycle. The analyses were performed in TPGY and type A Toxin Production Media (TPM). The mRNA transcript levels encoding the proteins of the neurotoxin complex were determined using Northern analyses. Neurotoxin concentrations in culture supernatants and lysed cell pellets were assayed using ELISA, Western blots, and mouse bioassay. Proteolytic activation of botulinum neurotoxin during the growth cycle was evaluated by Western blots. For all three strains, mRNA transcripts for the toxin complex genes were initially detected in early log phase, reached peak levels in early stationary phase, and rapidly decreased in mid-to-late stationary phase and during lysis. Toxin expression varied depending on the strain and growth medium. Toxin production was highest in strain Hall A-hyper, followed by NCTC 2916 and 62A. For C. botulinum strain Hall A-hyper, cell

lysis and toxin release into the supernatant occurred rapidly for cells grown in TPM, while cells grown in TPGY remained in stationary phase with minimal lysis and toxin release through 96 h of growth. In contrast, strains 62A and NCTC 2916 lysed more extensively than Hall A-hyper in TPGY. TPM supported higher toxin production and activation than TPGY in strains 62A and Hall A-hyper. These data support that the genes of the botulinum neurotoxin complex are temporally expressed during late-log and early stationary phase and that toxin complex formation depends on the strain and growth medium. Botulinum toxin synthesis and activation appears to be a complex process that is highly regulated by nutritional and environmental conditions. Further research is needed to elucidate the sensing mechanisms and genetic regulatory factors controlling these processes.

Duhamel, G. E., C. J. Stryker, et al. (2003). "Colonic spirochetosis of colony-raised rhesus macaques associated with Brachyspira and Helicobacter." Anaerobe **9**(1): 45.

http://www.sciencedirect.com/science/article/B6W9T-48VWDSG-7/2/9bb8c6c7da2e86f805b029c00f86d5fa

Colonic spirochetosis is an inflammatory bowel disease that affects a broad range of hosts, including human and non-human primates. The disease in humans and non-human primates is characterized by intimate attachment of the anaerobic spirochetes Brachyspira aalborgi and B. pilosicoli, and some unclassified flagellated microbes along the apical membrane of colonic enterocytes. Although the presence of spiral-shaped bacteria with single polar flagella and blunted ends in colonic spirochetosis is well established, the identities of many of these organisms is still unknown. Recently, Helicobacter species with a morphology similar to the flagellated bacteria present in colonic spirochetosis have been cultured from intestinal specimens obtained from rhesus macaques, some with idiopathic colitis. The purpose of the present study was to determine whether or not the flagellated bacteria seen in the colons of rhesus macaques with colonic spirochetosis are Helicobacter. The presence of flagellated bacteria alone (n=2) or together with spirochetes (n=1) in formalin-fixed and paraffin-embedded colons of three rhesus macaques with the naturally occurring disease was demonstrated by immunohistochemical staining and ultrastructural examination. Total DNA extracted from affected and control intestinal specimens was amplified by polymerase chain reaction (PCR) using Helicobacter 16S rRNA gene-specific primers. Comparative nucleotide sequence analysis of PCR products cloned from positive reactions indicated that two distinct Helicobacter genomospecies were present either alone or in combination with Brachyspira in the colons of rhesus macaques with microscopic lesions indicative of colonic spirochetosis.

Rafii, F. and M. Park "Effects of gyrase mutation on the growth kinetics of ciprofloxacin-resistant strains of Clostridium perfringens." Anaerobe **In Press, Corrected Proof**
http://www.sciencedirect.com/science/article/B6W9T-4FM5D5P-1/2/08ed73e354cd6d8e8e9a240a323a6a49

To investigate the effect of gyrA mutation on resistance of Clostridium perfringens to fluoroquinolones, a ciprofloxacin-resistant mutant was developed. The mutant had a single substitution in gyrA at position 87 (Asp to Tyr) and no additional mutations in gyrB, parC or parE. The MIC values of gatifloxacin and ciprofloxacin for this strain were 16 and 32-fold higher than those for the wild type, which were 0.125 and 0.250 [mu]g/mL, respectively. The resistant mutant grew equally well in the presence or absence of 5 [mu]g/mL of ciprofloxacin or 1 [mu]g/mL of gatifloxacin and grew to lower cell densities with up to 30 [mu]g/mL of ciprofloxacin or 5 [mu]g/mL of gatifloxacin. Higher concentrations of fluoroquinolones resulted in increases in the time required to reach the end of the exponential phase and in lower cell densities at the end. The efflux pump inhibitor reserpine did not affect susceptibility to fluoroquinolones. The substitution of

Asp 87 to Tyr in gyrA may have protected C. perfringens from low concentrations of ciprofloxacin and gatifloxacin and enabled survival and growth at higher concentrations.

Animal Behaviour (1)

Klein, S. L., M. C. Zink, et al. (2004). "Seoul virus infection increases aggressive behaviour in male Norway rats." Animal Behaviour **67**(3): 421.

http://www.sciencedirect.com/science/article/B6W9W-4BMJVGF-3/2/92f2358ca837d6dbc64800bfd2dd8b2b

In natural populations of rodents, males are more likely to engage in aggression and be infected with hantaviruses than females. Whether the relationship between hantavirus infection and aggression is due to host- or parasite-mediated mechanisms is unknown. The aim of this study was to determine whether hantavirus infection causes an increase in aggression in male rats and whether these behavioural changes are due to infection of the central nervous system or peripheral tissues. Male laboratory rats were infected with Seoul virus and tested for aggression in a resident-intruder paradigm 15 and 30 days postinoculation (p.i.). Males tested 30 days p.i. (i.e. during the persistent phase of infection) spent more time engaged in aggression than either uninfected males or males tested during the acute phase of infection (i.e. 15 days p.i.). Males that engaged in aggression for a longer duration had more virus present in lung, kidney and testis than males that spent less time engaged in aggression. Infected males shed virus in saliva, faeces, and urine; virus shedding, however, was not correlated with aggression and neither wounding nor transmission of virus to intruder males occurred during behavioural tests. Infection with Seoul virus did not alter either testosterone or corticosterone concentrations. Seoul virus antigens were not detected in the brains of infected rats. These data suggest that hantavirus infection leads to elevated aggression in infected males and may be a by-product of increased virus replication in peripheral tissues.

Animal Reproduction Science (2)

Engel, E., R. Klein, et al. "Investigations on the expression of cytokines in the canine corpus luteum in relation to dioestrus." Animal Reproduction Science **In Press, Corrected Proof**
http://www.sciencedirect.com/science/article/B6T43-4F3NXY2-1/2/c67b8a0651bb36a9ff4902d752efe068

Control of luteal regression in the dog is still poorly understood. Unlike other domestic animal species, luteolysis is not prevented by hysterectomy. Indications that PGF2[alpha] may act as an endogenous luteolytic agent have been found only in pregnant animals during the prepartal decline of progesterone. Evidence from several species indicates that the immune system plays an important role in corpus luteum (CI) function, possibly by the release of cytokines from immigrant immune cells. Hence, in the present experiment we attempted to examine the expression of cytokines in the canine CI during the course of dioestrus (formation and regression

of the CI), using RT-PCR. Groups of 4-5 bitches were ovario-hysterectomised on days 5, 15, 25, 35, 45 and 60-80 after ovulation. Canine-specific primers for IL-1[beta], IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, TNF[alpha], IFN[gamma] and TGF[beta]1 were used. Positive and negative controls were included in all tests. Adequate expression was confirmed by sequencing selective samples of positive PCR products. The expression of mRNA for IL-8, IL-10, IL-12, TNF-[alpha] and TGF-[beta]1 was detected in all samples at each stage of dioestrus, without any obvious variations indicating a likely modulatory function of these cytokines in differentiation, maintenance or regression of the canine CI. All tests for the expression of mRNA for IL-4, IL-1[beta] and IL-2 were negative. More negative than positive results were obtained when testing for the expression of mRNA for IL-6 and IFN-[gamma], leading to the conclusion that expression of these two cytokines is at a low level, and no conclusion can be drawn as to their involvement in control of CI function.

Makondo, K., G. S. Amiridis, et al. (1997). "Use of the polymerase chain reaction to sex the bovine fetus using cells recovered by ultrasound-guided fetal fluid aspiration." Animal Reproduction Science **49**(2-3): 125.

http://www.sciencedirect.com/science/article/B6T43-3S53VPS-5/2/3a75a6a9cf652ce34f06b0bc659d2357

The aim of this study was to carry out first trimester fetal sex diagnosis using the polymerase chain reaction (PCR) to amplify DNA from bovine fetal cells recovered by transvaginal ultrasound-guided uterine puncture and fetal fluid aspiration. For sex determination, a nested, allele-specific, PCR amplification of the bovine zfx and zfx gene fragments was utilised. The PCR assay was validated using fetal fluids recovered from uteri post mortem. Cells were harvested from the fetal fluids, genomic DNA extracted and the PCR assay applied. A technique which was developed for transvaginal ultrasound-guided follicle aspiration was modified to recover fetal fluid from live animals. Small volumes of fetal fluid (0.5-5 ml) were recovered between days 61-97 of gestation and the PCR assay applied. The gender determined by PCR of fetal fluid cells was in all cases confirmed by visual inspection (n = 15 abattoir specimens) or ultrasound scanning (n = 7 live animals). Fetal death, attributed to the introduction of intrauterine infection, occurred in 4/4 cows in the first series of aspirations but in only 1/3 heifers in the second series of aspirations.

Ann. Bot. (2)

Nieto Feliner, G., B. Gutierrez Larena, et al. (2004). "Fine-scale Geographical Structure, Intra-individual Polymorphism and Recombination in Nuclear Ribosomal Internal Transcribed Spacers in Armeria (Plumbaginaceae)." Ann. Bot. **93**(2): 189-200.

http://aob.oupjournals.org/cgi/content/abstract/93/2/189

* Background and Aims Isolation and drift are the main causes for geographic structure of molecular variation. In contrast, the one found in a previous survey in Armeria (Plumbaginaceae) for nuclear ribosomal ITS multicopy regions was species-independent and has been hypothesized to be due to extensive gene-flow and biased concerted evolution. Since this was inferred from a genus-level phylogenetic analysis, the aim of this study was to check for the occurrence of such structure and the validity of the proposed model at a local scale, in a southern Spanish massif (Sierra Nevada), as well as to examine the evolutionary implications at the

organism level. * Methods In addition to 117 sequences of direct PCR products from genomic DNA, 50 sequences of PCR products from cloned DNA were obtained to analyse cases of intragenomic polymorphisms for the ITS regions. * Key Results Sequence data confirm the occurrence of a species-independent structure at a local scale and reveal insights through the analysis of contact areas between different ITS copies (ribotypes). A comparison between cloned and direct sequences (a) confirms that, within these contact areas, ITS copies co-occur both in different individuals and within single genomes; and (b) reveals recombination between different copies. * Conclusions This study supports the utility of direct sequences for detecting intra-individual polymorphism and for partially inferring the ITS copies involved, given previous knowledge of the variability. The main evolutionary implication at the organism level is that gene-flow and concerted evolution shape the geographic structure of ITS variation.

Skipper, M. (2002). "Genes from the APETALA3 and PISTILLATA Lineages are Expressed in Developing Vascular Bundles of the Tuberous Rhizome, Flowering Stem and Flower Primordia of Eranthis hyemalis." Ann. Bot. **89**(1): 83-88.

http://aob.oupjournals.org/cgi/content/abstract/89/1/83

In Arabidopsis thaliana expression of the B-class MADS-box genes APETALA3 (AP3) and PISTILLATA (PI) is confined to petals and stamens but in other plant species these genes are also transcribed in non-flower tissues; in Solanum tuberosum they are transcribed specifically in vascular bundles leading to petals and stamens. Transcription analysis of B-class genes in Eranthis hyemalis using reverse transcribed in situ PCR revealed that both AP3 and PI are expressed in developing vascular bundles in the tuberous rhizome, flowering stem and floral primordia. In addition, AP3 and PI transcripts are also found in stems and leaves. These results suggest a more complex role of B-class genes in Eranthis and possible involvement in the development of vascular tissue.

Antimicrob. Agents Chemother. (40)

Arpin, C., V. Dubois, et al. (2003). "Extended-Spectrum {beta}-Lactamase-Producing Enterobacteriaceae in Community and Private Health Care Centers." Antimicrob. Agents Chemother. **47**(11): 3506-3514.

http://aac.asm.org/cgi/content/abstract/47/11/3506

In 1999, 39 of 2,599 isolates of the family Enterobacteriaceae (1.5%) collected by eight private laboratories in the Aquitaine region in France produced an extended-spectrum {beta}-lactamase (ESBL). Among these were 19 Enterobacter aerogenes isolates; 8 Klebsiella pneumoniae isolates; 6 Escherichia coli isolates; 3 Proteus mirabilis isolates; and 1 isolate each of Serratia marcescens, Morganella morganii, and Providencia stuartii. ESBL producers were isolated from 38 patients, including 33 residents of 11 clinics or nursing homes and 5 ambulatory patients. Seven different ESBLs were characterized. These mainly consisted of TEM-24 (25 isolates) and TEM-21 (9 isolates), but TEM-15 (2 isolates) and TEM-3, TEM-19, SHV-4, and CTX-M-1 (1 isolate each) were also characterized. Seven strains showed the coexistence of different TEM- and/or SHV-encoding genes, including a new SHV-1 variant, SHV-44, defined by the substitution R205L previously reported for SHV-3 in association with S238G. The epidemiology of the ESBL

producers was investigated by random amplification of polymorphic DNA, typing by enterobacterial repetitive intergenic consensus PCR, analysis of resistance cotransferred with the ESBL, and analysis of the restriction profiles of the ESBL-encoding plasmids. Of the TEM-24-expressing strains, 18 were E. aerogenes isolates, including 9 from the same clinic, that were representatives of the epidemic clone disseminating in France. Of the TEM-21-producing strains that belonged to different species of the family Enterobacteriaceae (E. coli, K. pneumoniae, and P. mirabilis), 8 were isolated in the same nursing home. Outbreaks due to strain and/or plasmid dissemination in these clinic and nursing home were demonstrated. The presence of ESBL producers in five ambulatory patients probably resulted from nosocomial acquisition. Our data highlight the serious need to monitor patients for ESBL-producing Enterobacteriaceae in general practice.

Arpin, C., R. Labia, et al. (2002). "TEM-80, a Novel Inhibitor-Resistant {beta}-Lactamase in a Clinical Isolate of Enterobacter cloacae." Antimicrob. Agents Chemother. **46**(5): 1183-1189.

http://aac.asm.org/cgi/content/abstract/46/5/1183

Enterobacter cloacae Ecl261 was isolated with Escherichia coli Ec257 from the urine of a patient living in a nursing home. Both isolates were resistant to ticarcillin (MICs, 1,024 {micro}g/ml), without significant potentiation of its activity by 2 {micro}g of clavulanate per ml (MICs, 512 {micro}g/ml), and susceptible to naturally active cephalosporins. This inhibitor-resistant phenotype was conferred in both strains by similar conjugative plasmids of 40 kb (Ecl261) and 30 kb (Ec257), which also conveyed resistance to sulfonamides and trimethoprim. Clinical and transconjugant strains produced a {beta}-lactamase with a pI of 5.2 which belonged to the TEM family, as indicated by specific PCR amplification. Compared with TEM-1, this enzyme exhibited lower catalytic efficiencies (14- and 120-fold less for amoxicillin and ticarcillin, respectively), and higher concentrations of {beta}-lactamase inhibitors were required to yield a 50% reduction in benzylpenicillin hydrolysis (750-, 82-, and 50-fold higher concentrations for clavulanate, sulbactam, and tazobactam, respectively). Gene sequencing revealed four nucleotide differences with the nucleotide sequence of blaTEM-1A. The first replacement (T32C), located in the promoter region, was described as being responsible for the increase in the level of {beta}-lactamase production. The three other changes led to amino acid substitutions that define a new inhibitor-resistant TEM (IRT) {beta}-lactamase, TEM-80 (alternate name, IRT-24). Two of them, Met69Leu and Asn276Asp, have previously been related to inhibitor resistance. The additional mutation, Ile127Val, was demonstrated by site-directed mutagenesis to have a very weak effect, at least alone, on the IRT phenotype. This is the first description of an IRT {beta}-lactamase in E. cloacae. The horizontal transfer of blaTEM-80 may have occurred either from Ec257 to Ecl261 or in the reverse order.

Ballard, S. A., E. A. Grabsch, et al. (2005). "Comparison of Three PCR Primer Sets for Identification of vanB Gene Carriage in Feces and Correlation with Carriage of Vancomycin-Resistant Enterococci: Interference by vanB-Containing Anaerobic Bacilli." Antimicrob. Agents Chemother. **49**(1): 77-81.

http://aac.asm.org/cgi/content/abstract/49/1/77

We assessed the sensitivities and specificities of three previously described PCR primers on enrichment broth cultures of feces for the accurate detection of fecal carriage of vancomycin-resistant enterococci (VRE). In addition, we investigated specimens that were vanB PCR positive but VRE culture negative for the presence of other vanB-containing pathogens. Feces from 59 patients (12 patients carrying vanB Enterococcus faecium strains and 47 patients negative for VRE carriage) were cultured for 36 h in aerobic brain heart infusion (BHI) broth, anaerobic BHI

(AnO2BHI) broth, or aerobic Enterococcosel (EC) broth. DNA was extracted from the cultures and tested for the presence of vanB by using the PCR primers of Dutka-Malen et al. (S. Dutka-Malen, S. Evers, and P. Courvalin, J. Clin. Microbiol. 33:24-27, 1995), Bell et al. (J. M. Bell, J. C. Paton, and J. Turnidge, J. Clin. Microbiol. 36:2187-2190, 1998), and Stinear et al. (T. P. Stinear, D. C. Olden, P. D. R. Johnson, J. K. Davies, and M. L. Grayson, Lancet 357:855-856, 2001). The sensitivity (specificity) of PCR compared with the results of culture on BHI, AnO2BHI, and EC broths were 67% (96%), 50% (94%), and 17% (100%), respectively, with the primers of Dutka-Malen et al.; 92% (60%), 92% (45%), and 92% (83%), respectively, with the primers of Bell et al.; and 92% (49%), 92% (43%), and 100% (51%) respectively, with the primers of Stinear et al. The primers of both Bell et al. and Stinear et al. were significantly more sensitive than those of Dutka-Malen et al. in EC broth ($P = 0.001$ and $P < 0.001$, respectively). The poor specificities for all primer pairs were due in part to the isolation and identification of six anaerobic gram-positive bacilli, Clostridium hathewayi (n = 3), a Clostridium innocuum-like organism (n = 1), Clostridium bolteae (n = 1), and Ruminococcus lactaris-like (n = 1), from five fecal specimens that were vanB positive but VRE culture negative. All six organisms were demonstrated to contain a vanB gene identical to that of VRE. VanB-containing bowel anaerobes may result in false-positive interpretation of PCR-positive fecal enrichment cultures as VRE, regardless of the primers and protocols used.

Brueggemann, A. B., S. L. Coffman, et al. (2002). "Fluoroquinolone Resistance in Streptococcus pneumoniae in United States since 1994-1995." Antimicrob. Agents Chemother. **46**(3): 680-688.

http://aac.asm.org/cgi/content/abstract/46/3/680

The in vitro activities of ciprofloxacin, levofloxacin, gatifloxacin, and moxifloxacin against a large collection of clinical isolates of Streptococcus pneumoniae (n = 4,650) obtained over a 5-year period, 1994-1995 through 1999-2000, were assessed as part of a longitudinal multicenter U.S. surveillance study of antimicrobial resistance. Three sampling periods were used during this investigation, the winter seasons of 1994-1995, 1997-1998, and 1999-2000; and 1,523, 1,596 and 1,531 isolates were collected during these three periods, respectively. The overall rank order of activity of the four fluoroquinolones examined in this study was moxifloxacin > gatifloxacin > levofloxacin = ciprofloxacin, in which moxifloxacin (MIC at which 90% of isolates are inhibited [MIC90], 0.25 {micro}g/ml; modal MIC, 0.12 {micro}g/ml) was twofold more active than gatifloxacin (MIC90, 0.5 {micro}g/ml; modal MIC, 0.25 {micro}g/ml), which in turn was fourfold more active than either levofloxacin (MIC90, 1 {micro}g/ml; modal MIC, 1 {micro}g/ml) or ciprofloxacin (MIC90, 2 {micro}g/ml; modal MIC, 1 {micro}g/ml). Changes in the in vitro activities of fluoroquinolones against S. pneumoniae strains in the United States over the 5-year period of the survey were assessed by comparing the MIC frequency distributions of the study drugs against the isolates obtained during the three sampling periods encompassing this investigation. These comparisons revealed no evidence of changes in the in vitro activities of the fluoroquinolones. In addition, the percentages of isolates in the three sampling periods for which MICs were above the resistance breakpoints were compared. Low percentages of resistant strains were detected, and there was no evidence of resistance rate changes over time. For example, by use of a ciprofloxacin MIC of [>=]4 {micro}g/ml to define resistance, the proportions of isolates from the three sampling periods for which MICs were at or above this breakpoint were 1.2, 1.6, and 1.4%, respectively. A total of 164 unique isolates (n = 58 from 1994-1995, 65 from 1997-1998, and 42 from 1999-2000) were examined for evidence of mutations in the quinolone resistance-determining regions (QRDRs) of the parC and the gyrA genes. Forty-nine isolates harbored at least one mutation in the QRDRs of one or both genes (1994-1995, n = 15; 1997-1998, n = 19; 1999-2000, n = 15). Among the 4,650 isolates of S. pneumoniae examined in the study, we estimated that 0.3% had mutations in both the parC and gyrA loci. The majority of mutations (67.3% of the mutations in 49 isolates with mutations) were amino acid substitutions in the parC locus only. Four isolates had a mutation in the gyrA locus only, and 12 isolates had mutations in both genes (8.2 and 24.5% of isolates with mutations,

respectively). There was no significant difference in the number of isolates with parC and/or gyrA mutations detected during each study period. Finally, because of the magnitude of the study, we had reasonably large numbers of pneumococcal isolates with genotypically defined mechanisms of fluoroquinolone resistance and were thus able to determine the effects of specific resistance mutations on the activities of different fluoroquinolones. In general, isolates with mutations in parC only were resistant to ciprofloxacin but remained susceptible to levofloxacin, gatifloxacin, and moxifloxacin, whereas isolates with mutations in gyrA only and isolates with mutations in both parC and gyrA were resistant to all four fluoroquinolones tested.

Bulatovic, V. M., N. L. Wengenack, et al. (2002). "Oxidative Stress Increases Susceptibility of Mycobacterium tuberculosis to Isoniazid." Antimicrob. Agents Chemother. **46**(9): 2765-2771.

http://aac.asm.org/cgi/content/abstract/46/9/2765

Isoniazid is a first-line antibiotic used in the treatment of infections caused by Mycobacterium tuberculosis. Isoniazid is a prodrug requiring oxidative activation by the catalase-peroxidase hemoprotein, KatG. Resistance to isoniazid can be obtained by point mutations in the katG gene, with one of the most common being a threonine-for-serine substitution at position 315 (S315T). The S315T mutation is found in more than 50% of isoniazid-resistant clinical isolates and results in an {approx}200-fold increase in the MIC of isoniazid compared to that for M. tuberculosis H37Rv. In the present study we investigated the hypothesis that superoxide plays a role in KatG-mediated isoniazid activation. Plumbagin and clofazimine, compounds capable of generating superoxide anion, resulted in a lower MIC of isoniazid for M. tuberculosis H37Rv and a strain carrying the S315T mutation. These agents did not cause as great of an increase in isoniazid susceptibility in the mutant strain when the susceptibilities were assessed by using the inhibitory concentration that causes a 50% decrease in growth. These results provide evidence that superoxide can play a role in isoniazid activation. Since clofazimine alone has antitubercular activity, the observation of synergism between clofazimine and isoniazid raises the interesting possibility of using both drugs in combination to treat M. tuberculosis infections.

Campion, J. J., P. J. McNamara, et al. (2004). "Evolution of Ciprofloxacin-Resistant Staphylococcus aureus in In Vitro Pharmacokinetic Environments." Antimicrob. Agents Chemother. **48**(12): 4733-4744.

http://aac.asm.org/cgi/content/abstract/48/12/4733

The development of novel antibacterial agents is decreasing despite increasing resistance to presently available agents among common pathogens. Insights into relationships between pharmacodynamics and resistance may provide ways to optimize the use of existing agents. The evolution of resistance was examined in two ciprofloxacin-susceptible Staphylococcus aureus strains exposed to in vitro-simulated clinical and experimental ciprofloxacin pharmacokinetic profiles for 96 h. As the average steady-state concentration (Cavg ss) increased, the rate of killing approached a maximum, and the rate of regrowth decreased. The enrichment of subpopulations with mutations in grlA and low-level ciprofloxacin resistance also varied depending on the pharmacokinetic environment. A regimen producing values for Cavg ss slightly above the MIC selected resistant variants with grlA mutations that did not evolve to higher levels of resistance. Clinical regimens which provided values for Cavg ss intermediate to the MIC and mutant prevention concentration (MPC) resulted in the emergence of subpopulations with gyrA mutations and higher levels of resistance. A regimen producing values for Cavg ss close to the MPC selected grlA mutants, but the appearance of subpopulations with higher levels of resistance was diminished. A regimen designed to maintain ciprofloxacin concentrations entirely above the MPC appeared to eradicate low-level resistant variants in the inoculum and prevent the emergence of

higher levels of resistance. There was no relationship between the time that ciprofloxacin concentrations remained between the MIC and the MPC and the degree of resistance or the presence or type of ciprofloxacin-resistance mutations that appeared in grlA or gyrA. Regimens designed to eradicate low-level resistant variants in S. aureus populations may prevent the emergence of higher levels of fluoroquinolone resistance.

Chen, N., D. E. Kyle, et al. (2003). "pfcrt Allelic Types with Two Novel Amino Acid Mutations in Chloroquine-Resistant Plasmodium falciparum Isolates from the Philippines." Antimicrob. Agents Chemother. **47**(11): 3500-3505.

http://aac.asm.org/cgi/content/abstract/47/11/3500

Mutations in the pfcrt and pfmdr1 genes have been associated with chloroquine resistance in Plasmodium falciparum. Ten and five mutations, respectively, have been identified in these genes from chloroquine-resistant parasites worldwide. Mutation patterns in pfcrt revealed that chloroquine resistance evolved independently in southeast Asia, South America, and Papua New Guinea. However, the evolution of chloroquine resistance in the rest of the Pacific region is unclear. In this study, we examined sequence polymorphisms in these genes in isolates from Morong, Philippines, and compared them to known chloroquine resistance sequences. Two novel mutations, A144T and L160Y, were identified outside of the 10 known mutations in pfcrt in Morong isolates. These novel mutations were identified only in parasites with K76T and N326D but without the common A220S mutation found in most chloroquine-resistant isolates. This represents a unique chloroquine resistance allelic type (K76T/A144T/L160Y/N326D) not previously found elsewhere in the world. One Morong isolate also had an additional C72S mutation, whereas only one isolate possessed an allelic type typical of chloroquine resistance in Asia. Parasites with the novel pfcrt allelic types were resistant to chloroquine in vitro and were unresponsive to verapamil (0.9 {micro}M) chemosensitization, similar to chloroquine-resistant parasites from South America and Papua New Guinea. These results suggest that chloroquine resistance evolved independently in the Philippines and represents a second chloroquine resistance founder event in the South Pacific.

Dicuonzo, G., E. Fiscarelli, et al. (2002). "Erythromycin-Resistant Pharyngeal Isolates of Streptococcus pyogenes Recovered in Italy." Antimicrob. Agents Chemother. **46**(12): 3987-3990.

http://aac.asm.org/cgi/content/abstract/46/12/3987

Three classes of macrolide resistance phenotypes and three different erythromycin resistance determinants were found among 127 erythromycin-resistant group A streptococcal (GAS) isolates recovered from 355 (35.8%) pediatric pharyngitis patients in Rome, Italy. According to emm and sof sequence typing results, erythromycin-resistant isolates comprised 11 different clonal types. Remarkably, 126 of the 127 macrolide-resistant isolates were serum opacity factor (sof) gene positive. These data suggest a strong association between macrolide resistance and the presence of sof among GAS isolates recovered from Italian pediatric pharyngitis patients.

Drusano, G. L., P. A. Bilello, et al. (2002). "Pharmacodynamics of Abacavir in an In Vitro Hollow-Fiber Model System." Antimicrob. Agents Chemother. **46**(2): 464-470.

http://aac.asm.org/cgi/content/abstract/46/2/464

Abacavir is a potent new carbocyclic nucleoside analogue. We employed our hollow-fiber pharmacodynamic modeling system to examine the antiretroviral effects of different abacavir exposures, as well as the impact of the schedule of drug administration on efficacy. Dose ranging of abacavir revealed that a concentration of four times the 50% effective concentration (EC50) (approximately the EC95) was required to inhibit the replication of human immunodeficiency virus type 1 (HIV-1) (strain MN) either in a continuous-infusion hollow-fiber experiment or in a classical tissue culture flask experiment. In contrast to earlier work with another drug class (HIV-1 protease inhibitors), addition of physiological amounts of the human drug binding proteins albumin and {alpha}1 acid glycoprotein revealed that there was little impact on the antiviral effect of the drug. Comparison of equivalent exposures (an area under the concentration-time curve [AUC] developed by approximately 500 mg per day of orally administered abacavir), either in a continuous-infusion mode or as a single oral dose of abacavir, demonstrated no difference in the ability to suppress either strain IIIB or strain MN. Comparison of administration of 250 mg every 12 h (q12h) versus once-daily administration of 500 mg for strain MN again showed no significant difference in suppressive effect. These experiments were carried out over 8 to 15 days. Because of these promising initial results, we extended the experiment to 30 days and examined three different schedules of administration that generated the same AUC at 24 h (AUC24): 300 mg q12h, 600 mg q24h, and 1,200 mg q48h. The aim of the last of these regimens was to definitively demonstrate schedule failure. There was little difference between the 1,200-mg q48h treatment group and the untreated control at 30 days. Likewise, there was little difference between the 600-mg q24h and 300-mg q12h treatment groups. However, at circa day 18 of the experiment, there was a small increase in viral output of p24 in the once-daily dosing unit. Examination of virus from all groups demonstrated no phenotypic or genotypic differences. The small difference in hollow-fiber unit p24 in the once-daily dosing group was not due to emergence of resistance over the 30-day single-drug exposure. We conclude that the dose of abacavir currently being studied in clinical trials (300 mg orally q12h) will be efficacious for the majority of sensitive clinical isolates of HIV-1. These in vitro data also suggest that this drug may be able to be administered to patients on a once-daily basis at a dose of 600 mg.

Durand, R., V. Huart, et al. (2002). "Rapid Detection of a Molecular Marker for Chloroquine-Resistant Falciparum Malaria." Antimicrob. Agents Chemother. **46**(8): 2684-2686.

http://aac.asm.org/cgi/content/abstract/46/8/2684

A PCR-based technique using molecular beacons was developed to detect the chloroquine resistance-associated pfcrt K76T point mutation in Plasmodium falciparum. One hundred thirty African clinical isolates were tested by the new method in comparison with the PCR-restriction fragment length polymorphism method. This rapid and inexpensive genomic assay could expand the possibilities for monitoring chloroquine resistance.

Gomes, A. R., S. Vinga, et al. (2005). "Analysis of the Genetic Variability of Virulence-Related Loci in Epidemic Clones of Methicillin-Resistant Staphylococcus aureus." Antimicrob. Agents Chemother. **49**(1): 366-379.

http://aac.asm.org/cgi/content/abstract/49/1/366

Methicillin-resistant Staphylococcus aureus (MRSA) isolates have previously been classified into major epidemic clonal types by pulsed-field gel electrophoresis in combination with multilocus sequence typing (MLST) and staphylococcal cassette chromosome mec typing. We aimed to investigate whether genetic variability in potentially polymorphic domains of virulence-related factors could provide another level of differentiation in a diverse collection of epidemic MRSA clones. The target regions of strains representative of epidemic clones and genetically related

methicillin-susceptible S. aureus isolates from the 1960s that were sequenced included the R domains of clfA and clfB; the D, W, and M regions of fnbA and fnbB; and three regions in the agr operon. Sequence variation ranged from very conserved regions, such as those for RNAIII and the agr interpromoter region, to the highly polymorphic R regions of the clf genes. The sequences of the clf R domains could be grouped into six major sequence types on the basis of the sequences in their 3' regions. Six sequence types were also observed for the fnb sequences at the amino acid level. From an evolutionary point of view, it was interesting that a small DNA stretch at the 3' clf R-domain sequence and the fnb sequences agreed with the results of MLST for this set of strains. In particular, clfB R-domain sequences, which had a high discriminatory capacity and with which the types distinguished were congruent with those obtained by other molecular typing methods, have potential for use for the typing of S. aureus. Clone- and strain-specific sequence motifs in the clf and fnb genes may represent useful additions to a typing methodology with a DNA array.

Goni-Urriza, M., C. Arpin, et al. (2002). "Type II Topoisomerase Quinolone Resistance-Determining Regions of Aeromonas caviae, A. hydrophila, and A. sobria Complexes and Mutations Associated with Quinolone Resistance." Antimicrob. Agents Chemother. **46**(2): 350-359.

http://aac.asm.org/cgi/content/abstract/46/2/350

Most Aeromonas strains isolated from two European rivers were previously found to be resistant to nalidixic acid. In order to elucidate the mechanism of this resistance, 20 strains of Aeromonas caviae (n = 10), A. hydrophila (n = 5), and A. sobria (n = 5) complexes, including 3 reference strains and 17 environmental isolates, were investigated. Fragments of the gyrA, gyrB, parC, and parE genes encompassing the quinolone resistance-determining regions (QRDRs) were amplified by PCR and sequenced. Results obtained for the six sensitive strains showed that the GyrA, GyrB, ParC, and ParE QRDR fragments of Aeromonas spp. were highly conserved ([>=]96.1% identity), despite some genetic polymorphism; they were most closely related to those of Vibrio spp., Pseudomonas spp., and members of the family Enterobacteriaceae (72.4 to 97.1% homology). All 14 environmental resistant strains carried a point mutation in the GyrA QRDR at codon 83, leading to the substitution Ser-83[-&gt]Ile (10 strains) or Ser-83[-&gt]Arg. In addition, seven strains harbored a mutation in the ParC QRDR either at position 80 (five strains), generating a Ser-80[-&gt]Ile (three strains) or Ser-80[-&gt]Arg change, or at position 84, yielding a Glu-84[-&gt]Lys modification. No amino acid alterations were discovered in the GyrB and ParE QRDRs. Double gyrA-parC missense mutations were associated with higher levels of quinolone resistance compared with the levels associated with single gyrA mutations. The most resistant strains probably had an additional mechanism(s) of resistance, such as decreased accumulation of the drugs. Our data suggest that, in mesophilic Aeromonas spp., as in other gram-negative bacteria, gyrase and topoisomerase IV are the primary and secondary targets for quinolones, respectively.

Hasman, H. and F. M. Aarestrup (2002). "tcrB, a Gene Conferring Transferable Copper Resistance in Enterococcus faecium: Occurrence, Transferability, and Linkage to Macrolide and Glycopeptide Resistance." Antimicrob. Agents Chemother. **46**(5): 1410-1416.

http://aac.asm.org/cgi/content/abstract/46/5/1410

A newly discovered gene, designated tcrB, which is located on a conjugative plasmid conferring acquired copper resistance in Enterococcus faecium, was identified in an isolate from a pig. The tcrB gene encodes a putative protein belonging to the CPx-type ATPase family with homology (46%) to the CopB protein from Enterococcus hirae. The tcrB gene was found in E. faecium isolated from pigs (75%), broilers (34%), calves (16%), and humans (10%) but not in isolates

from sheep. Resistant isolates, containing the tcrB gene, grew on brain heart infusion agar plates containing up to 28 mM CuSO4 compared to only 4 mM for the susceptible isolates. Copper resistance, and therefore the presence of the tcrB gene, was strongly correlated to macrolide and glycopeptide resistance in isolates from pigs, and the tcrB gene was shown to be located on the same conjugative plasmid as the genes responsible for resistance to these two antimicrobial agents. The frequent occurrence of this new copper resistance gene in isolates from pigs, where copper sulfate is being used in large amounts as feed additive, suggests that the use of copper has selected for resistance.

Imwong, M., S. Pukrittayakamee, et al. (2003). "Novel Point Mutations in the Dihydrofolate Reductase Gene of Plasmodium vivax: Evidence for Sequential Selection by Drug Pressure." Antimicrob. Agents Chemother. **47**(5): 1514-1521.

http://aac.asm.org/cgi/content/abstract/47/5/1514

Mutations in the dihydrofolate reductase (dhfr) genes of Plasmodium falciparum and P. vivax are associated with resistance to the antifolate antimalarial drugs. P. vivax dhfr sequences were obtained from 55 P. vivax isolates (isolates Belem and Sal 1, which are established lines originating from Latin America, and isolates from patient samples from Thailand [n = 44], India [n = 5], Iran [n = 2], and Madagascar [n = 2]) by direct sequencing of both strands of the purified PCR product and were compared to the P. vivax dhfr sequence from a P. vivax parasite isolated in Pakistan (isolate ARI/Pakistan), considered to represent the wild-type sequence. In total, 144 P. vivax dhfr mutations were found at only 12 positions, of which 4 have not been described previously. An F[-&gt]L mutation at residue 57 had been observed previously, but a novel codon (TTA) resulted in a mutation in seven of the nine mutated variant sequences. A new mutation at residue 117 resulted in S[-&gt]T (S[-&gt]N has been described previously). These two variants are the same as those observed in the P. falciparum dhfr gene at residue 108, where they are associated with different levels of antifolate resistance. Two novel mutations, I[-&gt]L at residue 13 and T[-&gt]M at residue 61, appear to be unique to P. vivax. The clinical, epidemiological, and sequence data suggest a sequential pathway for the acquisition of the P. vivax dhfr mutations. Mutations at residues 117 and 58 arise first when drug pressure is applied. Highly mutated genes carry the S[-&gt]T rather than the S[-&gt]N mutation at residue 117. Mutations at residues 57 and 61 then occur, followed by a fifth mutation at residue 13.

Kantor, R., W. J. Fessel, et al. (2002). "Evolution of Primary Protease Inhibitor Resistance Mutations during Protease Inhibitor Salvage Therapy." Antimicrob. Agents Chemother. **46**(4): 1086-1092.

http://aac.asm.org/cgi/content/abstract/46/4/1086

In order to track the evolution of primary protease inhibitor (PI) resistance mutations in human immunodeficiency virus type 1 (HIV-1) isolates, baseline and follow-up protease sequences were obtained from patients undergoing salvage PI therapy who presented initially with isolates containing a single primary PI resistance mutation. Among 78 patients meeting study selection criteria, baseline primary PI resistance mutations included L90M (42% of patients), V82A/F/T (27%), D30N (21%), G48V (6%), and I84V (4%). Despite the switching of treatment to a new PI, primary PI resistance mutations present at the baseline persisted in 66 of 78 (85%) patients. D30N persisted less frequently than L90M (50% versus 100%, respectively; $P < 0.001$) and V82A/F/T (50% versus 81%, respectively; $P = 0.05$). HIV-1 isolates from 38 (49%) patients failing PI salvage therapy developed new primary PI resistance mutations including L90M, I84V, V82A, and G48V. Common combinations of primary and secondary PI resistance mutations after salvage therapy included mutations at amino acid positions 10, 82, and 46 and/or 54 in 16 patients; 10, 90, and 71 and/or 73 in 14 patients; 10, 73, 84, 90, and 46 and/or 54 in 5 patients;

10, 48, and 82 in 5 patients; and 30, 88 and 90 in 5 patients. In summary, during salvage PI therapy, most HIV-1 isolates with a single primary PI resistance mutation maintained their original mutations, and 49% developed additional primary PI resistance mutations. The persistence of L90M, V82A/F/T, G48V, and I84V during salvage therapy suggests that these mutations play a role in clinical resistance to multiple PIs.

Kruuner, A., P. Jureen, et al. (2003). "Discordant Resistance to Kanamycin and Amikacin in Drug-Resistant Mycobacterium tuberculosis." Antimicrob. Agents Chemother. **47**(9): 2971-2973.

http://aac.asm.org/cgi/content/abstract/47/9/2971

It is generally thought that there is full cross-resistance in Mycobacterium tuberculosis between the aminoglycoside drugs kanamycin and amikacin. However, kanamycin resistance and amikacin susceptibility were seen in 43 of 79 (54%) multidrug-resistant Estonian isolates, indicating that there might be a need to test the resistance of M. tuberculosis isolates to both drugs.

Levy, D. D., B. Sharma, et al. (2004). "Single-Nucleotide Polymorphism Mutation Spectra and Resistance to Quinolones in Salmonella enterica Serovar Enteritidis with a Mutator Phenotype." Antimicrob. Agents Chemother. **48**(7): 2355-2363.

http://aac.asm.org/cgi/content/abstract/48/7/2355

Resistance to quinolone antibiotics has been associated with single-nucleotide polymorphisms (SNPs) in the quinolone resistance-determining region (QRDR) of gyrA. Mutations in the gyrA gene were compared by using mutant populations derived from wild-type Salmonella enterica serovar Enteritidis and its isogenic mutS:Tn10 mutator counterpart. Spontaneous mutants arising during nonselective growth were isolated by selection with either nalidixic acid, enrofloxacin, or ciprofloxacin. QRDR SNPs were identified in approximately 70% (512 of 695) of the isolates via colony hybridization with radiolabeled oligonucleotide probes. Notably, transition base substitution SNPs in the QRDR were dramatically increased in mutants derived from the mutS strain. Some, but not all, antibiotic-resistant mutants lacking QRDR SNPs were resistant to tetracycline and chloramphenicol, consistent with alterations in nonspecific efflux pumps or other membrane transport mechanisms. Changing the selection conditions shifted the mutation spectrum. Selection with ciprofloxacin was least likely to yield a mutant harboring either a QRDR SNP or chloramphenicol resistance. Selection with enrofloxacin was more likely to yield mutants containing Ser83[-&gt]Phe mutations, whereas selection with ciprofloxacin or nalidixic acid favored recovery of Asp87[-&gt]Gly mutants. Fluoroquinolone-resistant Salmonella strains isolated from veterinary or clinical settings frequently display a mutational spectrum with a preponderance of transition SNPs in the QRDR, the pattern found in vitro among mutS mutator mutants reported here. Both the preponderance of transition mutations and the varied mutation spectra reported for veterinary and clinical isolates suggest that bacterial mutators defective in methyl-directed mismatch repair may play a role in the emergence of quinolone and fluoroquinolone resistance in feral settings.

Marcelin, A.-G., I. Cohen-Codar, et al. (2005). "Virological and Pharmacological Parameters Predicting the Response to Lopinavir-Ritonavir in Heavily Protease Inhibitor-Experienced Patients." Antimicrob. Agents Chemother. **49**(5): 1720-1726.

http://aac.asm.org/cgi/content/abstract/49/5/1720

The genotypic inhibitory quotient (GIQ) has been proposed as a way to integrate drug exposure and genotypic resistance to protease inhibitors and can be useful to enhance the predictivity of virologic response for boosted protease inhibitors. The aim of this study was to evaluate the predictivity of the GIQ in 116 protease inhibitor-experienced patients treated with lopinavir-ritonavir. The overall decrease in human immunodeficiency virus type 1 (HIV-1) RNA from baseline to month 6 was a median of -1.50 log10 copies/ml and 40% of patients had plasma HIV-1 RNA below 400 copies/ml at month 6. The overall median lopinavir study-state Cmin concentration was 5,856 ng/ml. Using univariate linear regression analyses, both lopinavir GIQ and the number of baseline lopinavir mutations were highly associated with virologic response through 6 months. In the multivariate analysis, only lopinavir GIQ, baseline HIV RNA, and the number of prior protease inhibitors were significantly associated with response. When the analysis was limited to patients with more highly mutant viruses (three or more lopinavir mutations), only lopinavir GIQ remained significantly associated with virologic response. This study suggests that GIQ could be a better predictor of the virologic response than virological (genotype) or pharmacological (minimal plasma concentration) approaches used separately, especially among patients with at least three protease inhibitor resistance mutations. Therapeutic drug monitoring for patients treated by lopinavir-ritonavir would likely be most useful in patients with substantially resistant viruses.

Marcelin, A.-G., C. Lamotte, et al. (2003). "Genotypic Inhibitory Quotient as Predictor of Virological Response to Ritonavir-Amprenavir in Human Immunodeficiency Virus Type 1 Protease Inhibitor-Experienced Patients." Antimicrob. Agents Chemother. **47**(2): 594-600.

http://aac.asm.org/cgi/content/abstract/47/2/594

Forty-nine protease inhibitor (PI)-experienced but amprenavir (APV)-naive patients experiencing virological failure were treated with ritonavir (RTV) (100 mg twice a day [b.i.d.]) plus APV (600 mg b.i.d.). Patients responded to therapy with a median viral load decrease of -1.32 log10 by week 12. The addition of low-dose RTV enhanced the minimal APV concentration in plasma (APV Cmin) up to 10-fold compared with that obtained with APV (1,200 mg b.i.d.) without RTV. Baseline PI resistance mutations (L10F/I/V, K20M/R, E35D, R41K, I54V, L63P, V82A/F/T/S, I84V) identified by univariate analysis and included in a genotypic score and APV Cmin at week 8 were predictive of the virological response at week 12. The response to APV plus RTV was significantly reduced in patients with six or more of the resistance mutations among the ones defined above. The genotypic inhibitory quotient, calculated as the ratio of the APV Cmin to the number of human immunodeficiency virus type 1 protease mutations, was a better predictor than the virological or pharmacological variables used alone. This genotypic inhibitory quotient could be used in therapeutic drug monitoring to define the concentrations in plasma needed to control replication of viruses with different levels of PI resistance, as measured by the number of PI resistance mutations.

Mariam, D. H., Y. Mengistu, et al. (2004). "Effect of rpoB Mutations Conferring Rifampin Resistance on Fitness of Mycobacterium tuberculosis." Antimicrob. Agents Chemother. **48**(4): 1289-1294.

http://aac.asm.org/cgi/content/abstract/48/4/1289

Rifampin is a major drug used in the treatment of tuberculosis infections, and increasing rifampin resistance represents a worldwide clinical problem. Resistance to rifampin is caused by mutations in the rpoB gene, encoding the {beta}-subunit of RNA polymerase. We examined the effect of

three different rpoB mutations on the fitness of Mycobacterium tuberculosis. Rifampin-resistant mutants were isolated from a virulent clinical isolate of M. tuberculosis (strain Harlingen) in vitro at a mutation frequency of 2.3 x 10-8. Mutations in the rpoB gene were identified, and the growth rates of three defined mutants were measured by competition with the susceptible parent strain in laboratory medium and by single cultures in a macrophage cell line and in laboratory medium. All of the mutants showed a decreased growth rate in the three assays. The relative fitness of the mutants varied between 0.29 and 0.96 (that of the susceptible strain was set to 1.0) depending on the specific mutant and assay system. Unexpectedly, the relative fitness ranking of the mutants differed between the different assays. In conclusion, rifampin resistance is associated with a cost that is conditional.

Martinez, M. A. and B. Clotet (2003). "Genetic Screen for Monitoring Hepatitis C Virus NS3 Serine Protease Activity." Antimicrob. Agents Chemother. **47**(5): 1760-1765.

http://aac.asm.org/cgi/content/abstract/47/5/1760

We have developed a genetic system to monitor the activity of the hepatitis C virus (HCV) NS3 serine protease. This genetic system is based on the bacteriophage lambda regulatory circuit where the viral repressor cI is specifically cleaved to initiate the switch from lysogeny to lytic infection. An HCV protease-specific target, NS5A-5B, was inserted into the lambda phage cI repressor. The target specificity of the HCV NS5A-5B repressor was evaluated by coexpression of this repressor with a {beta}-galactosidase ({beta}gal)-HCV NS32-181/421-34 protease construct. Upon infection of Escherichia coli cells containing the two plasmids encoding the cI.HCV5AB-cro and the {beta}gal-HCV NS32-181/421-34 protease constructs, lambda phage replicated up to 8,000-fold more efficiently than in cells that did not express the HCV NS32-181/421-34 protease. This simple, rapid, and highly specific assay can be used to monitor the activity of the HCV NS3 serine protease, and it has the potential to be used for screening specific inhibitors.

Mascher, T., S. L. Zimmer, et al. (2004). "Antibiotic-Inducible Promoter Regulated by the Cell Envelope Stress-Sensing Two-Component System LiaRS of Bacillus subtilis." Antimicrob. Agents Chemother. **48**(8): 2888-2896.

http://aac.asm.org/cgi/content/abstract/48/8/2888

Soil bacteria are among the most prodigious producers of antibiotics. The Bacillus subtilis LiaRS (formerly YvqCE) two-component system is one of several antibiotic-sensing systems that coordinate the genetic response to cell wall-active antibiotics. Upon the addition of vancomycin or bacitracin, LiaRS autoregulates the liaIHGFSR operon. We have characterized the promoter of the lia operon and defined the cis-acting sequences necessary for antibiotic-inducible gene expression. A survey for compounds that act as inducers of the lia promoter revealed that it responds strongly to a subset of cell wall-active antibiotics that interfere with the lipid II cycle in the cytoplasmic membrane (bacitracin, nisin, ramoplanin, and vancomycin). Chemicals that perturb the cytoplasmic membrane, such as organic solvents, are also weak inducers. Thus, the reporter derived from PliaI (the liaI promoter) provides a tool for the detection and classification of antimicrobial compounds.

Masselot, F., A. Boulos, et al. (2003). "Molecular Evaluation of Antibiotic Susceptibility: Tropheryma whipplei Paradigm." Antimicrob. Agents Chemother. **47**(5): 1658-1664.

http://aac.asm.org/cgi/content/abstract/47/5/1658

Tropheryma whipplei, the agent of Whipple's disease, grows fastidiously only in cell cultures without plaque production, and only three strains have been passaged. The formation of bacterial clumps in the supernatant precludes enumeration of viable bacteria and MIC determination. We evaluated the bacteriostatic effects of fluoroquinolones against two T. whipplei isolates by measuring the inhibition of the DNA copy number increase by real-time quantitative PCR. The analysis of the T. whipplei genome database allowed the identification not only of the gyrA gene but also the parC gene encoding the alpha subunit of the natural fluoroquinolone targets DNA gyrase (GyrA) and topoisomerase IV (ParC), respectively. The parC gene was detected in actinobacteria for the first time. High ciprofloxacin MICs (4 and 8 {micro}g/ml) were correlated with the presence in T. whipplei GyrA and ParC sequences with an alanine residue at positions 83 and 80 (Escherichia coli numbering), respectively. Alanines at these positions have previously been associated with increased fluoroquinolone resistance in E. coli and mycobacteria. However, the MIC of levofloxacin was low (0.25 {micro}g/ml). The same T. whipplei GyrA and ParC sequences were found in two other cultured strains and in nine uncultured tissue samples from Whipple's disease patients, allowing one to speculate that T. whipplei is naturally relatively resistant to fluoroquinolones.

Nascimento, A. M., G. H. Goldman, et al. (2003). "Multiple Resistance Mechanisms among Aspergillus fumigatus Mutants with High-Level Resistance to Itraconazole." Antimicrob. Agents Chemother. **47**(5): 1719-1726.

http://aac.asm.org/cgi/content/abstract/47/5/1719

A collection of Aspergillus fumigatus mutants highly resistant to itraconazole (RIT) at 100 {micro}g ml-1 were selected in vitro (following UV irradiation as a preliminary step) to investigate mechanisms of drug resistance in this clinically important pathogen. Eight of the RIT mutants were found to have a mutation at Gly54 (G54E, -K, or -R) in the azole target gene CYP51A. Primers designed for highly conserved regions of multidrug resistance (MDR) pumps were used in reverse transcriptase PCR amplification reactions to identify novel genes encoding potential MDR efflux pumps in A. fumigatus. Two genes, AfuMDR3 and AfuMDR4, showed prominent changes in expression levels in many RIT mutants and were characterized in more detail. Analysis of the deduced amino acid sequence encoded by AfuMDR3 revealed high similarity to major facilitator superfamily transporters, while AfuMDR4 was a typical member of the ATP-binding cassette superfamily. Real-time quantitative PCR with molecular beacon probes was used to assess expression levels of AfuMDR3 and AfuMDR4. Most RIT mutants showed either constitutive high-level expression of both genes or induction of expression upon exposure to itraconazole. Our results suggest that overexpression of one or both of these newly identified drug efflux pump genes of A. fumigatus and/or selection of drug target site mutations are linked to high-level itraconazole resistance and are mechanistic considerations for the emergence of clinical resistance to itraconazole.

Perilli, M., B. Segatore, et al. (2002). "Characterization of a New Extended-Spectrum {beta}-Lactamase (TEM-87) Isolated in Proteus mirabilis during an Italian Survey." Antimicrob. Agents Chemother. **46**(3): 925-928.

http://aac.asm.org/cgi/content/abstract/46/3/925

A new natural TEM derivative, named TEM-87, was identified in a Proteus mirabilis isolate from an Italian hospital. Compared to TEM-1, TEM-87 contains the following mutations: E104K,

R164C, and M182T. Kinetic analysis of TEM-87 revealed extended-spectrum activity against oxyimino cephalosporins (preferentially ceftazidime) and aztreonam. Expression of blaTEM-87 in Escherichia coli decreased the host susceptibility to these drugs.

Portugal, I., L. Barreiro, et al. (2004). "pncA Mutations in Pyrazinamide-Resistant Mycobacterium tuberculosis Isolates in Portugal." Antimicrob. Agents Chemother. **48**(7): 2736-2738.

http://aac.asm.org/cgi/content/abstract/48/7/2736

The nucleotide sequences of the pncA genes within 55 multidrug-resistant pyrazinamide-resistant Mycobacterium tuberculosis clinical isolates were determined. Fifty-three out of the 55 isolates were pyrazinamidase (PZase) negative. Four strains contained a wild-type pncA gene, and PZase activity was undetectable in two of these strains. Seven of the 18 identified pncA mutations found have not been described in previous studies.

Rafii, F., M. Park, et al. (2005). "Alterations in DNA Gyrase and Topoisomerase IV in Resistant Mutants of Clostridium perfringens Found after In Vitro Treatment with Fluoroquinolones." Antimicrob. Agents Chemother. **49**(2): 488-492.

http://aac.asm.org/cgi/content/abstract/49/2/488

To compare mutations in the DNA gyrase (gyrA and gyrB) and topoisomerase IV (parC and parE) genes of Clostridium perfringens, which are associated with in vitro exposure to fluoroquinolones, resistant mutants were selected from eight strains by serial passage in the presence of increasing concentrations of norfloxacin, ciprofloxacin, gatifloxacin, or trovafloxacin. The nucleotide sequences of the entire gyrA, gyrB, parC, and parE genes of 42 mutants were determined. DNA gyrase was the primary target for each fluoroquinolone, and topoisomerase IV was the secondary target. Most mutations appeared in the quinolone resistance-determining regions of gyrA (resulting in changes of Asp-87 to Tyr or Gly-81 to Cys) and parC (resulting in changes of Asp-93 or Asp-88 to Tyr or Ser-89 to Ile); only two mutations were found in gyrB, and only two mutations were found in parE. More mutants with multiple gyrA and parC mutations were produced with gatifloxacin than with the other fluoroquinolones tested. Allelic diversity was observed among the resistant mutants, for which the drug MICs increased 2- to 256-fold. Both the structures of the drugs and their concentrations influenced the selection of mutants.

Reinhardt, A. K., C. M. Bebear, et al. (2002). "Characterization of Mutations in DNA Gyrase and Topoisomerase IV Involved in Quinolone Resistance of Mycoplasma gallisepticum Mutants Obtained In Vitro." Antimicrob. Agents Chemother. **46**(2): 590-593.

http://aac.asm.org/cgi/content/abstract/46/2/590

Mycoplasma gallisepticum enrofloxacin-resistant mutants were generated by stepwise selection in increasing concentrations of enrofloxacin. Alterations were found in the quinolone resistance-determining regions of the four target genes encoding DNA gyrase and topoisomerase IV from these mutants. This is the first description of such mutations in an animal mycoplasma species.

Rodrigues, V. d. F. S., M. A. Telles, et al. (2005). "Characterization of pncA Mutations in Pyrazinamide-

Resistant Mycobacterium tuberculosis in Brazil." Antimicrob. Agents Chemother. **49**(1): 444-446.

http://aac.asm.org/cgi/content/abstract/49/1/444

In this study the nucleotide sequence of the pncA gene from 59 Mycobacterium tuberculosis clinical isolates was analyzed. Mutations in the pncA gene were identified in 29 of 40 pyrazinamide-resistant isolates, and no pyrazinamidase activity was detected in 39 of them. Twelve mutations found in this work have not been described previously.

Ruzin, A., M. A. Visalli, et al. (2005). "Influence of Transcriptional Activator RamA on Expression of Multidrug Efflux Pump AcrAB and Tigecycline Susceptibility in Klebsiella pneumoniae." Antimicrob. Agents Chemother. **49**(3): 1017-1022.

http://aac.asm.org/cgi/content/abstract/49/3/1017

Tigecycline is an expanded broad-spectrum antibacterial agent that is active against many clinically relevant species of bacterial pathogens, including Klebsiella pneumoniae. The majority of K. pneumoniae isolates are fully susceptible to tigecycline; however, a few strains that have decreased susceptibility have been isolated. One isolate, G340 (for which the tigecycline MIC is 4 {micro}g/ml and which displays a multidrug resistance [MDR] phenotype), was selected for analysis of the mechanism for this decreased susceptibility by use of transposon mutagenesis with IS903{phi}kan. A tigecycline-susceptible mutant of G340, GC7535, was obtained (tigecycline MIC, 0.25 {micro}g/ml). Analysis of the transposon insertion mapped it to ramA, a gene that was previously identified to be involved in MDR in K. pneumoniae. For GC7535, the disruption of ramA led to a 16-fold decrease in the MIC of tigecycline and also a suppression of MDR. Trans-complementation with plasmid-borne ramA restored the original parental phenotype of decreased susceptibility to tigecycline. Northern blot analysis revealed a constitutive overexpression of ramA that correlated with an increased expression of the AcrAB transporter in G340 compared to that in tigecycline-susceptible strains. Laboratory mutants of K. pneumoniae with decreased susceptibility to tigecycline could be selected at a frequency of approximately 4 x 10-8. These results suggest that ramA is associated with decreased tigecycline susceptibility in K. pneumoniae due to its role in the expression of the AcrAB multidrug efflux pump.

Simjee, S., D. G. White, et al. (2002). "Identification of vat(E) in Enterococcus faecalis Isolates from Retail Poultry and Its Transferability to Enterococcus faecium." Antimicrob. Agents Chemother. **46**(12): 3823-3828.

http://aac.asm.org/cgi/content/abstract/46/12/3823

Sixteen isolates of Enterococcus faecalis were recovered from retail poultry samples (seven chickens and nine turkeys) purchased from grocery stores in the greater Washington, D.C., area. PCR for known streptogramin resistance genes identified vat(E) in five E. faecalis isolates (three isolates from chickens and two isolates from turkeys). The vat(E) gene was transmissible on a ca. 70-kb plasmid, along with resistance to erythromycin, tetracycline, and streptomycin, by conjugation to E. faecalis and Enterococcus faecium recipient strains. DNA sequencing showed little variation between E. faecalis vat(E) genes from the chicken samples; however, one E. faecalis vat(E) gene from a turkey sample possessed 5 nucleotide changes that resulted in four amino acid substitutions. None of these substitutions in the vat(E) allele have previously been described. This is the first report of vat(E) in E. faecalis and its transferability to E. faecium, which indicates that E. faecalis can act as a reservoir for the dissemination of vat(E)-mediated streptogramin resistance to E. faecium.

Smith, R. P., A. L. Baltch, et al. (2004). "In Vitro Activities of Garenoxacin and Levofloxacin against Chlamydia pneumoniae Are Not Affected by Presence of Mycoplasma DNA." Antimicrob. Agents Chemother. **48**(6): 2081-2084.

http://aac.asm.org/cgi/content/abstract/48/6/2081

We studied 20 Chlamydia pneumoniae isolates obtained from respiratory sites and atheroma tissue of patients from various geographic areas to determine the susceptibilities of these isolates to a new des-fluoroquinolone, garenoxacin, and to levofloxacin. In addition, we assessed the cultures with these isolates by PCR for the presence or absence of Mycoplasma sp. DNA. Both the MIC at which 90% of isolates are inhibited (MIC90) and the minimal bactericidal concentration at which 90% of isolates are killed (MBC90) for garenoxacin were 0.06 {micro}g/ml, and both the MIC90 and the MBC90 for levofloxacin were 2.0 {micro}g/ml. The activity of garenoxacin against C. pneumoniae was 32-fold greater than that of levofloxacin. Mycoplasma sp. DNA was detected by PCR in 17 of 20 cultures. Mycoplasma amplicons from five Mycoplasma DNA-positive C. pneumoniae cultures were sequenced and found to represent four Mycoplasma species. Our data demonstrate that C. pneumoniae cultures frequently contain Mycoplasma DNA and that its presence in C. pneumoniae cultures does not appear to affect the susceptibility results for the two fluoroquinolones that we tested.

Spanu, T., F. Luzzaro, et al. (2002). "Occurrence of Extended-Spectrum {beta}-Lactamases in Members of the Family Enterobacteriaceae in Italy: Implications for Resistance to {beta}-Lactams and Other Antimicrobial Drugs." Antimicrob. Agents Chemother. **46**(1): 196-202.

http://aac.asm.org/cgi/content/abstract/46/1/196

An Italian nationwide survey was carried out to assess the prevalences and the antimicrobial susceptibilities of members of the family Enterobacteriaceae producing extended-spectrum {beta}-lactamases (ESBLs). Over a 6-month period, 8,015 isolates were obtained from hospitalized patients and screened for resistance to extended-spectrum cephalosporins and monobactams. On the basis of a synergistic effect between clavulanate and selected {beta}-lactams (ceftazidime, aztreonam, cefotaxime, cefepime, and ceftriaxone), 509 isolates were found to be ESBL positive (6.3%). Colony blot hybridization with blaTEM and blaSHV DNA probes allowed one to distinguish four different genotypes: TEM-positive, SHV-positive, TEM- and SHV-positive, and non-TEM, non-SHV ESBL types. MICs for each isolate (E-test) were obtained for widely used {beta}-lactams, combinations of {beta}-lactams with {beta}-lactamase inhibitors, aminoglycosides, and fluoroquinolones. Among ESBL-positive strains, Klebsiella pneumoniae, Proteus mirabilis, and Escherichia coli accounted for 73.6% of isolates. Overall, TEM-type ESBLs were more prevalent than SHV-type enzymes (234 versus 173), whereas the prevalence of strains producing both TEM- and SHV-type ESBLs was similar to that of isolates producing non-TEM, non-SHV enzymes (55 and 38, respectively). In vitro, all but one of the ESBL-producing isolates remained susceptible to imipenem. Susceptibility to other drugs varied: piperacillin-tazobactam, 91%; amoxicillin-clavulanic acid, 85%; cefoxitin, 78%; amikacin, 76%; ampicillin-sulbactam, 61%; ciprofloxacin, 58%; and gentamicin, 56%. Associated resistance to aminoglycosides and ciprofloxacin was observed most frequently among TEM-positive strains. Since therapeutic options for multiresistant Enterobacteriaceae are limited, combinations of {beta}-lactams and {beta}-lactamase inhibitors appear to represent an important alternative for treating infections caused by ESBL-producing Enterobacteriaceae.

Sullivan, A., A. Fianu-Jonasson, et al. (2005). "Ecological Effects of Perorally Administered Pivmecillinam on the Normal Vaginal Microflora." Antimicrob. Agents Chemother. **49**(1): 170-175.

http://aac.asm.org/cgi/content/abstract/49/1/170

The knowledge of the effects of antimicrobial agents on the normal vaginal microflora is limited. The objective of the present study was to study the ecological impact of pivmecillinam on the normal vaginal microflora. In 20 healthy women, the estimated day of ovulation was determined during three subsequent menstrual cycles. Microbiological and clinical examinations were performed on the estimated day of ovulation and on day 3 in all cycles and also on day 7 after ovulation in cycles 1 and 2. Anaerobic and facultative anaerobic gram-positive rods, mainly species of lactobacilli and actinomycetes, dominated the microflora. One woman was colonized on the third day of administration with a resistant Escherichia coli strain, and Candida albicans was detected in one woman on days 3 and 7 in cycle 2. No other major changes in the normal microflora occurred during the study. Administration of pivmecillinam had a minor ecological impact on the normal vaginal microflora.

Svicher, V., F. Ceccherini-Silberstein, et al. (2005). "Novel Human Immunodeficiency Virus Type 1 Protease Mutations Potentially Involved in Resistance to Protease Inhibitors." Antimicrob. Agents Chemother. **49**(5): 2015-2025.

http://aac.asm.org/cgi/content/abstract/49/5/2015

Plasma-derived sequences of human immunodeficiency virus type 1 (HIV-1) protease from 1,162 patients (457 drug-naive patients and 705 patients receiving protease inhibitor [PI]-containing antiretroviral regimens) led to the identification and characterization of 17 novel protease mutations potentially associated with resistance to PIs. Fourteen mutations were positively associated with PIs and significantly correlated in pairs and/or clusters with known PI resistance mutations, suggesting their contribution to PI resistance. In particular, E34Q, K43T, and K55R, which were associated with lopinavir treatment, correlated with mutations associated with lopinavir resistance (E34Q with either L33F or F53L, or K43T with I54A) or clustered with multi-PI resistance mutations (K43T with V82A and I54V or V82A, V32I, and I47V, or K55R with V82A, I54V, and M46I). On the other hand, C95F, which was associated with treatment with saquinavir and indinavir, was highly expressed in clusters with either L90M and I93L or V82A and G48V. K45R and K20T, which were associated with nelfinavir treatment, were specifically associated with D30N and N88D and with L90M, respectively. Structural analysis showed that several correlated positions were within 8 A of each other, confirming the role of the local environment for interactions among mutations. We also identified three protease mutations (T12A, L63Q, and H69N) whose frequencies significantly decreased in PI-treated patients compared with that in drug-naive patients. They never showed positive correlations with PI resistance mutations; if anything, H69N showed a negative correlation with the compensatory mutations M36I and L10I. These mutations may prevent the appearance of PI resistance mutations, thus increasing the genetic barrier to PI resistance. Overall, our study contributes to a better definition of protease mutational patterns that regulate PI resistance and strongly suggests that other (novel) mutations beyond those currently known to confer resistance should be taken into account to better predict resistance to antiretroviral drugs.

Tomasinsig, L., M. Scocchi, et al. (2004). "Genome-Wide Transcriptional Profiling of the Escherichia coli Response to a Proline-Rich Antimicrobial Peptide." Antimicrob. Agents Chemother. **48**(9): 3260-3267.

http://aac.asm.org/cgi/content/abstract/48/9/3260

Most antimicrobial peptides (AMPs) impair the viability of target bacteria by permeabilizing bacterial membranes. However, the proline-rich AMPs have been shown to kill susceptible organisms without causing significant membrane perturbation and may act by inhibiting the activity of bacterial targets. To gain initial insight into the events that follow interaction of a proline-rich peptide with bacterial cells, we used DNA macroarray technology to monitor transcriptional alterations of Escherichia coli in response to challenge with a subinhibitory concentration of the proline-rich Bac7(1-35). Substantial changes in the expression levels of 70 bacterial genes from various functional categories were detected. Among these, 26 genes showed decreased expression, while 44 genes, including genes that are potentially involved in bacterial resistance to antimicrobials, showed increased expression. The generation of a transcriptional response under the experimental conditions used is consistent with the ability of Bac7(1-35) to interact with bacterial components and affect biological processes in this organism.

Wain, J., L. T. Diem Nga, et al. (2003). "Molecular Analysis of incHI1 Antimicrobial Resistance Plasmids from Salmonella Serovar Typhi Strains Associated with Typhoid Fever." Antimicrob. Agents Chemother. **47**(9): 2732-2739.

http://aac.asm.org/cgi/content/abstract/47/9/2732

The first outbreak of multidrug-resistant (MDR) typhoid fever in Vietnam was in 1993, and by 1995 nearly 90% of cases were MDR. Plasmid HCM1, sequenced in full, is an incHI1 plasmid from Salmonella enterica serovar Typhi strain CT18, isolated in Vietnam in 1993. Restriction analysis shows that pHCM1 shares a restriction fragment length polymorphism (RFLP) pattern with plasmids isolated from the first outbreak and 10 of 17 MDR plasmids isolated from sporadic cases occurring at the same time in Vietnam. A core region of pHCM1 has significant DNA sequence similarity to plasmid R27, isolated in 1961 from S. enterica in the United Kingdom. There are five regions of DNA in pHCM1 which are not present in R27. Two of these are putative acquisition regions; the largest is 34.955 kbp in length and includes sequences of several antibiotic resistance genes and several insertion sequences. The borders of this region are defined by two identical IS10 left elements, associated with an inversion of DNA or with a truncated Tn10 element. The second, smaller region is 14.751 kbp and carries a trimethoprim resistance gene dfr14A cassette associated with a class 1 integrase. In 1993 to 1994, restriction analysis revealed some variations in the structures of Salmonella serovar Typhi MDR plasmids which were mapped to the two putative acquisition regions and three smaller variable regions. In 1996 a single RFLP type, RFLP7, was found to carry the dfrA7 and sul-1 genes, which were not present on R27 or pHCM1. This plasmid type appears to have a selective advantage over other plasmids with the same resistance phenotype.

Weigel, L. M., G. J. Anderson, et al. (2002). "DNA Gyrase and Topoisomerase IV Mutations Associated with Fluoroquinolone Resistance in Proteus mirabilis." Antimicrob. Agents Chemother. **46**(8): 2582-2587.

http://aac.asm.org/cgi/content/abstract/46/8/2582

Mutations associated with fluoroquinolone resistance in clinical isolates of Proteus mirabilis were determined by genetic analysis of the quinolone resistance-determining region (QRDR) of gyrA, gyrB, parC, and parE. This study included the P. mirabilis type strain ATCC 29906 and 29 clinical isolates with reduced susceptibility (MIC, 0.5 to 2 {micro}g/ml) or resistance (MIC, [>=]4 {micro}g/ml) to ciprofloxacin. Susceptibility profiles for ciprofloxacin, clinafloxacin, gatifloxacin,

gemifloxacin, levofloxacin, moxifloxacin, and trovafloxacin were correlated with amino acid changes in the QRDRs. Decreased susceptibility and resistance were associated with double mutations involving both gyrA (S83R or -I) and parC (S80R or -I). Among these double mutants, MICs of ciprofloxacin varied from 1 to 16 {micro}g/ml, indicating that additional factors, such as drug efflux or porin changes, also contribute to the level of resistance. For ParE, a single conservative change of V364I was detected in seven strains. An unexpected result was the association of gyrB mutations with high-level resistance to fluoroquinolones in 12 of 20 ciprofloxacin-resistant isolates. Changes in GyrB included S464Y (six isolates), S464F (three isolates), and E466D (two isolates). A three-nucleotide insertion, resulting in an additional lysine residue between K455 and A456, was detected in gyrB of one strain. Unlike any other bacterial species analyzed to date, mutation of gyrB appears to be a frequent event in the acquisition of fluoroquinolone resistance among clinical isolates of P. mirabilis.

Yu, W. L., P. L. Winokur, et al. (2002). "First Description of Klebsiella pneumoniae Harboring CTX-M {beta}-Lactamases (CTX-M-14 and CTX-M-3) in Taiwan." Antimicrob. Agents Chemother. **46**(4): 1098-1100.

http://aac.asm.org/cgi/content/abstract/46/4/1098

Klebsiella pneumoniae isolates from Taiwan medical centers (50 strains; 1998 to 2000) with a CTX-M resistance phenotype (ceftazidime susceptible and ceftriaxone or cefotaxime nonsusceptible) were selected for initial isoelectric focusing analysis. {beta}-Lactamases with pIs of 7.9 (n = 22) and 8.4 (n = 28) in addition to 5.4 and/or 7.6 were detected. DNA gene sequencing identified the {beta}-lactamases with pIs of 7.9 and 8.4 as CTX-M-14 and CTX-M-3, respectively. Molecular typing suggested inter- and intrahospital clonal dissemination of these Taiwanese CTX-M-producing Klebsiella strains.

Zwiers, L.-H., I. Stergiopoulos, et al. (2002). "ABC Transporters and Azole Susceptibility in Laboratory Strains of the Wheat Pathogen Mycosphaerella graminicola." Antimicrob. Agents Chemother. **46**(12): 3900-3906.

http://aac.asm.org/cgi/content/abstract/46/12/3900

Laboratory strains of Mycosphaerella graminicola with decreased susceptibilities to the azole antifungal agent cyproconazole showed a multidrug resistance phenotype by exhibiting cross-resistance to an unrelated chemical, cycloheximide or rhodamine 6G, or both. Decreased azole susceptibility was found to be associated with either decreased or increased levels of accumulation of cyproconazole. No specific relationship could be observed between azole susceptibility and the expression of ATP-binding cassette (ABC) transporter genes MgAtr1 to MgAtr5 and the sterol P450 14{alpha}-demethylase gene, CYP51. ABC transporter MgAtr1 was identified as a determinant in azole susceptibility since heterologous expression of the protein reduced the azole susceptibility of Saccharomyces cerevisiae and disruption of MgAtr1 in one specific M. graminicola laboratory strain with constitutive MgAtr1 overexpression restored the level of susceptibility to cyproconazole to wild-type levels. However, the level of accumulation in the mutant with an MgAtr1 disruption did not revert to the wild-type level. We propose that variations in azole susceptibility in laboratory strains of M. graminicola are mediated by multiple mechanisms.

Antiviral Research (19)

Anderson, B. D., T. Shirasaka, et al. (1994). "Identification of drug-related genotypic changes in HIV-1 from serum using the selective polymerase chain reaction." Antiviral Research **25**(3-4): 245.

http://www.sciencedirect.com/science/article/B6T2H-476MP7D-7/2/c7ad6e137668c707ff6c306070ebde34

We attempted to detect drug-related HIV-1 pol gene mutations by selective polymerase chain reaction (PCR) using both proviral DNA and viral RNA isolated from patients (pts) with AIDS or ARC receiving antiretroviral therapy. Peripheral blood mononuclear cell (PBM)-associated proviral DNA and serum-derived viral RNA were obtained from eight patients before and after receiving an alternating regimen of AZT and ddC for 15-41 months or ddI monotherapy for 12-26 months. These specimens were examined for the presence of mutations at positions 70, 74, 215 and 219. We noted that selective PCR results can be ambiguous depending on the quantity of DNA template employed. We, therefore, used the minimal quantity of DNA templates that yielded evaluable PCR products in this study. For all the eight pairs of pre- and post-therapy proviral DNA samples, selective PCR results agreed with independently determined nucleotide sequences. Results of reverse transcription of serum-derived viral RNA followed by selective PCR differed in some cases from those using the proviral DNA. In particular, the use of serum viral RNA appeared to allow earlier detection of changes in drug-related mutations than the use of PBM-associated proviral DNA. We conclude that (i) selective PCR using the minimum and sufficient number of PBM-associated proviral DNA and serum viral RNA copies successfully detects the presence of known pol gene mutations; (ii) drug-related mutations may be distinguished earlier in virions in serum (or plasma) than in proviral DNA in PBM; and (iii) quantification of HIV-1 prior to selective PCR may be an important component in monitoring the therapy of HIV-1 infection.

Chang, J.-S., H.-W. Liu, et al. (2005). "Ethanol extract of Polygonum cuspidatum inhibits hepatitis B virus in a stable HBV-producing cell line." Antiviral Research **66**(1): 29.

http://www.sciencedirect.com/science/article/B6T2H-4FCRC79-1/2/7d371194ec42a72a6ebe978b988f2c49

Chronic hepatitis B virus (HBV) infection is endemic in Asia and its consequences are among the major public health problems in the world. Unfortunately, the therapeutic efficacies of present strategies are still unsatisfactory with a major concern about viral mutation. In search of effective antiviral agent, we examined the efficacy of extracts of Polygonum cuspidatum Sieb. et Zucc. (P. cuspidatum) against HBV in HepG2 2.2.15 cells by quantitative real time polymerase chain reaction. The expressions of viral antigens, HBeAg and HBsAg, were also determined by enzyme linked immunosorbent assay. The ethanol extract of P. cuspidatum could inhibit dose-dependently the production of HBV (p P. cuspidatum might also inhibit the production of HBV at a higher dosage. The expression of HBsAg was significantly increased by both ethanol extract and water extract of P. cuspidatum dose-dependently (p p P. cuspidatum (30 [mu]g/ml) could inhibit the expression of HBeAg (p P. cuspidatum might contain compounds that would contribute to the control of HBV infection in the future. However, its promoting effect on the expression of HBsAg and its cytotoxicity should be monitored. Further purification of the active compounds, identification and modification of their structures to improve the efficacy and decrease the cytotoxicity are required.

Chen, S.-H., J. E. Oakes, et al. (1994). "Synergistic anti-herpes effect of TNF-[alpha] and IFN-[gamma] in human corneal epithelial cells compared with that in corneal fibroblasts." Antiviral Research **25**(3-4): 201.

http://www.sciencedirect.com/science/article/B6T2H-476MP7D-4/2/423fc428ad10b7f5c0b60a4f68a00a3b

In this study we compared how effectively the proinflammatory cytokines TNF-[alpha] and IFN-[beta] could inhibit HSV-1 replication in human corneal tissue fragments and monolayers of epithelial cells and fibroblasts derived from intact corneas, and investigated the mechanism responsible for the inhibition. Pretreatment of corneal tissue or cells derived therefrom with TNF-[alpha] (50 U/ml) and IFN-[gamma] (5 IU/ml) consistently induced a synergistic antiviral effect. Inhibition of HSV-1 growth was most evident in fibroblasts (> 1000-fold reduction) and less apparent (7-25-fold reduction) when epithelial cells were the target. Virus suppression was correlated with the induction of IFN-[beta] because antibody to this cytokine but not IFN-[alpha] abrogated synergism. The more modest synergistic effect in epithelial cells was associated with a >= 4-fold reduction in the synthesis of IFN-[beta] protein and mRNA, and decreased responsiveness of these cells to the antiviral effect of IFN-[beta]. We conclude that the combination of TNF-[alpha] and IFN-[gamma] induces a synergistic antiviral effect in corneal cells. The degree of synergism observed varies with the corneal cell type, and is correlated with the amount of IFN-[beta] induced and the target cell responsiveness to the antiviral action of this cytokine.

Duan, J., J. D. Marte, et al. (2004). "A novel model of HPV infection in meshed human foreskin grafts." Antiviral Research **64**(3): 179.

http://www.sciencedirect.com/science/article/B6T2H-4DGT6X5-1/2/0762770676a2b9c1440d032181ab4744

The present study describes a novel meshing procedure that provided successful low-risk papillomavirus propagation and reproducible wart induction in human foreskin xenografts. The initial HPV6 and/or 11 inocula were collected from clinically excised human wart tissues and confirmed to be free of HPV16, 18 and 31 by PCR analysis. Human foreskin grafts were collected from a circumcision clinic, and pre-inoculated with HPV virions by scarification. Meshing was carried out with a Zimmer Skin Grafter Mesher. Grafts were cut to appropriate size (1 cm X 1 cm or 5 mm X 5 mm) for cutaneous or subcutaneous grafting to NIH-nu-bg-xid mice under halothane anesthesia. Cutaneous xenografts were dressed with antibiotics and protective band-aids for 3 weeks. In the paralleled experiment using the same viral stock containing both HPV6 and 11, and matched grafts, no visible papillomas were observed in non-meshed cutaneous xenografts (n = 4 up to 6 months). In comparison, six of eight cutaneous xenografts treated with the meshing procedure formed visible papillomas within 4 months. This high frequency of distinct papilloma induction over the surface of meshed xenografts were reproduced in subsequent experiments with viral stocks containing both HPV11 and 6 (8 out of 10 grafts), or with a single-type HPV11 inoculum (80-100%). In contrast, an initial viral stock of single-type HPV6 provided lower frequency and more delayed papilloma induction. Serial passage of HPV6 in the meshed xenograft appeared to improve both the induction frequency and growth rate up to the 3rd generation. Histology, in situ hybridization, and immunohistochemical analysis revealed similarity of xenograft warts to those observed in the clinic. The highly reproducible papilloma induction rate and successful viral stock propagation associated with the meshing procedure provide a novel feature in the HPV xenograft model.

Groschel, B., J. Cinatl, et al. (2002). "S-acyl-2-thioethyl (SATE) pronucleotides are potent inhibitors of

HIV-1 replication in T-lymphoid cells cross-resistant to deoxycytidine and thymidine analogs." Antiviral Research **53**(2): 143.

http://www.sciencedirect.com/science/article/B6T2H-44NM21X-5/2/033bd8c1ba05fd1bff40ae2f80477e8f

The biological evaluation of mononucleotide prodrugs (pronucleotides) of various nucleoside reverse transcriptase inhibitors (NRTIs) such as zidovudine (AZT), zalcitabine (ddC) and lamivudine (3TC) was reported in human T-lymphoid MOLT-4/8 cells which were grown continuously for more than 1 year in a medium containing cytarabine (Ara-C). In this cell line, expression of deoxycytidine kinase (dCK) and thymidine kinase 1 (TK1) was decreased in comparison to parental cells (3.8 and 2.9-fold, respectively). The lower mRNA level of TK1 correlated significantly with lower enzyme activity, whereas no dCK activity was detectable. In Ara-C-resistant cells, anti-HIV-1 effects of ddC, 3TC and AZT were more than 100-fold lower compared with parental cells. In contrast, the corresponding mononucleoside phosphotriesters bearing S-acyl-2-thioethyl (SATE) groups as biolabile phosphate protection retained anti-HIV-1 activity due to their ability to bypass the first monophosphorylation step catalyzed by dCK or TK1. The results demonstrate that in vitro selection of T-lymphoid cells in the presence of Ara-C results in cross-resistance to deoxycytidine (ddC, 3TC) and thymidine (AZT) analogs and that these cellular resistance mechanisms can be bypassed by the use of bis(SATE) pronucleotides.

Heim, A., U. Pfetzing, et al. (1998). "Antiviral activity of WIN 54954 in coxsackievirus B2 carrier state infected human myocardial fibroblasts." Antiviral Research **37**(1): 47.

http://www.sciencedirect.com/science/article/B6T2H-3S0M9JD-5/2/2d54dc141e37da18b48b46dd73478116

Persistent infections with a cardiotropic enterovirus, e.g. coxsackievirus B2 (CVB2), cause chronic myocarditis and eventually congestive heart failure. Therefore, the antiviral activity of WIN 54954, a capsid binding antiviral agent that inhibits enterovirus uncoating, was studied in persistently CVB2-infected cultures of human myocardial fibroblasts. Cultures displayed a typical carrier state infection with virus titers of 3.9+/-1.6 x 105 plaque forming units (PFU)/ml and 0.99% infected cells. WIN 54954 (0.025-1 [mu]g/ml) application was started 7 days after infection of the cultures. Compared to the WIN 54954 concentration resulting in a 90% plaque number reduction (EC90=0.197 [mu]g/ml) in acutely infected Vero cells, WIN 54954 reduced virus yields of myocardial fibroblast cultures more efficiently, e.g. more than 100 fold (99%) with 0.025 [mu]g/ml after 4 days of application. Antiviral effects of WIN 54954 increased with application time and at 0.025 [mu]g/ml Win 54954 completely inhibited infectious virus progeny after 16 days. Increasing the WIN 54954 concentration up to 1 [mu]g/ml did not cause a greater inhibition of virus replication. In situ hybridization demonstrated that at 0.1 [mu]g/ml WIN 54954 reduced the number of infected cells from 0.99 to 0.18%, although a complete eradication of CVB2-infected cells was not achieved at concentrations as high as 1 [mu]g/ml. In conclusion, the results indicate that low concentrations of WIN 54954 are effective in treating persistent enterovirus infections of myocardial fibroblasts, although a complete eradication of the infection is not achieved with WIN 54954 as a single antiviral agent.

Ives, J. A. L., J. A. Carr, et al. (2002). "The H274Y mutation in the influenza A/H1N1 neuraminidase active site following oseltamivir phosphate treatment leave virus severely compromised both in vitro and in vivo." Antiviral Research **55**(2): 307.

http://www.sciencedirect.com/science/article/B6T2H-45TTWGM-

2/2/c5f7eb2452007ce8cc675a28c8edf956

Oseltamivir carboxylate is a potent and specific inhibitor of influenza A and B neuraminidase (NA). Oseltamivir phosphate, the ethyl ester prodrug of oseltamivir carboxylate, is the first orally active NA inhibitor available for the prophylaxis and treatment of influenza A and B. It offers an improvement over amantadine and rimantadine which are active only against influenza A and rapidly generate resistant virus. The emergence of virus resistant to oseltamivir carboxylate in the treatment of naturally acquired influenza infection is low (about 1%). The types of NA mutation to arise are sub-type specific and largely predicted from in vitro drug selection studies. A substitution of the conserved histidine at position 274 for tyrosine in the NA active site has been selected via site directed mutagenesis, serial passage in culture under drug pressure in H1N1 and during the treatment of experimental H1N1 infection in man. Virus carrying H274Y NA enzyme selected in vivo has reduced sensitivity to oseltamivir carboxylate. The replicative ability in cell culture was reduced up to 3 logs, as was infectivity in animal models of influenza virus infection. Additionally, pathogenicity of the mutant virus is significantly compromised in ferret, compared to the corresponding wild type virus. Virus carrying a H274Y mutation is unlikely to be of clinical consequence in man.

King, R. W., D. L. Winslow, et al. (1995). "Identification of a clinical isolate of HIV-1 with an isoleucine at position 82 of the protease which retains susceptibility to protease inhibitors." Antiviral Research **28**(1): 13.

http://www.sciencedirect.com/science/article/B6T2H-3YGV2M7-S/2/828da9e15bdbece5a05a2af540c21870

The HIV-1 protease (PR) is essential for the production of mature virions. As such, it has become a target for the development of anti-HIV chemotherapeutics. Multiple passages of virus in cell culture in the presence of PR inhibitors have resulted in the selection of variants with decreased sensitivity to inhibitors of the PR. The most common alteration observed is a single amino acid change at position 82. This particular position has been well characterized by several laboratories as being important for the susceptibility of the virus to inhibitors of PR function. Mutations which result in the substitution of the wild-type valine with alanine, phenylalanine, threonine or isoleucine at position 82 of the PR have been associated with decreased sensitivity to several PR inhibitors. We describe here a clinical strain of HIV-1 that contains an isoleucine at position 82 of the PR instead of the usual valine. This strain is unique in that it was isolated from a patient that was anti-retroviral naive, and in the past, variants at position 82 of the PR have only been found after treatment of patients or cell culture with PR inhibitors. Moreover, this virus remains sensitive to PR inhibitors of the cyclic urea and C-2 symmetrical diol classes.

Kodama, E., S. Mori, et al. (1995). "Analysis of mutations in the thymidine kinase gene of varicella zoster virus associated with resistance to 5-iodo-2'-deoxyuridine and 5-bromo-2'-deoxyuridine." Antiviral Research **27**(1-2): 165.

http://www.sciencedirect.com/science/article/B6T2H-3YGTTBB-14/2/1adea0125b229ec7e0f6e5f35130596e

We have analyzed mutations in the thymidine kinase (TK) gene of varicella zoster virus (VZV) which showed resistance to 5-iodo-2'-deoxyuridine (IDU) and 5-bromo-2'-deoxyuridine (BrDU). Through sequencing of the TK gene, we found three amino acids were exchanged (41 Asn -> Ser, 266 Cys -> Ile, 288 Ser -> Leu). These mutations were not located at either the nucleoside- or the ATP-binding site. This result suggests that the resistance to IDU and BrDU in this particular

strain is due to the change in conformation of TK rather than the replacement of amino acids in the binding sites.

Mayhew, C. N., L. J. Mampuru, et al. (2002). "Suppression of retrovirus-induced immunodeficiency disease (murine AIDS) by trimidox and didox: Novel ribonucleotide reductase inhibitors with less bone marrow toxicity than hydroxyurea." Antiviral Research **56**(2): 167.

http://www.sciencedirect.com/science/article/B6T2H-46W05D2-4/2/26c6d158754c9852d647787aae9f3138

Recently, the use of the ribonucleotide reductase (RR) inhibitor hydroxyurea (HU) in combination with nucleoside analogs has gained attention as a potential strategy for anti-HIV-1 therapy. However, appeal for the long-term use of HU in HIV-1 infection may be limited by its propensity to induce hematopoietic toxicity. We report a comparison of the efficacy and bone marrow toxicity of HU (400 and 200 mg/kg/day) with the novel RR inhibitors and free radical-scavenging compounds didox (DX; 3,4-dihydroxybenzohydroxamic acid; 350 mg/kg/day) and trimidox (TX; 3,4,5-trihydroxybenzamidoxime; 175 mg/kg/day) in the murine AIDS (LPBM5 MuLV) model of retrovirus infection. Infected mice received daily drug treatment for 8 weeks. Efficacy was determined by measuring drug effects on retroviral-induced disease progression (i.e. development of splenomegaly and hypergammaglobulinemia) and by evaluating splenic levels of proviral DNA. Bone marrow toxicity was evaluated by measuring peripheral blood indices (WBC, hematocrit and reticulocyte counts), femoral cellularity and by determining the numbers of hematopoietic progenitor cells (CFU-GM, BFU-E) per femur and spleen. Compared to infected controls receiving no drug treatment, disease progression was significantly suppressed by TX, DX and HU. However, HU was associated with mortality and induced significant hematopoietic toxicity in a time- and dose-dependent manner. Conversely, TX and DX effectively inhibited retrovirus-induced disease but did not induce hematopoietic toxicity. These results suggest that due to their reduced hematopoietic toxicity and ability to inhibit disease progression in murine AIDS, TX and DX may offer effective alternatives to HU therapy in HIV-1 infection.

Meerbach, A., R. Klocking, et al. (2000). "Inhibitory effect of cycloSaligenyl-nucleoside monophosphates (cycloSal-NMP) of acyclic nucleoside analogues on HSV-1 and EBV." Antiviral Research **45**(1): 69.

http://www.sciencedirect.com/science/article/B6T2H-3YHG1HV-7/2/b9d2ec457e34932e623b3733751ddccf

The in vitro antiviral activity of a new series of cycloSal-pro-nucleotides derived from the acyclic nucleoside analogues aciclovir and penciclovir against herpes simplex virus type 1 (HSV-1), thymidine kinase deficient (TK-) HSV-1, and Epstein-Barr virus (EBV) was evaluated. Using the XTT-based tetrazolium reduction assay EZ4U, the cycloSal derivatives were examined for their antiviral and cytotoxic effects in HSV-1 as well as HSV-1-TK--infected Vero cells. The anti-EBV activity was assessed by means of an EBV DNA hybridization assay using a digoxigenin-labeled probe specific for the Bam H1-W-fragment of the EBV genome and by measuring viral capsid antigen (VCA) expression in P3HR-1 cells by indirect immunofluorescence. Among the new cycloSal-phosphotriesters the three aciclovir monophosphates proved to be potent and selective inhibitors of HSV-1 replication, EBV DNA synthesis and EB-VCA expression. Of interest is the retention of activity of the aciclovir monophosphates in HSV-1-TK--infected cells. Particularly 3-methyl-cycloSal-aciclovir monophosphate retained the same effectiveness, as compared to the wild type virus strain. In contrast to the aciclovir pro-nucleotides the penciclovir cycloSal-phosphotriesters exhibited at best only a marginal antiviral effect on HSV and EBV replication.

Paolucci, S., F. Baldanti, et al. (2000). "Quantification of the impact of HIV-1 reverse transcriptase and protease mutations on the efficacy of rescue HAART." Antiviral Research **45**(2): 101.

http://www.sciencedirect.com/science/article/B6T2H-3YWXPMY-3/2/70a08fc2ab2b212eac28537e45570992

The reduction in the efficacy of rescue treatment (administered on a clinical basis) due to drug resistance was retrospectively quantified in 55 human immunodeficiency virus type 1 (HIV-1)-infected patients failing highly active antiretroviral therapy (HAART) by using a novel score calculation system based upon HIV-1 reverse transcriptase (RT) and protease (PR) mutations. Patients were all naive for nelfinavir (NFV) and efavirenz (EFV) and were assigned to one of the following rescue therapy schedules: (i) 17 patients received NFV+EFV+stavudine (d4T) (group A); (ii) 14 patients received NFV+saquinavir (SQV)+lamivudine (3TC)+d4T/zidovudine (AZT) (group B); (iii) 19 patients received NFV+d4T+didanosine (ddI)/3TC/zalcitabine (ddC) (group C); (iv) five patients received miscellaneous treatments including NFV (group D). Responders were considered patients showing a drop in HIV-1 RNA level>0.5 log10 after 3 months of therapy. Forty-eight (28 responders and 20 non-responders) out of 55 patients completed the first 3 months of rescue therapy and reduction in HIV-1 viral load was found to be significantly higher in group A compared to groups B and C (81.2% responders vs. 38.5 and 40.0%, respectively). At baseline, no patient carried EFV- or d4T-resistant HIV-1 strains, despite prolonged administration of d4T, while 41/48 (87.2%) patients had mutations conferring resistance to NFV in the absence of previous treatment with this drug. A significant inverse correlation between reduction in viral load and reduction in therapy efficacy due to drug resistance, as determined by the score calculation system, was found (r=0.62). A cut-off value of 36% reduction in therapy efficacy showed a positive predictive value (capacity to detect failure of rescue treatment) of 81.2% and a negative predictive value (ability to detect successful treatment) of 75.8%. In addition, 45 out of 48 patients completed also the 9-12 month period of rescue therapy and 10/28 responders had a rebound in HIV-1 viral load level detected after the first 3 months of rescue therapy. Of these, 5/7 (71.4%) showed a further reduction in rescue therapy efficacy due to the emergence of new mutations.

Pesce, C. D., F. Bolacchi, et al. (2005). "Anti-gene peptide nucleic acid targeted to proviral HIV-1 DNA inhibits in vitro HIV-1 replication." Antiviral Research **66**(1): 13.

http://www.sciencedirect.com/science/article/B6T2H-4F6K73J-1/2/77c5132a2b2915009b9012269c26e1bc

Highly active antiretroviral therapy (HAART) is unlikely to affect reservoirs of HIV in latently infected cells. Anti-gene compounds, such as peptide nucleic acids (PNAs), which block transcriptional activity via sequence-specific invasion of double-stranded DNA may be an effective strategy to target cells harbouring proviral HIV DNA. Here we show that a PNA oligomer (PNAHIV), 15 bases in length, linked to a nuclear localization signal (NLS), substantially suppressed HIV-1 replication in chronically infected lymphocytes and macrophages and efficiently prevented mitogen-induced HIV-1 reactivation in lymphocytes, as determined by HIV-p24 antigen production in supernatants and FACS analysis for intracellular HIV accumulation. In contrast, a mismatched PNA did not show any effect on HIV expression. Semi-quantitative RT-PCR and quantitative real-time RT-PCR demonstrated a decrease of HIV RNA expression in infected cells treated by PNAHIV indicating that inhibition of HIV-1 replication occurred at the transcription step. In conclusion, the use of anti-gene PNA to target the HIV-1 proviral DNA in the quest for new antiretroviral agents appears quite promising.

Simard, C., F. Nadon, et al. (1995). "Evidence that the amino acid region 124-203 of glycoprotein G from the respiratory syncytial virus (RSV) constitutes a major part of the polypeptide domain that is involved in the protection against RSV infection." Antiviral Research **28**(4): 303.

http://www.sciencedirect.com/science/article/B6T2H-3YGV2K1-3/2/575785ce3be67f567ec32f68b02ed432

The first 230 residues of the 298-amino acid glycoprotein G of respiratory syncytial virus (RSV) are sufficient to confer complete resistance to challenge with live RSV, whereas the first 180 residues completely failed (Olmsted et al. (1989) J. Virol. 63, 411-420). The characterization of a protective epitope corresponding to the amino acid region 174-187 of the G protein (Trudel et al. (1991) Virology 185, 749-757) suggests that interruption of this region in the 180 residue truncated polypeptide may be responsible for its inability to confer protection and consequently that the 174-187 region may play a major role in the protection effected by the protein G. To support these hypotheses, we examined the ability of the amino acid region 124-203 of glycoprotein G to confer protection. The corresponding peptide was expressed as a non-fusion protein in a recombinant vaccinia virus designated VG27. Immunization of BALB/c mice with this recombinant efficiently induced the production of antibodies capable of recognizing both the parental glycoprotein G and peptide 174-187. Furthermore, upon challenge with RSV, a significant decrease of infectious particles was found in the lungs of mice immunized with VG27 as compared with non-immunized mice. Our results suggest that the 124-203 amino acid region of the RSV G protein constitutes a major part of the domain involved in protection.

Song, W., Y. Maeda, et al. (2004). "Persistence of mutations during replication of an HIV library containing combinations of selected protease mutations." Antiviral Research **61**(3): 173.

http://www.sciencedirect.com/science/article/B6T2H-4B22RY0-2/2/490c103f6bffe03fef19e63b34c388a8

It has been known that, in some cases, accumulation of specific mutations in HIV-1 protease leads to multi-protease inhibitor (PI) resistance. We examined the persistence of mutations detected in HIV-1 clinical isolates cross-resistant to the current PIs using an HIV-1 protease restricted library (HXB2 protease in an HIV-1NL4-3 background) in the absence of protease inhibitors. The virus library contained combinations of 0-11 amino acid substitutions (4,096 possible combinations) in the protease-encoding region. We examined the frequency of each amino acid substitution in the library using a T cell line, MT-2. The frequency of the amino acid substitutions V82T/I and L90M decreased rapidly with a short half life (t1/2t1/2=34.2, 28.1 and 30.6 days, respectively. Other amino acid substitutions, i.e., L10I, I54V, L63P, A71V and V82A, were well retained (t1/2>36 days). By contrast, the half lives (t1/2) of the D30N and N88D mutations associated with nelfinavir (NFV) resistance were only 7.2 and 1.8 days, respectively. These results indicate that this type of the HIV-1 protease restricted library is useful to evaluate the persistence of PI resistance-associated mutations in the absence of drug selective pressure.

Spiga, M.-G., D. A. Weidner, et al. (1999). "Inhibition of [beta]-globin gene expression by 3'-azido-3'-deoxythymidine in human erythroid progenitor cells." Antiviral Research **44**(3): 167.

http://www.sciencedirect.com/science/article/B6T2H-3Y6Y3VD-3/2/01a80c66bdc2f081a6fb3bce3858cb8b

3'-Azido-3'-deoxythymidine (AZT) treatment in HIV-infected patients is limited by bone marrow suppression including neutropenia and anemia. Previous studies had shown a direct effect of

high concentrations of this drug on globin gene expression in K-562 erythroleukemia cells. To better define the mechanism(s) of AZT-induced bone marrow toxicity, the present study evaluates these effects in more relevant human erythroid progenitor liquid cultures, because AZT is 100 times more toxic to human bone marrow cells than K-562 cells. At a clinically relevant concentration of 1 [mu]M, AZT inhibited specifically erythroid cell growth by ~58% as compared with untreated cells. The percentage of cells synthesizing hemoglobin was decreased also by 47% in AZT-treated cells with [beta]-globin mRNA levels accounting for 0.27 pmol in treated cells as compared with 1.44 under control conditions while [beta]-actin levels remained unchanged. Under the same conditions, AZT inhibited the [beta]-globin chain synthesis by ~60% as compared with the control. Consistent with the data described above was the finding that a concentration as low as 0.1 [mu]M of AZT decreased by almost 40% the binding level of the erythroid-specific transcription factor GATA-1. These findings demonstrate that AZT, at clinical relevant concentrations, specifically inhibits [beta]-globin gene expression in human erythroid progenitor liquid cell culture.

Sumpter, L. R., M. S. Inayat, et al. (2004). "In vivo examination of hydroxyurea and the novel ribonucleotide reductase inhibitors trimidox and didox in combination with the reverse transcriptase inhibitor abacavir: suppression of retrovirus-induced immunodeficiency disease." Antiviral Research **62**(3): 111.

http://www.sciencedirect.com/science/article/B6T2H-4BFVFWT-1/2/a8e400415f594f1670518217b7057c4f

Inhibition of ribonucleotide reductase (RR) has gained attention as a potential strategy for HIV-1 therapy through the success of hydroxyurea (HU) to potentiate the activity of the nucleoside reverse transcriptase inhibitor (NRTI) didanosine (ddI) in clinical trials. However, the use of HU has been limited by its development of hematopoietic toxicity. In this study, the novel RR inhibitors didox (DX; 3,4-dihydroxybenzohydroxamic acid), and trimidox (TX; 3,4,5-trihydroxybenzamidoxime) were evaluated along with HU for anti-retroviral efficacy in LPBM5-induced retro-viral disease (MAIDS) both as monotherapeutic regimens and in combination with the guanine containing NRTI abacavir (ABC). Anti-retroviral drug efficacy was determined by measuring inhibition of splenomegaly, hypergammaglobulinemia, and splenic levels of proviral DNA. In this study, all RRIs tested showed the ability to improve the efficacy of ABC in the MAIDS model by reducing splenomegaly, hypergammaglobulinemia, and splenic proviral DNA levels.

Witvrouw, M., C. Pannecouque, et al. (2000). "In vitro evaluation of the effect of temporary removal of HIV drug pressure." Antiviral Research **46**(3): 215.

http://www.sciencedirect.com/science/article/B6T2H-40J7B7R-5/2/0dd50a0a370b34610b7a06ff76bfde62

We tried to establish whether MT-4 cells that were infected with HIV-1(HTLV-IIIB) at a high multiplicity of infection (m.o.i.=1), and subsequently treated with high concentrations of anti-HIV drugs for several days, would be able to resume virus production after the antivirals are washed away. The HIV inhibitors studied were the nucleoside reverse transcriptase inhibitors (NRTIs) zidovudine and lamivudine, the non-nucleoside reverse transcriptase inhibitors (NNRTIs) nevirapine, delavirdine and loviride, the acyclic nucleoside phosphonate RT inhibitor (R)-9-(2-phosphonylmethoxypropyl)adenine (tenofovir) and the protease inhibitors (PIs) saquinavir, indinavir and ritonavir. The compounds, at 50 and 500 times their 50% inhibitory concentration (IC50, determined at a m.o.i.=0.01), were added immediately after virus adsorption and removed after an incubation period of 0 (wash control), 24, 48 or 72 h. Virus breakthrough was monitored by microscopical examination of cytopathicity, viral infectivity (yield) and p24 levels in the

supernatant. The presence of HIV-1(HTLV-IIIB) proviral DNA was determined after a 72-h incubation period. None of the antiviral drugs studied was able to prevent resumption of viral growth after removal of the compound. Tenofovir, lamivudine and the NNRTIs nevirapine, delavirdine and loviride, at 500 times their respective IC50, were able to delay viral breakthrough for approximately 2-3 days. The NRTI zidovudine and the PIs saquinavir, indinavir and ritonavir, under the same conditions, were not able to delay viral breakthrough at all. Virus recovered upon treatment proved as sensitive to the anti-HIV drugs as wild-type virus. Our results suggest that viral replication at the cellular level was not completely inhibited by drug monotherapy. Consequently, virus rebounded when drug therapy stopped. In conclusion, our findings suggest that drug holidays would result in viral breakthrough, even after virus replication has been previously suppressed by adequate drug levels.

Ying, C., E. De Clercq, et al. (2000). "Ribavirin and mycophenolic acid potentiate the activity of guanine- and diaminopurine-based nucleoside analogues against hepatitis B virus." Antiviral Research **48**(2): 117.

http://www.sciencedirect.com/science/article/B6T2H-41WCDST-4/2/0b006a0b15d4007d7b19646d01cbe048

Mycophenolic acid [the active metabolite of the immunosuppressive agent mycophenolate mofetil (MMF)] and ribavirin were found to potentiate the anti-HBV activity of the guanine-based nucleoside analogues penciclovir (PCV), lobucavir (LBV) and 3'-fluorodideoxyguanosine (FLG) and diaminopurine dioxolane (DAPD). Ribavirin and mycophenolic acid are both inhibitors of inosine 5'-monophosphate dehydrogenase and cause a depletion of intracellular dGTP levels. It may be assumed that the 5'-triphosphorylated derivatives of the guanine-based nucleoside analogues, in the presence of reduced levels of dGTP, inhibit more efficiently the priming reaction as well as the reverse transcription and DNA-dependent DNA polymerase activity of the HBV polymerase. This assumption is corroborated by the observation that exogenously added guanosine reversed the potentiating effect of ribavirin and mycophenolic acid on the anti-HBV activity of the guanosine analogues. Our observations may have implications for those (liver) transplant recipients that receive MMF as (part of their) immunosuppressive regimen and that, because of de novo or persistent infection with HBV, need specific anti-HBV therapy.

Appl. Envir. Microbiol. (126)

Alice, A. F., G. Perez-Martinez, et al. (2002). "Existence of a True Phosphofructokinase in Bacillus sphaericus: Cloning and Sequencing of the pfk Gene." Appl. Envir. Microbiol. **68**(12): 6410-6415.

http://aem.asm.org/cgi/content/abstract/68/12/6410

Some strains of Bacillus sphaericus are entomopathogenic to mosquito larvae, which transmit diseases, such as filariasis and malaria, affecting millions of people worldwide. This species is unable to use hexoses and pentoses as unique carbon sources, which was proposed to be due to the lack of glycolytic enzymes, such as 6-phosphofructokinase (PFK). In this study, PFK activity was detected and the pfk gene was cloned and sequenced. Furthermore, this gene was shown to be present in strains belonging to all the homology groups of this heterogeneous species, in which PFK activity was also detected. A careful sequence analysis revealed the conservation of

different catalytic and regulatory residues, as well as the enzyme's phylogenetic affiliation with the family of allosteric ATP-PFK enzymes.

Avrahami, S. and R. Conrad (2003). "Patterns of Community Change among Ammonia Oxidizers in Meadow Soils upon Long-Term Incubation at Different Temperatures." Appl. Envir. Microbiol. **69**(10): 6152-6164.

http://aem.asm.org/cgi/content/abstract/69/10/6152

The effect of temperature on the community structure of ammonia-oxidizing bacteria was investigated in three different meadow soils. Two of the soils (OMS and GMS) were acidic (pH 5.0 to 5.8) and from sites in Germany with low annual mean temperature (about 10{degrees}C), while KMS soil was slightly alkaline (pH 7.9) and from a site in Israel with a high annual mean temperature (about 22{degrees}C). The soils were fertilized and incubated for up to 20 weeks in a moist state and as a buffered (pH 7) slurry amended with urea at different incubation temperatures (4 to 37{degrees}C). OMS soil was also incubated with less fertilizer than the other soils. The community structure of ammonia oxidizers was analyzed before and after incubation by denaturing gradient gel electrophoresis (DGGE) of the amoA gene, which codes for the {alpha} subunit of ammonia monooxygenase. All amoA gene sequences found belonged to the genus Nitrosospira. The analysis showed community change due to temperature both in moist soil and in the soil slurry. Two patterns of community change were observed. One pattern was a change between the different Nitrosospira clusters, which was observed in moist soil and slurry incubations of GMS and OMS. Nitrosospira AmoA cluster 1 was mainly detected below 30{degrees}C, while Nitrosospira cluster 4 was predominant at 25{degrees}C. Nitrosospira clusters 3a, 3b, and 9 dominated at 30{degrees}C. The second pattern, observed in KMS, showed a community shift predominantly within a single Nitrosospira cluster. The sequences of the individual DGGE bands that exhibited different trends with temperature belonged almost exclusively to Nitrosospira cluster 3a. We conclude that ammonia oxidizer populations are influenced by temperature. In addition, we confirmed previous observations that N fertilizer also influences the community structure of ammonia oxidizers. Thus, Nitrosospira cluster 1 was absent in OMS soil treated with less fertilizer, while Nitrosospira cluster 9 was only found in the sample given less fertilizer.

Avrahami, S., R. Conrad, et al. (2002). "Effect of Soil Ammonium Concentration on N2O Release and on the Community Structure of Ammonia Oxidizers and Denitrifiers." Appl. Envir. Microbiol. **68**(11): 5685-5692.

http://aem.asm.org/cgi/content/abstract/68/11/5685

The effect of ammonium addition (6.5, 58, and 395 {micro}g of NH4+-N g [dry weight] of soil-1) on soil microbial communities was explored. For medium and high ammonium concentrations, increased N2O release rates and a shift toward a higher contribution of nitrification to N2O release occurred after incubation for 5 days at 4{degrees}C. Communities of ammonia oxidizers were assayed after 4 weeks of incubation by denaturant gradient gel electrophoresis (DGGE) of the amoA gene coding for the small subunit of ammonia monooxygenase. The DGGE fingerprints were invariably the same whether the soil was untreated or incubated with low, medium, or high ammonium concentrations. Phylogenetic analysis of cloned PCR products from excised DGGE bands detected amoA sequences which probably belonged to Nitrosospira 16S rRNA clusters 3 and 4. Additional clones clustered with Nitrosospira sp. strains Ka3 and Ka4 and within an amoA cluster from unknown species. A Nitrosomonas-like amoA gene was detected in only one clone. In agreement with the amoA results, community profiles of total bacteria analyzed by terminal restriction fragment length polymorphism (T-RFLP) showed only minor differences. However, a

community shift occurred for denitrifier populations based on T-RFLP analysis of nirK genes encoding copper-containing nitrite reductase with incubation at medium and high ammonia concentrations. Major terminal restriction fragments observed in environmental samples were further described by correspondence to cloned nirK genes from the same soil. Phylogenetic analysis grouped these clones into clusters of soil nirK genes. However, some clones were also closely related to genes from known denitrifiers. The shift in the denitrifier community was probably the consequence of the increased supply of oxidized nitrogen through nitrification. Nitrification activity increased upon addition of ammonium, but the community structure of ammonium oxidizers did not change.

Avrova, A. O., L. J. Hyman, et al. (2002). "Application of Amplified Fragment Length Polymorphism Fingerprinting for Taxonomy and Identification of the Soft Rot Bacteria Erwinia carotovora and Erwinia chrysanthemi." Appl. Envir. Microbiol. **68**(4): 1499-1508.

http://aem.asm.org/cgi/content/abstract/68/4/1499

The soft rot bacteria Erwinia carotovora and Erwinia chrysanthemi are important pathogens of potato and other crops. However, the taxonomy of these pathogens, particularly at subspecies level, is unclear. An investigation using amplified fragment length polymorphism (AFLP) fingerprinting was undertaken to determine the taxonomic relationships within this group based on their genetic relatedness. Following cluster analysis on the similarity matrices derived from the AFLP gels, four clusters (clusters 1 to 4) resulted. Cluster 1 contained Erwinia carotovora subsp. carotovora (subclusters 1a and 1b) and Erwinia carotovora subsp. odorifera (subcluster 1c) strains, while cluster 2 contained Erwinia carotovora subsp. atroseptica (subcluster 2a) and Erwinia carotovora subsp. betavasculorum (subcluster 2b) strains. Clusters 3 and 4 contained Erwinia carotovora subsp. wasabiae and E. chrysanthemi strains, respectively. While E. carotovora subsp. carotovora and E. chrysanthemi showed a high level of molecular diversity (23 to 38% mean similarity), E. carotovora subsp. odorifera, E. carotovora subsp. betavasculorum, E. carotovora subsp. atroseptica, and E. carotovora subsp. wasabiae showed considerably less (56 to 76% mean similarity), which may reflect their limited geographical distributions and/or host ranges. The species- and subspecies-specific banding profiles generated from the AFLPs allowed rapid identification of unknown isolates and the potential for future development of diagnostics. AFLP fingerprinting was also found to be more differentiating than other techniques for typing the soft rot erwinias and was applicable to all strain types, including different serogroups.

Baker, B. J., M. A. Lutz, et al. (2004). "Metabolically Active Eukaryotic Communities in Extremely Acidic Mine Drainage." Appl. Envir. Microbiol. **70**(10): 6264-6271.

http://aem.asm.org/cgi/content/abstract/70/10/6264

Acid mine drainage (AMD) microbial communities contain microbial eukaryotes (both fungi and protists) that confer a biofilm structure and impact the abundance of bacteria and archaea and the community composition via grazing and other mechanisms. Since prokaryotes impact iron oxidation rates and thus regulate AMD generation rates, it is important to analyze the fungal and protistan populations. We utilized 18S rRNA and beta-tubulin gene phylogenies and fluorescent rRNA-specific probes to characterize the eukaryotic diversity and distribution in extremely acidic (pHs 0.8 to 1.38), warm (30 to 50{degrees}C), metal-rich (up to 269 mM Fe2+, 16.8 mM Zn, 8.5 mM As, and 4.1 mM Cu) AMD solutions from the Richmond Mine at Iron Mountain, Calif. A Rhodophyta (red algae) lineage and organisms from the Vahlkampfiidae family were identified. The fungal 18S rRNA and tubulin gene sequences formed two distinct phylogenetic groups associated with the classes Dothideomycetes and Eurotiomycetes. Three fungal isolates that

were closely related to the Dothideomycetes clones were obtained. We suggest the name "Acidomyces richmondensis" for these isolates. Since these ascomycete fungi were morphologically indistinguishable, rRNA-specific oligonucleotide probes were designed to target the Dothideomycetes and Eurotiomycetes via fluorescent in situ hybridization (FISH). FISH analyses indicated that Eurotiomycetes are generally more abundant than Dothideomycetes in all of the seven locations studied within the Richmond Mine system. This is the first study to combine the culture-independent detection of fungi with in situ detection and a demonstration of activity in an acidic environment. The results expand our understanding of the subsurface AMD microbial community structure.

Berg, G. M., D. J. Repeta, et al. (2002). "Dissolved Organic Nitrogen Hydrolysis Rates in Axenic Cultures of Aureococcus anophagefferens (Pelagophyceae): Comparison with Heterotrophic Bacteria." Appl. Envir. Microbiol. **68**(1): 401-404.

http://aem.asm.org/cgi/content/abstract/68/1/401

The marine autotroph Aureococcus anophagefferens (Pelagophyceae) was rendered axenic in order to investigate hydrolysis rates of peptides, chitobiose, acetamide, and urea as indicators of the ability to support growth on dissolved organic nitrogen. Specific rates of hydrolysis varied between 8 and 700% of rates observed in associated heterotrophic marine bacteria.

Bergsma-Vlami, M., M. E. Prins, et al. (2005). "Assessment of Genotypic Diversity of Antibiotic-Producing Pseudomonas Species in the Rhizosphere by Denaturing Gradient Gel Electrophoresis." Appl. Envir. Microbiol. **71**(2): 993-1003.

http://aem.asm.org/cgi/content/abstract/71/2/993

The genotypic diversity of antibiotic-producing Pseudomonas spp. provides an enormous resource for identifying strains that are highly rhizosphere competent and superior for biological control of plant diseases. In this study, a simple and rapid method was developed to determine the presence and genotypic diversity of 2,4-diacetylphloroglucinol (DAPG)-producing Pseudomonas strains in rhizosphere samples. Denaturing gradient gel electrophoresis (DGGE) of 350-bp fragments of phlD, a key gene involved in DAPG biosynthesis, allowed discrimination between genotypically different phlD+ reference strains and indigenous isolates. DGGE analysis of the phlD fragments provided a level of discrimination between phlD+ genotypes that was higher than the level obtained by currently used techniques and enabled detection of specific phlD+ genotypes directly in rhizosphere samples with a detection limit of approximately 5 x 103 CFU/g of root. DGGE also allowed simultaneous detection of multiple phlD+ genotypes present in mixtures in rhizosphere samples. DGGE analysis of 184 indigenous phlD+ isolates obtained from the rhizospheres of wheat, sugar beet, and potato plants resulted in the identification of seven phlD+ genotypes, five of which were not described previously based on sequence and phylogenetic analyses. Subsequent bioassays demonstrated that eight genotypically different phlD+ genotypes differed substantially in the ability to colonize the rhizosphere of sugar beet seedlings. Collectively, these results demonstrated that DGGE analysis of the phlD gene allows identification of new genotypic groups of specific antibiotic-producing Pseudomonas with different abilities to colonize the rhizosphere of sugar beet seedlings.

Bertin, Y., K. Boukhors, et al. (2004). "Localization of the Insertion Site and Pathotype Determination of the Locus of Enterocyte Effacement of Shiga Toxin-Producing Escherichia coli Strains." Appl.

Envir. Microbiol. **70**(1): 61-68.

http://aem.asm.org/cgi/content/abstract/70/1/61

Of 220 Shiga toxin-producing Escherichia coli (STEC) strains collected in central France from healthy cattle, food samples, and asymptomatic children, 12 possessed the eae gene included in the locus of enterocyte effacement (LEE) pathogenicity island. Based on gene typing, we observed 7 different eae espA espB tir pathotypes among the 12 STEC strains and described the new espA{beta}v variant. As previously observed, the O157 serogroup is associated with eae{gamma}, O26 is associated with eae{beta}, and O103 is associated with eae{varepsilon}. However, the unexpected eae{zeta} allele was detected in 5 of the 12 isolates. PCR amplification and pulsed-field gel electrophoresis using the I-CeuI endonuclease followed by Southern hybridization indicated that the LEE was inserted in the vicinity of the selC (three isolates), pheU (two isolates), or pheV (six isolates) tRNA gene. Six isolates harbored two or three of these tRNA loci altered by the insertion of integrase genes (CP4-int and/or int-phe), suggesting the insertion of additional foreign DNA fragments at these sites. In spite of great genetic diversity of LEE pathotypes and LEE insertion sites, bovine strains harbor alleles of LEE genes that are frequently found in clinical STEC strains isolated from outbreaks and sporadic cases around the world, underscoring the potential risk of the bovine strains on human health.

Bird, C. and M. Wyman (2003). "Nitrate/Nitrite Assimilation System of the Marine Picoplanktonic Cyanobacterium Synechococcus sp. Strain WH 8103: Effect of Nitrogen Source and Availability on Gene Expression." Appl. Envir. Microbiol. **69**(12): 7009-7018.

http://aem.asm.org/cgi/content/abstract/69/12/7009

The genes encoding the structural components of the nitrate/nitrite assimilation system of the oceanic cyanobacterium Synechococcus sp. strain WH 8103 were cloned and characterized. The genes encoding nitrate reductase (narB) and nitrite reductase (nirA) are clustered on the chromosome but are organized in separate transcriptional units. Upstream of narB is a homologue of nrtP that encodes a nitrate/nitrite-bispecific permease rather than the components of an ABC-type nitrate transporter found in freshwater cyanobacteria. Unusually, neither nirA nor ntcA (encoding a positive transcription factor of genes subject to nitrogen control) were found to be tightly regulated by ammonium. Furthermore, transcription of glnA (encoding glutamine synthetase) is up-regulated in ammonium-grown cells, highlighting significant differences in nitrogen control in this cyanobacterium. Nitrogen depletion led to the transient up-regulation of ntcA, nirA, nrtP, narB, and glnA in what appears to be an NtcA-dependent manner. The NtcA-like promoters found upstream of nirA, nrtP, and narB all differ in sequence from the canonical NtcA promoter established for other cyanobacteria, and in the case of nirA, the NtcA-like promoter was functional only in cells deprived of combined nitrogen. The ecological implications of these findings are discussed in the context of the oligotrophic nature of oceanic surface waters in which Synechococcus spp. thrive.

Blackwood, C. B., T. Marsh, et al. (2003). "Terminal Restriction Fragment Length Polymorphism Data Analysis for Quantitative Comparison of Microbial Communities." Appl. Envir. Microbiol. **69**(2): 926-932.

http://aem.asm.org/cgi/content/abstract/69/2/926

Terminal restriction fragment length polymorphism (T-RFLP) is a culture-independent method of obtaining a genetic fingerprint of the composition of a microbial community. Comparisons of the

utility of different methods of (i) including peaks, (ii) computing the difference (or distance) between profiles, and (iii) performing statistical analysis were made by using replicated profiles of eubacterial communities. These samples included soil collected from three regions of the United States, soil fractions derived from three agronomic field treatments, soil samples taken from within one meter of each other in an alfalfa field, and replicate laboratory bioreactors. Cluster analysis by Ward's method and by the unweighted-pair group method using arithmetic averages (UPGMA) were compared. Ward's method was more effective at differentiating major groups within sets of profiles; UPGMA had a slightly reduced error rate in clustering of replicate profiles and was more sensitive to outliers. Most replicate profiles were clustered together when relative peak height or Hellinger-transformed peak height was used, in contrast to raw peak height. Redundancy analysis was more effective than cluster analysis at detecting differences between similar samples. Redundancy analysis using Hellinger distance was more sensitive than that using Euclidean distance between relative peak height profiles. Analysis of Jaccard distance between profiles, which considers only the presence or absence of a terminal restriction fragment, was the most sensitive in redundancy analysis, and was equally sensitive in cluster analysis, if all profiles had cumulative peak heights greater than 10,000 fluorescence units. It is concluded that T-RFLP is a sensitive method of differentiating between microbial communities when the optimal statistical method is used for the situation at hand. It is recommended that hypothesis testing be performed by redundancy analysis of Hellinger-transformed data and that exploratory data analysis be performed by cluster analysis using Ward's method to find natural groups or by UPGMA to identify potential outliers. Analyses can also be based on Jaccard distance if all profiles have cumulative peak heights greater than 10,000 fluorescence units.

Brinig, M. M., P. W. Lepp, et al. (2003). "Prevalence of Bacteria of Division TM7 in Human Subgingival Plaque and Their Association with Disease." Appl. Envir. Microbiol. **69**(3): 1687-1694.

http://aem.asm.org/cgi/content/abstract/69/3/1687

Members of the uncultivated bacterial division TM7 have been detected in the human mouth, but little information is available regarding their prevalence and diversity at this site. Human subgingival plaque samples from healthy sites and sites exhibiting various stages of periodontal disease were analyzed for the presence of TM7 bacteria. TM7 ribosomal DNA (rDNA) was found in 96% of the samples, and it accounted for approximately 0.3%, on average, of all bacterial rDNA in the samples as determined by real-time quantitative PCR. Two new phylotypes of this division were identified, and members of the division were found to exhibit filamentous morphology by fluorescence in situ hybridization. The abundance of TM7 rDNA relative to total bacterial rDNA was higher in sites with mild periodontitis (0.54% {+/-} 0.1%) than in either healthy sites (0.21% {+/-} 0.05%, $P < 0.01$) or sites with severe periodontitis (0.29% {+/-} 0.06%, $P < 0.05$). One division subgroup, the I025 phylotype, was detected in 1 of 18 healthy samples and 38 of 58 disease samples. These data suggest that this phylotype, and the TM7 bacterial division in general, may play a role in the multifactorial process leading to periodontitis.

Brinkman, N. E., R. A. Haugland, et al. (2003). "Evaluation of a Rapid, Quantitative Real-Time PCR Method for Enumeration of Pathogenic Candida Cells in Water." Appl. Envir. Microbiol. **69**(3): 1775-1782.

http://aem.asm.org/cgi/content/abstract/69/3/1775

Quantitative PCR (QPCR) technology, incorporating fluorigenic 5' nuclease (TaqMan) chemistry, was utilized for the specific detection and quantification of six pathogenic species of Candida (C. albicans, C. tropicalis, C. krusei, C. parapsilosis, C. glabrata and C. lusitaniae) in water. Known numbers of target cells were added to distilled and tap water samples, filtered, and disrupted

directly on the membranes for recovery of DNA for QPCR analysis. The assay's sensitivities were between one and three cells per filter. The accuracy of the cell estimates was between 50 and 200% of their true value (95% confidence level). In similar tests with surface water samples, the presence of PCR inhibitory compounds necessitated further purification and/or dilution of the DNA extracts, with resultant reductions in sensitivity but generally not in quantitative accuracy. Analyses of a series of freshwater samples collected from a recreational beach showed positive correlations between the QPCR results and colony counts of the corresponding target species. Positive correlations were also seen between the cell quantities of the target Candida species detected in these analyses and colony counts of Enterococcus organisms. With a combined sample processing and analysis time of less than 4 h, this method shows great promise as a tool for rapidly assessing potential exposures to waterborne pathogenic Candida species from drinking and recreational waters and may have applications in the detection of fecal pollution.

Brussaard, C. P. D., S. M. Short, et al. (2004). "Isolation and Phylogenetic Analysis of Novel Viruses Infecting the Phytoplankton Phaeocystis globosa (Prymnesiophyceae)." Appl. Envir. Microbiol. **70**(6): 3700-3705.

http://aem.asm.org/cgi/content/abstract/70/6/3700

Viruses infecting the harmful bloom-causing alga Phaeocystis globosa (Prymnesiophyceae) were readily isolated from Dutch coastal waters (southern North Sea) in 2000 and 2001. Our data show a large increase in the abundance of putative P. globosa viruses during blooms of P. globosa, suggesting that viruses are an important source of mortality for this alga. In order to examine genetic relatedness among viruses infecting P. globosa and other phytoplankton, DNA polymerase gene (pol) fragments were amplified and the inferred amino acid sequences were phylogenetically analyzed. The results demonstrated that viruses infecting P. globosa formed a closely related monophyletic group within the family Phycodnaviridae, with at least 96.9% similarity to each other. The sequences grouped most closely with others from viruses that infect the prymnesiophyte algae Chrysochromulina brevifilum and Chrysochromulina strobilus. Whether the P. globosa viruses belong to the genus Prymnesiovirus or form a separate group needs further study. Our data suggest that, like their phytoplankton hosts, the Chrysochromulina and Phaeocystis viruses share a common ancestor and that these prymnesioviruses and their algal host have coevolved.

Bustamante, W., A. Alpizar, et al. (2003). "Predominance of vanA Genotype among Vancomycin-Resistant Enterococcus Isolates from Poultry and Swine in Costa Rica." Appl. Envir. Microbiol. **69**(12): 7414-7419.

http://aem.asm.org/cgi/content/abstract/69/12/7414

The use of avoparcin as a growth promoter is considered to have selected for vancomycin-resistant enterococci (VRE). In Costa Rica, the use of avoparcin for poultry and swine was intensive until the product was withdrawn from the market in 2000. We evaluated the presence of VRE in poultry, swine, and cattle fecal samples obtained during 1998 and 1999. A total of 185 VRE isolates were recovered from 116 out of 893 samples. Enterococcus faecium was the most frequently isolated species (50.8%), being predominant among poultry (71.6%) and swine (37.7%) isolates, but it was not recovered from the bovine samples. The second-most-frequently-isolated species from poultry and swine, respectively, were E. durans (23.2%) and E. faecalis (21.7%). E. casseliflavus was the only species obtained from bovine samples, but it was not found among the avian isolates. An evident predominance of the vanA determinant among vancomycin-resistant enterococcal species from poultry and swine, but not from cattle, was observed and was similar to the situation in European countries before avoparcin was forbidden.

The diversity of the vanA determinant in the isolates was assessed by detection of the IS1251 insertion in the vanSH intergenic region and of the IS1476 insertion in the vanXY intergenic region. However, in none of the 154 vanA+ isolates recovered in this study were those insertions detected.

Buttner, M. P., P. Cruz, et al. (2004). "Evaluation of the Biological Sampling Kit (BiSKit) for Large-Area Surface Sampling." Appl. Envir. Microbiol. **70**(12): 7040-7045.

http://aem.asm.org/cgi/content/abstract/70/12/7040

Current surface sampling methods for microbial contaminants are designed to sample small areas and utilize culture analysis. The total number of microbes recovered is low because a small area is sampled, making detection of a potential pathogen more difficult. Furthermore, sampling of small areas requires a greater number of samples to be collected, which delays the reporting of results, taxes laboratory resources and staffing, and increases analysis costs. A new biological surface sampling method, the Biological Sampling Kit (BiSKit), designed to sample large areas and to be compatible with testing with a variety of technologies, including PCR and immunoassay, was evaluated and compared to other surface sampling strategies. In experimental room trials, wood laminate and metal surfaces were contaminated by aerosolization of Bacillus atrophaeus spores, a simulant for Bacillus anthracis, into the room, followed by settling of the spores onto the test surfaces. The surfaces were sampled with the BiSKit, a cotton-based swab, and a foam-based swab. Samples were analyzed by culturing, quantitative PCR, and immunological assays. The results showed that the large surface area (1 m2) sampled with the BiSKit resulted in concentrations of B. atrophaeus in samples that were up to 10-fold higher than the concentrations obtained with the other methods tested. A comparison of wet and dry sampling with the BiSKit indicated that dry sampling was more efficient (efficiency, 18.4%) than wet sampling (efficiency, 11.3%). The sensitivities of detection of B. atrophaeus on metal surfaces were 42 {+/-} 5.8 CFU/m2 for wet sampling and 100.5 {+/-} 10.2 CFU/m2 for dry sampling. These results demonstrate that the use of a sampling device capable of sampling larger areas results in higher sensitivity than that obtained with currently available methods and has the advantage of sampling larger areas, thus requiring collection of fewer samples per site.

Campbell, M. S. and A. C. Wright (2003). "Real-Time PCR Analysis of Vibrio vulnificus from Oysters." Appl. Envir. Microbiol. **69**(12): 7137-7144.

http://aem.asm.org/cgi/content/abstract/69/12/7137

Vibrio vulnificus is an opportunistic human pathogen commonly found in estuarine environments. Infections are associated with raw oyster consumption and can produce rapidly fatal septicemia in susceptible individuals. Standard enumeration of this organism in shellfish or seawater is laborious and inaccurate; therefore, more efficient assays are needed. An oligonucleotide probe derived from the cytolysin gene, vvhA, was previously used for colony hybridizations to enumerate V. vulnificus. However, this method requires overnight growth, and vibrios may lack culturability under certain conditions. In the present study, we targeted the same locus for development of a TaqMan real-time PCR assay. Probe specificity was confirmed by amplification of 28 V. vulnificus templates and by the lack of a PCR product with 22 non-V. vulnificus strains. Detection of V. vulnificus in pure cultures was observed over a 6-log-unit linear range of concentration (102 to 108 CFU ml-1), with a lower limit of 72 fg of genomic DNA {micro}l of PCR mixture-1 or the equivalent of six cells. Similar sensitivity was observed in DNA extracted from mixtures of V. vulnificus and V. parahaemolyticus cells. Real-time PCR enumeration of artificially inoculated oyster homogenates correlated well with colony hybridization counts (r2 = 0.97). Numbers of indigenous V. vulnificus cells in oysters by real-time PCR showed no significant

differences from numbers from plate counts with probe (t test; P = 0.43). Viable but nonculturable cells were also enumerated by real-time PCR and confirmed by the BacLight viability assay. These data indicate that real-time PCR can provide sensitive species-specific detection and enumeration of V. vulnificus in seafood.

Casavant, N. C., G. A. Beattie, et al. (2002). "Site-Specific Recombination-Based Genetic System for Reporting Transient or Low-Level Gene Expression." Appl. Envir. Microbiol. **68**(7): 3588-3596.

http://aem.asm.org/cgi/content/abstract/68/7/3588

We report here the construction, characterization, and application of a plasmid-based genetic system that reports the expression of a target promoter by effecting an irreversible, heritable change in a bacterial cell. This system confers strong repression of the reporter gene gfp in the absence of target promoter expression and utilizes the site-specific recombination machinery of bacteriophage P22 to trigger high-level reporter gene expression in the original cell and its progeny after target gene induction. We demonstrate the effectiveness of this genetic system by tailoring it to indicate the availability of arabinose to the biological control agent Enterobacter cloacae JL1157 in culture and in the barley rhizosphere. The presence of bioavailable arabinose triggered the production of P22 excisionase and integrase from the reporter plasmid pAraLHB in JL1157, and this led to excision of the cI repressor gene, which is flanked by att sites, and the subsequent irreversible expression of gfp in the original cell and in its progeny. In culture, nearly 100% of an E. cloacae JL1157(pAraLHB) population expressed gfp after exposure to 6.5 to 65 {micro}M arabinose for 3 h. We used this biosensor to demonstrate that arabinose was released from the seeds of several legumes and grass species during germination and from roots of barley seedlings grown hydroponically or in soil. When introduced into microcosms containing barley, the biosensor permitted the localization of arabinose along the roots. Arabinose was present near the root-seed junction and on the seminal roots but was not detected at the root tips. This recombination-based reporter system should be useful for monitoring bacterial exposure to transient or low levels of specific molecules directly in the environment.

Chang, Y.-H., Y.-H. Shangkuan, et al. (2003). "PCR Assay of the groEL Gene for Detection and Differentiation of Bacillus cereus Group Cells." Appl. Envir. Microbiol. **69**(8): 4502-4510.

http://aem.asm.org/cgi/content/abstract/69/8/4502

Strains of species in the Bacillus cereus group are potentially enterotoxic. Thus, the detection of all B. cereus group strains is important. As 16S ribosomal DNA sequence analysis cannot adequately differentiate species of the B. cereus group, we explored the potential of the groEL gene as a phylogenetic marker. A phylogenetic analysis of the groEL sequences of 78 B. cereus group strains revealed that the B. cereus group strains were split into two major clusters, one including six B. mycoides and one B. pseudomycoides (cluster II) and the other including two B. mycoides and the rest of the B. cereus group strains (cluster I). Cluster I was further differentiated into two subclusters, Ia and Ib. The sodA gene sequences of representative strains from different clusters were also compared. The phylogenetic tree constructed from the sodA sequences showed substantial similarity to the tree constructed from the groEL sequences. Based on the groEL sequences, a PCR assay for detection and identification of B. cereus group strains was developed. Subsequent restriction fragment length polymorphism (RFLP) analysis verified the PCR amplicons and the differentiation of the B. cereus group strains. RFLP with MboI was identical for all the B. cereus group strains analyzed, while RFLP with MfeI or PstI classified all B. cereus and B. thuringiensis strains into two groups. All cluster II B. mycoides and B. pseudomycoides strains could be discriminated from other B. cereus group bacteria by restriction analysis with TspRI.

Choi, Y. J., C. B. Miguez, et al. (2004). "Characterization and Heterologous Gene Expression of a Novel Esterase from Lactobacillus casei CL96." Appl. Envir. Microbiol. **70**(6): 3213-3221.

http://aem.asm.org/cgi/content/abstract/70/6/3213

A novel esterase gene (estI) of Lactobacillus casei CL96 was localized on a 3.3-kb BamHI DNA fragment containing an open reading frame (ORF) of 1,800 bp. The ORF of estI was isolated by PCR and expressed in Escherichia coli, the methylotrophic bacterium Methylobacterium extorquens, and the methylotrophic yeast Pichia pastoris under the control of T7, methanol dehydrogenase (PmxaF), and alcohol oxidase (AOX1) promoters, respectively. The amino acid sequence of EstI indicated that the esterase is a novel member of the GHSMG family of lipolytic enzymes and that the enzyme contains a lipase-like catalytic triad, consisting of Ser325, Asp516, and His558. E. coli BL21(DE3)/pLysS containing estI expressed a novel 67.5-kDa protein corresponding to EstI in an N-terminal fusion with the S {middle dot} tag peptide. The recombinant L. casei CL96 EstI protein was purified to electrophoretic homogeneity in a one-step affinity chromatography procedure on S-protein agarose. The optimum pH and temperature of the purified enzyme were 7.0 and 37{degrees}C, respectively. Among the pNP (p-nitrophenyl) esters tested, the most selective substrate was pNP-caprylate (C8), with Km and kcat values of 14 {+/-} 1.08 {micro}M and 1,245 {+/-} 42.3 S-1, respectively.

Corbella, M. E. and A. Puyet (2003). "Real-Time Reverse Transcription-PCR Analysis of Expression of Halobenzoate and Salicylate Catabolism-Associated Operons in Two Strains of Pseudomonas aeruginosa." Appl. Envir. Microbiol. **69**(4): 2269-2275.

http://aem.asm.org/cgi/content/abstract/69/4/2269

Pseudomonas aeruginosa JB2 can use 2-chlorobenzoate (2-CBa), 3-CBa, 2,3-dichlorobenzoate (2,3-DCBa), and 2,5-DCBa as sole carbon and energy sources, whereas strain 142 can only grow on 2-CBa and 2,4-DCBa. Both strains, however, harbor the same halobenzoate 1,2-dioxygenase (ohbAB) and chlorocatechol (clcABD) degradation genes necessary for the metabolism of ortho-CBas. In addition, the hybABCD operon, encoding a salicylate 5-hydroxylase, is also found in both strains. The expression of ohbAB, hybABCD, and clcABD operons was measured in cultures grown on different CBas as the sole carbon source and also in glucose-grown cells supplemented with CBas as inducers. A method to standardize real-time reverse transcription-PCR experimental data was used that allows the comparison of semiquantitative mRNA accumulation in different strains and culture conditions. In both strains, the ohb and hyb systems were induced in cells grown on 2-CBa or DCBas, whereas clc was induced only by DCBas. Repression by catabolite was observed both on ohb and clc systems in glucose-grown cells. Chlorocatechol 1,2-dioxygenase activity in JB2 was detected even in clc-repressed conditions, confirming the presence of additional isofunctional genes previously detected in P. aeruginosa 142. Although similar levels of induction of ohbAB were observed in strain JB2 grown on either benzoate, monochlorobenzoates, or DCBas, the ohbAB operon of strain 142 was only strongly induced by growth on 2-CBa and, to a lesser extent, on 2,4-DCBa. This observation suggests that regulation of the ohbAB operon may be different in both strains. The concomitant induction of ohb and hyb by CBas may allow the formation of hybrid halobenzoate dioxygenase(s) composed of Ohb/Hyb dioxygenase subunits and Hyb ferredoxin/ferredoxin reductase components.

de la Torre, J. R., B. M. Goebel, et al. (2003). "Microbial Diversity of Cryptoendolithic Communities from

the McMurdo Dry Valleys, Antarctica." Appl. Envir. Microbiol. **69**(7): 3858-3867.

http://aem.asm.org/cgi/content/abstract/69/7/3858

In the McMurdo Dry Valleys of Antarctica, microorganisms colonize the pore spaces of exposed rocks and are thereby protected from the desiccating environmental conditions on the surface. These cryptoendolithic communities have received attention in microscopy and culture-based studies but have not been examined by molecular approaches. We surveyed the microbial biodiversity of selected cryptoendolithic communities by analyzing clone libraries of rRNA genes amplified from environmental DNA. Over 1,100 individual clones from two types of cryptoendolithic communities, cyanobacterium dominated and lichen dominated, were analyzed. Clones fell into 51 relatedness groups (phylotypes) with >=98% rRNA sequence identity (46 bacterial and 5 eucaryal). No representatives of Archaea were detected. No phylotypes were shared between the two classes of endolithic communities studied. Clone libraries based on both types of communities were dominated by a relatively small number of phylotypes that, because of their relative abundance, presumably represent the main primary producers in these communities. In the lichen-dominated community, three rRNA sequences, from a fungus, a green alga, and a chloroplast, of the types known to be associated with lichens, accounted for over 70% of the clones. This high abundance confirms the dominance of lichens in this community. In contrast, analysis of the supposedly cyanobacterium-dominated community indicated, in addition to cyanobacteria, at least two unsuspected organisms that, because of their abundance, may play important roles in the community. These included a member of the {alpha} subdivision of the Proteobacteria that potentially is capable of aerobic anoxygenic photosynthesis and a distant relative of Deinococcus that defines, along with other Deinococcus-related sequences from Antarctica, a new clade within the Thermus-Deinococcus bacterial phylogenetic division.

de las Rivas, B., A. Marcobal, et al. (2004). "Allelic Diversity and Population Structure in Oenococcus oeni as Determined from Sequence Analysis of Housekeeping Genes." Appl. Envir. Microbiol. **70**(12): 7210-7219.

http://aem.asm.org/cgi/content/abstract/70/12/7210

Oenococcus oeni is the organism of choice for promoting malolactic fermentation in wine. The population biology of O. oeni is poorly understood and remains unclear. For a better understanding of the mode of genetic variation within this species, we investigated by using multilocus sequence typing (MLST) with the gyrB, pgm, ddl, recP, and mleA genes the genetic diversity and genetic relationships among 18 O. oeni strains isolated in various years from wines of the United States, France, Germany, Spain, and Italy. These strains have also been characterized by ribotyping and restriction fragment length polymorphism (RFLP) analysis of the PCR-amplified 16S-23S rRNA gene intergenic spacer region (ISR). Ribotyping grouped the strains into two groups; however, the RFLP analysis of the ISRs showed no differences in the strains analyzed. In contrast, MLST in oenococci had a good discriminatory ability, and we have found a higher genetic diversity than indicated by ribotyping analysis. All sequence types were represented by a single strain, and all the strains could be distinguished from each other because they had unique combinations of alleles. Strains assumed to be identical showed the same sequence type. Phylogenetic analyses indicated a panmictic population structure in O. oeni. Sequences were analyzed for evidence of recombination by split decomposition analysis and analysis of clustered polymorphisms. All results indicated that recombination plays a major role in creating the genetic heterogeneity of O. oeni. A low standardized index of association value indicated that the O. oeni genes analyzed are close to linkage equilibrium. This study constitutes the first step in the development of an MLST method for O. oeni and the first example of the application of MLST to a nonpathogenic food production bacteria.

de Lipthay, J. R., N. Tuxen, et al. (2002). "In Situ Exposure to Low Herbicide Concentrations Affects Microbial Population Composition and Catabolic Gene Frequency in an Aerobic Shallow Aquifer." Appl. Envir. Microbiol. **69**(1): 461-467.

http://aem.asm.org/cgi/content/abstract/69/1/461

The aim of this study was to evaluate how the in situ exposure of a Danish subsurface aquifer to phenoxy acid herbicides at low concentrations (<40 {micro}g l-1) changes the microbial community composition. Sediment and groundwater samples were collected inside and outside the herbicide-exposed area and were analyzed for the presence of general microbial populations, Pseudomonas bacteria, and specific phenoxy acid degraders. Both culture-dependent and culture-independent methods were applied. The abundance of microbial phenoxy acid degraders (100 to 104 g-1 sediment) was determined by most probable number assays, and their presence was only detected in herbicide-exposed sediments. Similarly, PCR analysis showed that the 2,4-dichlorophenoxyacetic acid degradation pathway genes tfdA and tfdB (102 to 103 gene copies g-1 sediment) were only detected in sediments from contaminated areas of the aquifer. PCR-restriction fragment length polymorphism measurements demonstrated the presence of different populations of tfd genes, suggesting that the in situ herbicide degradation was caused by the activity of a heterogeneous population of phenoxy acid degraders. The number of Pseudomonas bacteria measured by either PCR or plating on selective agar media was higher in sediments subjected to high levels of phenoxy acid. Furthermore, high numbers of CFU compared to direct counting of 4',6-diamidino-2-phenylindole-stained cells in the microscope suggested an increased culturability of the indigenous microbial communities from acclimated sediments. The findings of this study demonstrate that continuous exposure to low herbicide concentrations can markedly change the bacterial community composition of a subsurface aquifer.

Di Giovanni, G. D. and M. W. LeChevallier (2005). "Quantitative-PCR Assessment of Cryptosporidium parvum Cell Culture Infection." Appl. Envir. Microbiol. **71**(3): 1495-1500.

http://aem.asm.org/cgi/content/abstract/71/3/1495

A quantitative TaqMan PCR method was developed for assessing the Cryptosporidium parvum infection of in vitro cultivated human ileocecal adenocarcinoma (HCT-8) cell cultures. This method, termed cell culture quantitative sequence detection (CC-QSD), has numerous applications, several of which are presented. CC-QSD was used to investigate parasite infection in cell culture over time, the effects of oocyst treatment on infectivity and infectivity assessment of different C. parvum isolates. CC-QSD revealed that cell culture infection at 24 and 48 h postinoculation was approximately 20 and 60%, respectively, of the endpoint 72-h postinoculation infection. Evaluation of three different lots of C. parvum Iowa isolate oocysts revealed that the mean infection of 0.1 N HCl-treated oocysts was only 36% of the infection obtained with oocysts treated with acidified Hanks' balanced salt solution containing 1% trypsin. CC-QSD comparison of the C. parvum Iowa and TAMU isolates revealed significantly higher levels of infection for the TAMU isolate, which agrees with and supports previous human, animal, and cell culture studies. CC-QSD has the potential to aid in the optimization of Cryptosporidium cell culture methods and facilitate quantitative evaluation of cell culture infectivity experiments.

Dick, L. K. and K. G. Field (2004). "Rapid Estimation of Numbers of Fecal Bacteroidetes by Use of a Quantitative PCR Assay for 16S rRNA Genes." Appl. Envir. Microbiol. **70**(9): 5695-5697.

http://aem.asm.org/cgi/content/abstract/70/9/5695

Assessment of health risk associated with fecal pollution requires a reliable fecal indicator and a rapid quantification method. We report the development of a Taq nuclease assay for enumeration of 16S rRNA genes of Bacteroidetes. Sensitivity and correlation with standard fecal indicators provide experimental evidence for application of the assay in monitoring fecal pollution.

Distel, D. L., D. J. Beaudoin, et al. (2002). "Coexistence of Multiple Proteobacterial Endosymbionts in the Gills of the Wood-Boring Bivalve Lyrodus pedicellatus (Bivalvia: Teredinidae)." Appl. Envir. Microbiol. **68**(12): 6292-6299.

http://aem.asm.org/cgi/content/abstract/68/12/6292

Wood-boring bivalves of the family Teredinidae (commonly called shipworms) are known to harbor dense populations of gram-negative bacteria within specialized cells (bacteriocytes) in their gills. These symbionts are thought to provide enzymes, e.g., cellulase and dinitrogenase, which assist the host in utilizing wood as a primary food source. A cellulolytic, dinitrogen-fixing bacterium, Teredinibacter turnerae, has been isolated from the gill tissues of numerous teredinid bivalves and has been proposed to constitute the sole or predominant symbiont of this bivalve family. Here we demonstrate that one teredinid species, Lyrodus pedicellatus, contains at least four distinct bacterial 16S rRNA types within its gill bacteriocytes, one of which is identical to that of T. turnerae. Phylogenetic analyses indicate that the three newly detected ribotypes are derived from gamma proteobacteria that are related to but distinct (>6.5% sequence divergence) from T. turnerae. In situ hybridizations with 16S rRNA-directed probes demonstrated that the pattern of occurrence of symbiont ribotypes within bacteriocytes was predictable and specific, with some bacteriocytes containing two symbiont ribotypes. However, only two of the six possible pairwise combinations of the four ribotypes were observed to cooccur within the same host cells. The results presented here are consistent with the existence of a complex multiple symbiosis in this shipworm species.

Duizer, E., P. Bijkerk, et al. (2004). "Inactivation of Caliciviruses." Appl. Envir. Microbiol. **70**(8): 4538-4543.

http://aem.asm.org/cgi/content/abstract/70/8/4538

The viruses most commonly associated with food- and waterborne outbreaks of gastroenteritis are the noroviruses. The lack of a culture method for noroviruses warrants the use of cultivable model viruses to gain more insight on their transmission routes and inactivation methods. We studied the inactivation of the reported enteric canine calicivirus no. 48 (CaCV) and the respiratory feline calicivirus F9 (FeCV) and correlated inactivation to reduction in PCR units of FeCV, CaCV, and a norovirus. Inactivation of suspended viruses was temperature and time dependent in the range from 0 to 100{degrees}C. UV-B radiation from 0 to 150 mJ/cm2 caused dose-dependent inactivation, with a 3 D (D = 1 log10) reduction in infectivity at 34 mJ/cm2 for both viruses. Inactivation by 70% ethanol was inefficient, with only 3 D reduction after 30 min. Sodium hypochlorite solutions were only effective at >300 ppm. FeCV showed a higher stability at pH <3 and pH >7 than CaCV. For all treatments, detection of viral RNA underestimated the reduction in viral infectivity. Norovirus was never more sensitive than the animal caliciviruses and profoundly more resistant to low and high pH. Overall, both animal viruses showed similar inactivation profiles when exposed to heat or UV-B radiation or when incubated in ethanol or hypochlorite. The low stability of CaCV at low pH suggests that this is not a typical enteric (calici-) virus. The incomplete inactivation by ethanol and the high hypochlorite concentration needed for sufficient virus inactivation point to a concern for decontamination of fomites and surfaces contaminated with noroviruses and virus-safe water.

Duthoit, F., J.-J. Godon, et al. (2003). "Bacterial Community Dynamics during Production of Registered Designation of Origin Salers Cheese as Evaluated by 16S rRNA Gene Single-Strand Conformation Polymorphism Analysis." Appl. Envir. Microbiol. **69**(7): 3840-3848.

http://aem.asm.org/cgi/content/abstract/69/7/3840

Microbial dynamics during processing and ripening of traditional cheeses such as registered designation of origin Salers cheese, an artisanal cheese produced in France, play an important role in the elaboration of sensory qualities. The aim of the present study was to obtain a picture of the dynamics of the microbial ecosystem of RDO Salers cheese by using culture-independent methods. This included DNA extraction, PCR, and single-strand conformation polymorphism (SSCP) analysis. Bacterial and high-GC% gram-positive bacterial primers were used to amplify V2 or V3 regions of the 16S rRNA gene. SSCP patterns revealed changes during the manufacturing of the cheese. Patterns of the ecosystems of cheeses that were provided by three farmers were also quite different. Cloning and sequencing of the 16S rRNA gene revealed sequences related to lactic acid bacteria (Lactococcus lactis, Streptococcus thermophilus, Enterococcus faecium, Leuconostoc mesenteroides, Leuconostoc pseudomesenteroides, Lactobacillus plantarum, and Lactobacillus pentosus), which were predominant during manufacturing and ripening. Bacteria belonging to the high-GC% gram-positive group (essentially corynebacteria) were found by using specific primers. The present molecular approach can effectively describe the ecosystem of artisanal dairy products.

Egert, M., B. Wagner, et al. (2003). "Microbial Community Structure in Midgut and Hindgut of the Humus-Feeding Larva of Pachnoda ephippiata (Coleoptera: Scarabaeidae)." Appl. Envir. Microbiol. **69**(11): 6659-6668.

http://aem.asm.org/cgi/content/abstract/69/11/6659

The guts of soil-feeding macroinvertebrates contain a complex microbial community that is involved in the transformation of ingested soil organic matter. In a companion paper (T. Lemke, U. Stingl, M. Egert, M. W. Friedrich, and A. Brune, Appl. Environ. Microbiol. 69:6650-6658, 2003), we show that the gut of our model organism, the humivorous larva of the cetoniid beetle Pachnoda ephippiata, is characterized by strong midgut alkalinity, high concentrations of microbial fermentation products, and the presence of a diverse, yet unstudied microbial community. Here, we report on the community structure of bacteria and archaea in the midgut, hindgut, and food soil of P. ephippiata larvae, determined with cultivation-independent techniques. Clone libraries and terminal restriction fragment length polymorphism analysis of 16S rRNA genes revealed that the intestines of P. ephippiata larvae contain a complex gut microbiota that differs markedly between midgut and hindgut and that is clearly distinct from the microbiota in the food soil. The bacterial community is dominated by phylogenetic groups with a fermentative metabolism (Lactobacillales, Clostridiales, Bacillales, and Cytophaga-Flavobacterium-Bacteroides [CFB] phylum), which is corroborated by high lactate and acetate concentrations in the midgut and hindgut and by the large numbers of lactogenic and acetogenic bacteria in both gut compartments reported in the companion paper. Based on 16S rRNA gene frequencies, Actinobacteria dominate the alkaline midgut, while the hindgut is dominated by members of the CFB phylum. The archaeal community, however, is less diverse. 16S rRNA genes affiliated with mesophilic Crenarchaeota, probably stemming from the ingested soil, were most frequent in the midgut, whereas Methanobacteriaceae-related 16S rRNA genes were most frequent in the hindgut. These findings agree with the reported restriction of methanogenesis to the hindgut of Pachnoda larvae.

Ehrlich, K. C., P.-K. Chang, et al. (2004). "Aflatoxin Biosynthesis Cluster Gene cypA Is Required for G Aflatoxin Formation." Appl. Envir. Microbiol. **70**(11): 6518-6524.

http://aem.asm.org/cgi/content/abstract/70/11/6518

Aspergillus flavus isolates produce only aflatoxins B1 and B2, while Aspergillus parasiticus and Aspergillus nomius produce aflatoxins B1, B2, G1, and G2. Sequence comparison of the aflatoxin biosynthesis pathway gene cluster upstream from the polyketide synthase gene, pksA, revealed that A. flavus isolates are missing portions of genes (cypA and norB) predicted to encode, respectively, a cytochrome P450 monooxygenase and an aryl alcohol dehydrogenase. Insertional disruption of cypA in A. parasiticus yielded transformants that lack the ability to produce G aflatoxins but not B aflatoxins. The enzyme encoded by cypA has highest amino acid identity to Gibberella zeae Tri4 (38%), a P450 monooxygenase previously shown to be involved in trichodiene epoxidation. The substrate for CypA may be an intermediate formed by oxidative cleavage of the A ring of O-methylsterigmatocystin by OrdA, the P450 monooxygenase required for formation of aflatoxins B1 and B2.

Eiler, A., S. Langenheder, et al. (2003). "Heterotrophic Bacterial Growth Efficiency and Community Structure at Different Natural Organic Carbon Concentrations." Appl. Envir. Microbiol. **69**(7): 3701-3709.

http://aem.asm.org/cgi/content/abstract/69/7/3701

Batch cultures of aquatic bacteria and dissolved organic matter were used to examine the impact of carbon source concentration on bacterial growth, biomass, growth efficiency, and community composition. An aged concentrate of dissolved organic matter from a humic lake was diluted with organic compound-free artificial lake water to obtain concentrations of dissolved organic carbon (DOC) ranging from 0.04 to 2.53 mM. The bacterial biomass produced in the cultures increased linearly with the DOC concentration, indicating that bacterial biomass production was limited by the supply of carbon. The bacterial growth rate in the exponential growth phase exhibited a hyperbolic response to the DOC concentration, suggesting that the maximum growth rate was constrained by the substrate concentration at low DOC concentrations. Likewise, the bacterial growth efficiency calculated from the production of biomass and CO2 increased asymptotically from 0.4 to 10.4% with increasing DOC concentration. The compositions of the microbial communities that emerged in the cultures were assessed by separation of PCR-amplified 16S rRNA fragments by denaturing gradient gel electrophoresis. Nonmetric multidimensional scaling of the gel profiles showed that there was a gradual change in the community composition along the DOC gradient; members of the {beta} subclass of the class Proteobacteria and members of the Cytophaga-Flavobacterium group were well represented at all concentrations, whereas members of the {alpha} subclass of the Proteobacteria were found exclusively at the lowest carbon concentration. The shift in community composition along the DOC gradient was similar to the patterns of growth efficiency and growth rate. The results suggest that the bacterial growth efficiencies, the rates of bacterial growth, and the compositions of bacterial communities are not constrained by substrate concentrations in most natural waters, with the possible exception of the most oligotrophic environments.

Fach, P., S. Perelle, et al. (2002). "Detection by PCR-Enzyme-Linked Immunosorbent Assay of Clostridium botulinum in Fish and Environmental Samples from a Coastal Area in Northern France." Appl. Envir. Microbiol. **68**(12): 5870-5876.

http://aem.asm.org/cgi/content/abstract/68/12/5870

The prevalence of Clostridium botulinum types A, B, E, and F was determined in 214 fresh fish and environmental samples collected in Northern France. A newly developed PCR-enzyme-linked immunosorbent assay (ELISA) used in this survey detected more than 80% of samples inoculated with fewer than 10 C. botulinum spores per 25 g and 100% of samples inoculated with more than 30 C. botulinum spores per 25 g. The percent agreement between PCR-ELISA and mouse bioassay was 88.9%, and PCR-ELISA detected more positive samples than the mouse bioassay did. The prevalence of C. botulinum in seawater fish and sediment was 16.6 and 4%, respectively, corresponding to 3.5 to 7 and 1 to 2 C. botulinum most-probable-number counts, respectively, and is in the low range of C. botulinum contamination reported elsewhere. The toxin type identification of the 31 naturally contaminated samples was 71% type B, 22.5% type A, and 9.6% type E. Type F was not detected. The high prevalence of C. botulinum type B in fish samples is relatively unusual compared with the high prevalence of C. botulinum type E reported in many worldwide and northern European surveys. However, fish processing and fish preparation in France have not been identified as a significant hazard for human type B botulism.

Ferris, M. J., M. Kuhl, et al. (2003). "Cyanobacterial Ecotypes in Different Optical Microenvironments of a 68{degrees}C Hot Spring Mat Community Revealed by 16S-23S rRNA Internal Transcribed Spacer Region Variation." Appl. Envir. Microbiol. **69**(5): 2893-2898.

http://aem.asm.org/cgi/content/abstract/69/5/2893

We examined the population of unicellular cyanobacteria (Synechococcus) in the upper 3-mm vertical interval of a 68{degrees}C region of a microbial mat in a hot spring effluent channel (Yellowstone National Park, Wyoming). Fluorescence microscopy and microsensor measurements of O2 and oxygenic photosynthesis demonstrated the existence of physiologically distinct Synechococcus populations at different depths along a light gradient quantified by scalar irradiance microprobes. Molecular methods were used to evaluate whether physiologically distinct populations could be correlated with genetically distinct populations over the vertical interval. We were unable to identify patterns in genetic variation in Synechococcus 16S rRNA sequences that correlate with different vertically distributed populations. However, patterns of variation at the internal transcribed spacer locus separating 16S and 23S rRNA genes suggested the existence of closely related but genetically distinct populations corresponding to different functional populations occurring at different depths.

Fischer-Le Saux, M., D. Hervio-Heath, et al. (2002). "Detection of Cytotoxin-Hemolysin mRNA in Nonculturable Populations of Environmental and Clinical Vibrio vulnificus Strains in Artificial Seawater." Appl. Envir. Microbiol. **68**(11): 5641-5646.

http://aem.asm.org/cgi/content/abstract/68/11/5641

The objective of this study was to develop a molecular detection method that better estimates the potential risk associated with the presence of Vibrio vulnificus. For that purpose, we applied seminested reverse transcription-PCR (RT-PCR) to viable but nonculturable (VBNC) populations of V. vulnificus and targeted the cytotoxin-hemolysin virulence gene vvhA. Three strains, two environmental, IF Vv10 and IF Vv18, and one clinical, C7184, were used in this study. Artificial seawater, inoculated with mid-log-phase cells, was maintained at 4{degrees}C. VBNC cells resulted after 3, 6, and 14 days for C7184, IF Vv18, and IF Vv10, respectively. Our data indicate that seminested RT-PCR is sensitive for the detection of vvhA mRNA in artificial seawater when exclusively nonculturable bacteria are present. This is the first report of the expression of a toxin gene in VBNC V. vulnificus. Moreover, vvhA transcripts were shown to persist in nonculturable populations over a 4.5-month period, with a progressive decline of the signal over time. This result indicates that special attention should be given to the presence of potentially pathogenic

VBNC cells in environmental samples when assessing public health risk.

Formiga-Cruz, M., G. Tofino-Quesada, et al. (2002). "Distribution of Human Virus Contamination in Shellfish from Different Growing Areas in Greece, Spain, Sweden, and the United Kingdom." Appl. Envir. Microbiol. **68**(12): 5990-5998.

http://aem.asm.org/cgi/content/abstract/68/12/5990

Viral pollution in shellfish has been analyzed simultaneously across a wide range of geographical regions, with emphasis on the concomitant variations in physicochemical characteristics and social features. The methods for sample treatment and for the detection of human enteric viruses were optimized by the participating laboratories. The second part of this study involves the selection of a protocol for virus detection, which was validated by analyzing the distribution and concentration of human viral pathogens under diverse conditions during an 18-month period in four European countries. Shellfish-growing areas from diverse countries in the north and south of Europe were defined and studied, and the microbiological quality of the shellfish was analyzed. Human adenovirus, Norwalk-like virus, and enterovirus were identified as contaminants of shellfish in all the participating countries. Hepatitis A virus was also isolated in all areas except Sweden. The seasonal distribution of viral contamination was also described. Norwalk-like virus appeared to be the only group of viruses that demonstrated seasonal variation, with lower concentrations occurring during warm months. The depuration treatments currently applied were shown to be adequate for reducing Escherichia coli levels but ineffective for the elimination of viral particles. The human adenoviruses detected by PCR correlate with the presence of other human viruses and could be useful as a molecular index of viral contamination in shellfish.

Franciosa, G., M. Pourshaban, et al. (2004). "Identification of Type A, B, E, and F Botulinum Neurotoxin Genes and of Botulinum Neurotoxigenic Clostridia by Denaturing High-Performance Liquid Chromatography." Appl. Envir. Microbiol. **70**(7): 4170-4176.

http://aem.asm.org/cgi/content/abstract/70/7/4170

Denaturing high-performance liquid chromatography (DHPLC) is a recently developed technique for rapid screening of nucleotide polymorphisms in PCR products. We used this technique for the identification of type A, B, E, and F botulinum neurotoxin genes. PCR products amplified from a conserved region of the type A, B, E, and F botulinum toxin genes from Clostridium botulinum, neurotoxigenic C. butyricum type E, and C. baratii type F strains were subjected to both DHPLC analysis and sequencing. Unique DHPLC peak profiles were obtained with each different type of botulinum toxin gene fragment, consistent with nucleotide differences observed in the related sequences. We then evaluated the ability of this technique to identify botulinal neurotoxigenic organisms at the genus and species level. A specific short region of the 16S rRNA gene which contains genus-specific and in some cases species-specific heterogeneity was amplified from botulinum neurotoxigenic clostridia and from different food-borne pathogens and subjected to DHPLC analysis. Different peak profiles were obtained for each genus and species, demonstrating that the technique could be a reliable alternative to sequencing for the rapid identification of food-borne pathogens, specifically of botulinal neurotoxigenic clostridia most frequently implicated in human botulism.

Futamata, H., Y. Nagano, et al. (2005). "Unique Kinetic Properties of Phenol-Degrading Variovorax Strains Responsible for Efficient Trichloroethylene Degradation in a Chemostat Enrichment

Culture." Appl. Envir. Microbiol. **71**(2): 904-911.

http://aem.asm.org/cgi/content/abstract/71/2/904

A chemostat enrichment of soil bacteria growing on phenol as the sole carbon source has been shown to exhibit quite high trichloroethylene (TCE)-degrading activities (H. Futamata, S. Harayama, and K. Watanabe, Appl. Environ. Microbiol. 67:4671-4677, 2001). To identify the bacterial populations responsible for the high TCE-degrading activity, a multidisciplinary survey of the chemostat enrichment was conducted by employing molecular-ecological and culture-dependent approaches. Three chemostat enrichment cultures were newly developed under different phenol-loading conditions (0.25, 0.75, and 1.25 g liter-1 day-1) in this study, and the TCE-degrading activities of the enrichments were measured. Among them, the enrichment at 0.75 g liter-1 day-1 (enrichment 0.75) expressed the highest activity. Denaturing gradient gel electrophoresis of PCR-amplified 16S rRNA gene fragments detected a Variovorax ribotype as the strongest band in enrichment 0.75; however, it was not a major ribotype in the other samples. Bacteria were isolated from enrichment 0.75 by direct plating, and their 16S rRNA genes and genes encoding the largest subunit of phenol hydroxylase (LmPHs) were analyzed. Among the bacteria isolated, several strains were affiliated with the genus Variovorax and were shown to have high-affinity-type LmPHs. The LmPH of the Variovorax strains was also detected as the major genotype in enrichment 0.75. Kinetic analyses of phenol and TCE degradation revealed, however, that these strains exhibited quite low affinity for phenol compared to other phenol-degrading bacteria, while they showed quite high specific TCE-degrading activities and relatively high affinity for TCE. Owing to these unique kinetic traits, the Variovorax strains can obviate competitive inhibition of TCE degradation by the primary substrate of the catabolic enzyme (i.e., phenol), contributing to the high TCE-degrading activity of the chemostat enrichments. On the basis of physiological information, mechanisms accounting for the way the Variovorax population overgrew the chemostat enrichment are discussed.

Gevers, D., M. Danielsen, et al. (2003). "Molecular Characterization of tet(M) Genes in Lactobacillus Isolates from Different Types of Fermented Dry Sausage." Appl. Envir. Microbiol. **69**(2): 1270-1275.

http://aem.asm.org/cgi/content/abstract/69/2/1270

The likelihood that products prepared from raw meat and milk may act as vehicles for antibiotic-resistant bacteria is currently of great concern in food safety issues. In this study, a collection of 94 tetracycline-resistant (Tcr) lactic acid bacteria recovered from nine different fermented dry sausage types were subjected to a polyphasic molecular study with the aim of characterizing the host organisms and the tet genes, conferring tetracycline resistance, that they carry. With the (GTG)5-PCR DNA fingerprinting technique, the Tcr lactic acid bacterial isolates were identified as Lactobacillus plantarum, L. sakei subsp. carnosus, L. sakei subsp. sakei, L. curvatus, and L. alimentarius and typed to the intraspecies level. For a selection of 24 Tcr lactic acid bacterial isolates displaying unique (GTG)5-PCR fingerprints, tet genes were determined by means of PCR, and only tet(M) was detected. Restriction enzyme analysis with AccI and ScaI revealed two different tet(M) allele types. This grouping was confirmed by partial sequencing of the tet(M) open reading frame, which indicated that the two allele types displayed high sequence similarities (>99.6%) with tet(M) genes previously reported in Staphylococcus aureus MRSA 101 and in Neisseria meningitidis, respectively. Southern hybridization with plasmid profiles revealed that the isolates contained tet(M)-carrying plasmids. In addition to the tet(M) gene, one isolate also contained an erm(B) gene on a different plasmid from the one encoding the tetracycline resistance. Furthermore, it was also shown by PCR that the tet(M) genes were not located on transposons of the Tn916/Tn1545 family. To our knowledge, this is the first detailed molecular study demonstrating that taxonomically and genotypically diverse Lactobacillus strains from different types of fermented meat products can be a host for plasmid-borne tet genes.

Gibello, A., M. C. Porrero, et al. (2004). "Analysis of the gyrA Gene of Clinical Yersinia ruckeri Isolates with Reduced Susceptibility to Quinolones." Appl. Envir. Microbiol. **70**(1): 599-602.

http://aem.asm.org/cgi/content/abstract/70/1/599

Antimicrobial susceptibility of seven clinical strains of Yersinia ruckeri representative of those isolated between 1994 and 2002 from a fish farm with endemic enteric redmouth disease was studied. All isolates displayed indistinguishable pulsed-field gel electrophoresis restriction patterns, indicating that they represented a single strain. However, considering both inhibition zone diameters (IZD) and MICs, the isolates recovered in 2001-2002 formed a separate cluster with lower levels of susceptibility to all the quinolones tested, especially nalidixic acid (NA) and oxolinic acid (OA), compared with the isolates recovered between 1994 and 1998. Analysis of the PCR product of the quinolone resistance-determining region of the gyrA gene from clinical isolates of Y. ruckeri with reduced susceptibility to OA and NA revealed a single amino acid substitution, Ser-83 to Arg-83 (Escherichia coli numbering). Identical substitution was observed in induced OA-resistant mutant strains, which displayed IZD and MICs of quinolones similar to those of the clinical isolates of Y. ruckeri with reduced susceptibility to these antimicrobial agents. These data indicate in that for Y. ruckeri, the substitution of Ser by Arg at position 83 of the gyrA gene is associated with reduced susceptibility to quinolones.

Girguis, P. R., V. J. Orphan, et al. (2003). "Growth and Methane Oxidation Rates of Anaerobic Methanotrophic Archaea in a Continuous-Flow Bioreactor." Appl. Envir. Microbiol. **69**(9): 5472-5482.

http://aem.asm.org/cgi/content/abstract/69/9/5472

Anaerobic methanotrophic archaea have recently been identified in anoxic marine sediments, but have not yet been recovered in pure culture. Physiological studies on freshly collected samples containing archaea and their sulfate-reducing syntrophic partners have been conducted, but sample availability and viability can limit the scope of these experiments. To better study microbial anaerobic methane oxidation, we developed a novel continuous-flow anaerobic methane incubation system (AMIS) that simulates the majority of in situ conditions and supports the metabolism and growth of anaerobic methanotrophic archaea. We incubated sediments collected from within and outside a methane cold seep in Monterey Canyon, Calif., for 24 weeks on the AMIS system. Anaerobic methane oxidation was measured in all sediments after incubation on AMIS, and quantitative molecular techniques verified the increases in methane-oxidizing archaeal populations in both seep and nonseep sediments. Our results demonstrate that the AMIS system stimulated the maintenance and growth of anaerobic methanotrophic archaea, and possibly their syntrophic, sulfate-reducing partners. Our data demonstrate the utility of combining physiological and molecular techniques to quantify the growth and metabolic activity of anaerobic microbial consortia. Further experiments with the AMIS system should provide a better understanding of the biological mechanisms of methane oxidation in anoxic marine environments. The AMIS may also enable the enrichment, purification, and isolation of methanotrophic archaea as pure cultures or defined syntrophic consortia.

Greub, G. and D. Raoult (2003). "History of the ADP/ATP-Translocase-Encoding Gene, a Parasitism Gene Transferred from a Chlamydiales Ancestor to Plants 1 Billion Years Ago." Appl. Envir. Microbiol. **69**(9): 5530-5535.

http://aem.asm.org/cgi/content/abstract/69/9/5530

Nonmitochondrial ADP/ATP translocase is an energy parasite enzyme. Its encoding gene, tlc, is found only in Rickettsiales, Chlamydiales, and plant and alga plastids. We demonstrate the presence of tlc in Parachlamydia acanthamoebae. This gene shares more similarity with the tlc1 gene of Chlamydiaceae and the tlc of plant and alga plastids than with the tlc2 gene of Chlamydiaceae. Phylogenetic analysis, including all other tlc homologs found in GenBank, showed that tlc was duplicated in a Chlamydiales ancestor before the appearance of multicellular eukaryotes. A time scale, calibrated with seven independent time points obtained from fossil estimates and from the 16S rRNA molecular clock, was congruent with the molecular clock provided by tlc. Plant and alga plastids acquired tlc approximately when Parachlamydiaceae and Chlamydiaceae diverged, at the eucaryotic radiation time, ca. 1 billion years ago.

Gueimonde, M., S. Tolkko, et al. (2004). "New Real-Time Quantitative PCR Procedure for Quantification of Bifidobacteria in Human Fecal Samples." Appl. Envir. Microbiol. **70**(7): 4165-4169.

http://aem.asm.org/cgi/content/abstract/70/7/4165

The application of a real-time quantitative PCR method (5' nuclease assay), based on the use of a probe labeled at its 5' end with a stable, fluorescent lanthanide chelate, for the quantification of human fecal bifidobacteria was evaluated. The specificities of the primers and the primer-probe combination were evaluated by conventional PCR and real-time PCR, respectively. The results obtained by real-time PCR were compared with those obtained by fluorescent in situ hybridization, the current gold standard for intestinal microbiota quantification. In general, a good correlation between the two methods was observed. In order to determine the detection limit and the accuracy of the real-time PCR procedure, germfree rat feces were spiked with known amounts of bifidobacteria and analyzed by both methods. The detection limit of the method used in this study was found to be about 5 x 104 cells per g of feces. Both methods, real-time PCR and fluorescent in situ hybridization, led to an accurate quantification of the spiked samples with high levels of bifidobacteria, but real-time PCR was more accurate for samples with low levels. We conclude that the real-time PCR procedure described here is a specific, accurate, rapid, and easy method for the quantification of bifidobacteria in feces.

Harris, J. K., S. T. Kelley, et al. (2004). "New Perspective on Uncultured Bacterial Phylogenetic Division OP11." Appl. Envir. Microbiol. **70**(2): 845-849.

http://aem.asm.org/cgi/content/abstract/70/2/845

Organisms belonging to the OP11 candidate phylogenetic division of Bacteria have been detected only in rRNA-based sequence surveys of environmental samples. Preliminary studies indicated that such organisms represented by the sequences are abundant and widespread in nature and highly diverse phylogenetically. In order to document more thoroughly the phylogenetic breadth and environmental distribution of this diverse group of organisms, we conducted further molecular analyses on environmental DNAs. Using PCR techniques and primers directed toward each of the five described subdivisions of OP11, we surveyed 17 environmental DNAs and analyzed rRNA gene sequences in 27 clonal libraries from 14 environments. Ninety-nine new and unique sequences were determined completely, and approximately 200 additional clones were subjected to partial sequencing. Extensive phylogenetic comparisons of the new sequences to those representing other bacterial divisions further resolved the phylogeny of the bacterial candidate division OP11 and identified two new candidate bacterial divisions, OP11-derived 1 (OD1) and Sulphur River 1 (SR1). The widespread

environmental distribution of representatives of the bacterial divisions OD1, OP11, and SR1 suggests potentially conspicuous biogeochemical roles for these organisms in their respective environments. The information on environmental distribution offers clues for attempts to culture landmark representatives of these novel bacterial divisions, and the sequences are specific molecular signatures that provide for their identification in other contexts.

Hietala, A. M., M. Eikenes, et al. (2003). "Multiplex Real-Time PCR for Monitoring Heterobasidion annosum Colonization in Norway Spruce Clones That Differ in Disease Resistance." Appl. Envir. Microbiol. **69**(8): 4413-4420.

http://aem.asm.org/cgi/content/abstract/69/8/4413

A multiplex real-time PCR assay was developed to monitor the dynamics of the Picea abies-Heterobasidion annosum pathosystem. Tissue cultures and 32-year-old trees with low or high resistance to this pathogen were used as the host material. Probes and primers were based on a laccase gene for the pathogen and a polyubiquitin gene for the host. The real-time PCR procedure was compared to an ergosterol-based quantification method in a tissue culture experiment, and there was a strong correlation (product moment correlation coefficient, 0.908) between the data sets. The multiplex real-time PCR procedure had higher resolution and sensitivity during the early stages of colonization and also could be used to monitor the host. In the tissue culture experiment, host DNA was degraded more rapidly in the clone with low resistance than in the clone with high resistance. In the field experiment, the lesions elicited were not strictly proportional to the area colonized by the pathogen. Fungal colonization was more restricted and localized in the lesion in the clone with high resistance, whereas in the clone with low resistance, the fungus could be detected until the visible end of the lesion. Thus, the real-time PCR assay gives better resolution than does the traditionally used lesion length measurement when screening host clones for resistance.

Hoffman, R. M., M. M. Marshall, et al. (2003). "Identification and Characterization of Two Subpopulations of Encephalitozoon intestinalis." Appl. Envir. Microbiol. **69**(8): 4966-4970.

http://aem.asm.org/cgi/content/abstract/69/8/4966

Microsporidia are obligate intracellular protozoa that have been shown to be pathogenic to most living creatures. The development of in vitro cell culture propagation methods has provided researchers with large numbers of spores and facilitated the study of these organisms. Here, we describe heterogeneity within cell culture-propagated Encephalitozoon intestinalis suspensions. Flow cytometer histograms depicting the log side scatter and forward-angle light scatter of spores from nine suspensions produced over 12 months consistently showed two populations differing in size. The suspensions were composed primarily of the smaller-spore subpopulation (76.4% {+/-} 5.1%). The presence of two subpopulations was confirmed by microscopic examination and image analysis ($P < 0.001$). Small subpopulation spores were noninfectious in rabbit kidney (RK13) cell culture infectivity assays, while the large spores were infectious when inocula included [IMG]=" BORDER="0">25 spores. The small spores stained brilliantly with fluorescein isothiocyanate-conjugated monoclonal antibody against Encephalitozoon genus spore wall antigen, while the large spores stained poorly. There was no difference in staining intensities using commercial (MicroSporFA) and experimental polyclonal antibodies. Vital-dye (DAPI [4',6'-diamidino-2-phenylindole], propidium iodide, or SYTOX Green) staining showed the spores of the small subpopulation to be permeable to all vital dyes tested, while spores of the large subpopulation were not permeable in the absence of ethanol pretreatment. PCR using primers directed to the 16S rRNA or {beta}-tubulin genes and subsequent sequence analysis confirmed both subpopulations as E. intestinalis. Our data suggest that existing cell culture propagation

methods produce two types of spores differing in infectivity, and the presence of these noninfective spores in purified spore suspensions should be considered when designing disinfection and drug treatment studies.

Horie, H., H. Yoshida, et al. (2002). "Neurovirulence of Type 1 Polioviruses Isolated from Sewage in Japan." Appl. Envir. Microbiol. **68**(1): 138-142.

http://aem.asm.org/cgi/content/abstract/68/1/138

Sixteen type 1 poliovirus strains were isolated from a sewage disposal plant located downstream of the Oyabe River in Japan between October 1993 and September 1995. The isolates were intratypically differentiated as vaccine-derived strains. Neutralizing antigenicity analysis with monoclonal antibodies and estimation of neurovirulence by mutant analysis by PCR and restriction enzyme cleavage (MAPREC) were performed for 13 type 1 strains of these isolates. The isolates were classified into three groups. Group I (five strains) had a variant type of antigenicity and neurovirulent phenotype. Group II (four strains) had the vaccine type of antigenicity and neurovirulent phenotype. Group III (four strains) had the vaccine type of antigenicity and an attenuated phenotype. Furthermore, it was demonstrated that the virulent isolates were neutralized by human sera obtained after oral poliomyelitis vaccine (OPV) administration, and the sera of rats immunized with inactivated poliovirus vaccine. Although vaccination was effective against virulent polioviruses, virulent viruses will continue to exist in the environment as long as OPV is in use.

Horz, H.-P., V. Rich, et al. (2005). "Methane-Oxidizing Bacteria in a California Upland Grassland Soil: Diversity and Response to Simulated Global Change." Appl. Envir. Microbiol. **71**(5): 2642-2652.

http://aem.asm.org/cgi/content/abstract/71/5/2642

We investigated the diversity of methane-oxidizing bacteria (i.e., methanotrophs) in an annual upland grassland in northern California, using comparative sequence analysis of the pmoA gene. In addition to identifying type II methanotrophs commonly found in soils, we discovered three novel pmoA lineages for which no cultivated members have been previously reported. These novel pmoA clades clustered together either with clone sequences related to "RA 14" or "WB5FH-A," which both represent clusters of environmentally retrieved sequences of putative atmospheric methane oxidizers. Conservation of amino acid residues and rates of nonsynonymous versus synonymous nucleotide substitution in these novel lineages suggests that the pmoA genes in these clades code for functionally active methane monooxygenases. The novel clades responded to simulated global changes differently than the type II methanotrophs. We observed that the relative abundance of type II methanotrophs declined in response to increased precipitation and increased atmospheric temperature, with a significant antagonistic interaction between these factors such that the effect of both together was less than that expected from their individual effects. Two of the novel clades were not observed to respond significantly to these environmental changes, while one of the novel clades had an opposite response, increasing in relative abundance in response to increased precipitation and atmospheric temperature, with a significant antagonistic interaction between these factors.

Huys, G., K. D'Haene, et al. (2004). "Prevalence and Molecular Characterization of Tetracycline Resistance in Enterococcus Isolates from Food." Appl. Envir. Microbiol. **70**(3): 1555-1562.

http://aem.asm.org/cgi/content/abstract/70/3/1555

In the present study, a collection of 187 Enterococcus food isolates mainly originating from European cheeses were studied for the phenotypic and genotypic assessment of tetracycline (TC) resistance. A total of 45 isolates (24%) encompassing the species Enterococcus faecalis (n = 33), E. durans (n = 7), E. faecium (n = 3), E. casseliflavus (n = 1), and E. gallinarum (n = 1) displayed phenotypic resistance to TC with MIC ranges of 16 to 256 {micro}g/ml. Eight of these strains exhibited multiresistance to TC, erythromycin, and chloramphenicol. By PCR detection, TC resistance could be linked to the presence of the tet(M) (n = 43), tet(L) (n = 16), and tet(S) (n = 1) genes. In 15 isolates, including all of those for which the MIC was 256 {micro}g/ml, both tet(M) and tet(L) were found. Furthermore, all tet(M)-containing enterococci also harbored a member of the Tn916-Tn1545 conjugative transposon family, of which 12 erythromycin-resistant isolates also contained the erm(B) gene. Filter mating experiments revealed that 10 E. faecalis isolates, 3 E. durans isolates, and 1 E. faecium isolate could transfer either tet(M), tet(L), or both of these genes to E. faecalis recipient strain JH2-2. In most cases in which only tet(M) was transferred, no detectable plasmids were acquired by JH2-2 but instead all transconjugants contained a member of the Tn916-Tn1545 family. Sequencing analysis of PCR amplicons and evolutionary modeling showed that a subset of the transferable tet(M) genes belonged to four sequence homology groups (SHGs) showing an internal homology of [IMG]=" BORDER="0">99.6%. Two of these SHGs contained tet(M) mosaic structures previously found in Tn916 elements and on Lactobacillus and Neisseria plasmids, respectively, whereas the other two SHGs probably represent new phylogenetic lineages of this gene.

Hwang, Y.-S., E.-S. Kim, et al. (2003). "Cloning and Analysis of a DNA Fragment Stimulating Avermectin Production in Various Streptomyces avermitilis Strains." Appl. Envir. Microbiol. **69**(2): 1263-1269.

http://aem.asm.org/cgi/content/abstract/69/2/1263

To isolate a gene for stimulating avermectin production, a genomic library of Streptomyces avermitilis ATCC 31267 was constructed in Streptomyces lividans TK21 as the host strain. An 8.0-kb DNA fragment that significantly stimulated actinorhodin and undecylprodigiosin production was isolated. When wild-type S. avermitilis was transformed with the cloned fragment, avermectin production increased approximately 3.5-fold. The introduction of this fragment into high-producer (ATCC 31780) and semi-industrial (L-9) strains also resulted in an increase of avermectin production by more than 2.0- and 1.4-fold, respectively. Subclones were studied to locate the minimal region involved in stimulation of pigmented-antibiotic and avermectin production. An analysis of the nucleotide sequence of the entire DNA fragment identified eight complete and one incomplete open reading frame. All but one of the deduced proteins exhibited strong homology (68 to 84% identity) to the hypothetical proteins of Streptomyces coelicolor A3(2). The orfX gene product showed no significant similarity to any other protein in the databases, and an analysis of its sequence suggested that it was a putative membrane protein. Although the nature of the stimulatory effect is still unclear, the disruption of orfX revealed that this gene was intrinsically involved in the stimulation of avermectin production in S. avermitilis.

Ibekwe, A. M., C. M. Grieve, et al. (2003). "Characterization of Microbial Communities and Composition in Constructed Dairy Wetland Wastewater Effluent." Appl. Envir. Microbiol. **69**(9): 5060-5069.

http://aem.asm.org/cgi/content/abstract/69/9/5060

Constructed wetlands have been recognized as a removal treatment option for high concentrations of contaminants in agricultural waste before land application. The goal of this

study was to characterize microbial composition in two constructed wetlands designed to remove contaminants from dairy washwater. Water samples were collected weekly for 11 months from two wetlands to determine the efficiency of the treatment system in removal of chemical contaminants and total and fecal coliforms. The reduction by the treatment was greatest for biological oxygen demand, suspended solids, chemical oxygen demand, nitrate, and coliforms. There was only moderate removal of total nitrogen and phosphorus. Changes in the total bacterial community and ammonia-oxidizing bacterial composition were examined by using denaturing gradient gel electrophoresis (DGGE) and sequencing of PCR-amplified fragments of the gene carrying the {alpha} subunit of the ammonia monooxygenase gene (amoA) recovered from soil samples and DGGE bands. DGGE analysis of wetlands and manure samples revealed that the total bacterial community composition was dominated by bacteria from phylogenetic clusters related to Bacillus, Clostridium, Mycoplasma, Eubacterium, and Proteobacteria originally retrieved from the gastrointestinal tracts of mammals. The population of ammonia-oxidizing bacteria showed a higher percentage of Nitrosospira-like sequences from the wetland samples, while a higher percentage of Nitrosomonas-like sequences from manure, feces, raw washwater, and facultative pond was found. These results show that the wetland system is a natural process dependent upon the development of healthy microbial communities for optimal wastewater treatment.

Ibekwe, A. M., P. M. Watt, et al. (2002). "Multiplex Fluorogenic Real-Time PCR for Detection and Quantification of Escherichia coli O157:H7 in Dairy Wastewater Wetlands." Appl. Envir. Microbiol. **68**(10): 4853-4862.

http://aem.asm.org/cgi/content/abstract/68/10/4853

Surface water and groundwater are continuously used as sources of drinking water in many metropolitan areas of the United States. The quality of water from these sources may be reduced due to increases in contaminants such as Escherichia coli from urban and agricultural runoffs. In this study, a multiplex fluorogenic PCR assay was used to quantify E. coli O157:H7 in soil, manure, cow and calf feces, and dairy wastewater in an artificial wetland. Primers and probes were designed to amplify and quantify the Shiga-like toxin 1 (stx1) and 2 (stx2) genes and the intimin (eae) gene of E. coli O157:H7 in a single reaction. Primer specificity was confirmed with DNA from 33 E. coli O157:H7 and related strains with and without the three genes. A direct correlation was determined between the fluorescence threshold cycle (CT) and the starting quantity of E. coli O157:H7 DNA. A similar correlation was observed between the CT and number of CFU per milliliter used in the PCR assay. A detection limit of 7.9 x 10-5 pg of E. coli O157:H7 DNA ml-1 equivalent to approximately 6.4 x 103 CFU of E. coli O157:H7 ml-1 based on plate counts was determined. Quantification of E. coli O157:H7 in soil, manure, feces, and wastewater was possible when cell numbers were [>=]3.5 x 104 CFU g-1. E. coli O157:H7 levels detected in wetland samples decreased by about 2 logs between wetland influents and effluents. The detection limit of the assay in soil was improved to less than 10 CFU g-1 with a 16-h enrichment. These results indicate that the developed PCR assay is suitable for quantitative determination of E. coli O157:H7 in environmental samples and represents a considerable advancement in pathogen quantification in different ecosystems.

Jellison, K. L., D. L. Distel, et al. (2004). "Phylogenetic Analysis of the Hypervariable Region of the 18S rRNA Gene of Cryptosporidium Oocysts in Feces of Canada Geese (Branta canadensis): Evidence for Five Novel Genotypes." Appl. Envir. Microbiol. **70**(1): 452-458.

http://aem.asm.org/cgi/content/abstract/70/1/452

To assess genetic diversity in Cryptosporidium oocysts from Canada geese, 161 fecal samples

from Canada geese in the United States were analyzed. Eleven (6.8%) were positive for Cryptosporidium spp. following nested PCR amplification of the hypervariable region of the 18S rRNA gene. Nine PCR products from geese were cloned and sequenced, and all nine diverged from previously reported Cryptosporidium 18S rRNA gene sequences. Five sequences were very similar or identical to each other but genetically distinct from that of Cryptosporidium baileyi; two were most closely related to, but genetically distinct from, the first five; and two were distinct from any other sequence analyzed. One additional sequence in the hypervariable region of the 18S rRNA gene isolated from a cormorant was identical to that of C. baileyi. Phylogenetic analysis provided evidence for new genotypes of Cryptosporidium species in Canada geese. Results of this study suggest that the taxonomy of Cryptosporidium species in geese is complex and that a more complete understanding of genetic diversity among these parasites will facilitate our understanding of oocyst sources and species in the environment.

Jellison, K. L., H. F. Hemond, et al. (2002). "Sources and Species of Cryptosporidium Oocysts in the Wachusett Reservoir Watershed." Appl. Envir. Microbiol. **68**(2): 569-575.

http://aem.asm.org/cgi/content/abstract/68/2/569

Understanding the behavior of Cryptosporidium oocysts in the environment is critical to developing improved watershed management practices for protection of the public from waterborne cryptosporidiosis. Analytical methods of improved specificity and sensitivity are essential to this task. We developed a nested PCR-restriction fragment length polymorphism assay that allows detection of a single oocyst in environmental samples and differentiates the human pathogen Cryptosporidium parvum from other Cryptosporidium species. We tested our method on surface water and animal fecal samples from the Wachusett Reservoir watershed in central Massachusetts. We also directly compared results from our method with those from the immunofluorescence microscopy assay recommended in the Information Collection Rule. Our results suggest that immunofluorescence microscopy may not be a reliable indicator of public health risk for waterborne cryptosporidiosis. Molecular and environmental data identify both wildlife and dairy farms as sources of oocysts in the watershed, implicate times of cold water temperatures as high-risk periods for oocyst contamination of surface waters, and suggest that not all oocysts in the environment pose a threat to public health.

Jinneman, K. C., K. J. Yoshitomi, et al. (2003). "Multiplex Real-Time PCR Method To Identify Shiga Toxin Genes stx1 and stx2 and Escherichia coli O157:H7/H- Serotype." Appl. Envir. Microbiol. **69**(10): 6327-6333.

http://aem.asm.org/cgi/content/abstract/69/10/6327

A multiplex real-time PCR method to simultaneously detect the stx1 and stx2 genes of Shiga toxin-producing Escherichia coli and a unique conserved single-nucleotide polymorphism in the E. coli O157:H7/H- uidA gene has been developed. There is more than 98.6% sensitivity and 100% specificity for all three gene targets based on a panel of 138 isolates. The PCR efficiencies were [IMG]=" BORDER="0">1.89, and as few as 6 CFU/reaction could be detected.

Johnston, L. M. and L.-A. Jaykus (2004). "Antimicrobial Resistance of Enterococcus Species Isolated from Produce." Appl. Envir. Microbiol. **70**(5): 3133-3137.

http://aem.asm.org/cgi/content/abstract/70/5/3133

The purpose of this study was to characterize the antibiotic resistance profiles of Enterococcus species isolated from fresh produce harvested in the southwestern United States. Among the 185 Enterococcus isolates obtained, 97 (52%) were Enterococcus faecium, 38 (21%) were Enterococcus faecalis, and 50 (27%) were other Enterococcus species. Of human clinical importance, E. faecium strains had a much higher prevalence of resistance to ciprofloxacin, tetracycline, and nitrofurantoin than E. faecalis. E. faecalis strains had a low prevalence of resistance to antibiotics used to treat E. faecalis infections of both clinical and of agricultural relevance, excluding its intrinsic resistance patterns. Thirty-four percent of the isolates had multiple-drug-resistance patterns, excluding intrinsic resistance. Data on the prevalence and types of antibiotic resistance in Enterococcus species isolated from fresh produce may be used to describe baseline antibiotic susceptibility profiles associated with Enterococcus spp. isolated from the environment. The data collected may also help elucidate the role of foods in the transmission of antibiotic-resistant strains to human populations.

Jothikumar, N., J. A. Lowther, et al. (2005). "Rapid and Sensitive Detection of Noroviruses by Using TaqMan-Based One-Step Reverse Transcription-PCR Assays and Application to Naturally Contaminated Shellfish Samples." Appl. Envir. Microbiol. **71**(4): 1870-1875.

http://aem.asm.org/cgi/content/abstract/71/4/1870

Noroviruses (NoV), which are members of the family Caliciviridae, are the most important cause of outbreaks of acute gastroenteritis worldwide and are commonly found in shellfish grown in polluted waters. In the present study, we developed broadly reactive one-step TaqMan reverse transcription (RT)-PCR assays for the detection of genogroup I (GI) and GII NoV in fecal samples, as well as shellfish samples. The specificity and sensitivity of all steps of the assays were systematically evaluated, and in the final format, the monoplex assays were validated by using RNA extracted from a panel of 84 stool specimens, which included NoV strains representing 19 different genotypes (7 GI, 11 GII, and 1 GIV strains). The assays were further validated with 38 shellfish cDNA extracts previously tested by nested PCR. Comparison with a recently described real-time assay showed that our assay had significantly higher sensitivity and was at least as sensitive as the nested PCR. For stool specimens, a one-step duplex TaqMan RT-PCR assay performed as well as individual genogroup-specific monoplex assays. All other enteric viruses examined were negative, and no cross-reaction between genogroups was observed. These TaqMan RT-PCR assays provide rapid (less than 90 min), sensitive, and reliable detection of NoV and should prove to be useful for routine monitoring of both clinical and shellfish samples.

Kalanetra, K. M., S. L. Huston, et al. (2004). "Novel, Attached, Sulfur-Oxidizing Bacteria at Shallow Hydrothermal Vents Possess Vacuoles Not Involved in Respiratory Nitrate Accumulation." Appl. Envir. Microbiol. **70**(12): 7487-7496.

http://aem.asm.org/cgi/content/abstract/70/12/7487

Novel, vacuolate sulfur bacteria occur at shallow hydrothermal vents near White Point, Calif. There, these filaments are attached densely to diverse biotic and abiotic substrates and extend one to several centimeters into the surrounding environment, where they are alternately exposed to sulfidic and oxygenated seawater. Characterizations of native filaments collected from this location indicate that these filaments possess novel morphological and physiological properties compared to all other vacuolate bacteria characterized to date. Attached filaments, ranging in diameter from 4 to 100 {micro}m or more, were composed of cylindrical cells, each containing a thin annulus of sulfur globule-filled cytoplasm surrounding a large central vacuole. A near-complete 16S rRNA gene sequence was obtained and confirmed by fluorescent in situ

hybridization to be associated only with filaments having a diameter of 10 {micro}m or more. Phylogenetic analysis indicates that these wider, attached filaments form within the gamma proteobacteria a monophyletic group that includes all previously described vacuolate sulfur bacteria (the genera Beggiatoa, Thioploca, and Thiomargarita) and no nonvacuolate genera. However, unlike for all previously described vacuolate bacteria, repeated measurements of cell lysates from samples collected over 2 years indicate that the attached White Point filaments do not store internal nitrate. It is possible that these vacuoles are involved in transient storage of oxygen or contribute to the relative buoyancy of these filaments.

Kanaly, R. A., S. Harayama, et al. (2002). "Rhodanobacter sp. Strain BPC1 in a Benzo[a]pyrene-Mineralizing Bacterial Consortium." Appl. Envir. Microbiol. **68**(12): 5826-5833.

http://aem.asm.org/cgi/content/abstract/68/12/5826

A bacterial consortium which rapidly mineralizes benzo[a]pyrene when it is grown on a high-boiling-point diesel fuel distillate (HBD) was recovered from soil and maintained for approximately 3 years. Previous studies have shown that mobilization of benzo[a]pyrene into the supernatant liquid precedes mineralization of this compound (R. Kanaly, R. Bartha, K. Watanabe, and S. Harayama, Appl. Environ. Microbiol. 66:4205-4211, 2000). In the present study, we found that sterilized supernatant liquid filtrate (SSLF) obtained from the growing consortium stimulated mineralization of benzo[a]pyrene when it was readministered to a consortium inoculum without HBD. Following this observation, eight bacterial strains were isolated from the consortium, and SSLF of each of them was assayed for the ability to stimulate benzo[a]pyrene mineralization by the original consortium. The SSLF obtained from one strain, designated BPC1, most vigorously stimulated benzo[a]pyrene mineralization by the original consortium; its effect was more than twofold greater than the effect of the SSLF obtained from the original consortium. A 16S rRNA gene sequence analysis and biochemical tests identified strain BPC1 as a member of the genus Rhodanobacter, whose type strain, Rhodanobacter lindaniclasticus RP5557, which was isolated for its ability to grow on the pesticide lindane, is not extant. Strain BPC1 could not grow on lindane, benzo[a]pyrene, simple hydrocarbons, and HBD in pure culture. In contrast, a competitive PCR assay indicated that strain BPC1 grew in the consortium fed only HBD and benzo[a]pyrene. This growth of BPC1 was concomitant with growth of the total bacterial consortium and preceded the initiation of benzo[a]pyrene mineralization. These results suggest that strain BPC1 has a specialized niche in the benzo[a]pyrene-mineralizing consortium; namely, it grows on metabolites produced by fellow members and contributes to benzo[a]pyrene mineralization by increasing the bioavailability of this compound.

Kashefi, K., D. E. Holmes, et al. (2002). "Use of Fe(III) as an Electron Acceptor To Recover Previously Uncultured Hyperthermophiles: Isolation and Characterization of Geothermobacterium ferrireducens gen. nov., sp. nov." Appl. Envir. Microbiol. **68**(4): 1735-1742.

http://aem.asm.org/cgi/content/abstract/68/4/1735

It has recently been recognized that the ability to use Fe(III) as a terminal electron acceptor is a highly conserved characteristic in hyperthermophilic microorganisms. This suggests that it may be possible to recover as-yet-uncultured hyperthermophiles in pure culture if Fe(III) is used as an electron acceptor. As part of a study of the microbial diversity of the Obsidian Pool area in Yellowstone National Park, Wyo., hot sediment samples were used as the inoculum for enrichment cultures in media containing hydrogen as the sole electron donor and poorly crystalline Fe(III) oxide as the electron acceptor. A pure culture was recovered on solidified, Fe(III) oxide medium. The isolate, designated FW-1a, is a hyperthermophilic anaerobe that grows exclusively by coupling hydrogen oxidation to the reduction of poorly crystalline Fe(III) oxide.

Organic carbon is not required for growth. Magnetite is the end product of Fe(III) oxide reduction under the culture conditions evaluated. The cells are rod shaped, about 0.5 {micro}m by 1.0 to 1.2 {micro}m, and motile and have a single flagellum. Strain FW-1a grows at circumneutral pH, at freshwater salinities, and at temperatures of between 65 and 100{degrees}C with an optimum of 85 to 90{degrees}C. To our knowledge this is the highest temperature optimum of any organism in the Bacteria. Analysis of the 16S ribosomal DNA (rDNA) sequence of strain FW-1a places it within the Bacteria, most closely related to abundant but uncultured microorganisms whose 16S rDNA sequences have been previously recovered from Obsidian Pool and a terrestrial hot spring in Iceland. While previous studies inferred that the uncultured microorganisms with these 16S rDNA sequences were sulfate-reducing organisms, the physiology of the strain FW-1a, which does not reduce sulfate, indicates that these organisms are just as likely to be Fe(III) reducers. These results further demonstrate that Fe(III) may be helpful for recovering as-yet-uncultured microorganisms from hydrothermal environments and illustrate that caution must be used in inferring the physiological characteristics of at least some thermophilic microorganisms solely from 16S rDNA sequences. Based on both its 16S rDNA sequence and physiological characteristics, strain FW-1a represents a new genus among the Bacteria. The name Geothermobacterium ferrireducens gen. nov., sp. nov., is proposed (ATCC BAA-426).

Keegan, A. R., S. Fanok, et al. (2003). "Cell Culture-Taqman PCR Assay for Evaluation of Cryptosporidium parvum Disinfection." Appl. Envir. Microbiol. **69**(5): 2505-2511.

http://aem.asm.org/cgi/content/abstract/69/5/2505

Cryptosporidium parvum represents a challenge to the water industry and a threat to public health. In this study, we developed a cell culture-quantitative PCR assay to evaluate the inactivation of C. parvum with disinfectants. The assay was validated by using a range of disinfectants in common use in the water industry, including low-pressure UV light (LP-UV), ozone, mixed oxidants (MIOX), and chlorine. The assay was demonstrated to be reliable and sensitive, with a lower detection limit of a single infectious oocyst. Effective oocyst inactivation was achieved (>2 log10 units) with LP-UV (20 mJ/cm2) or 2 mg of ozone/liter (for 10 min). MIOX and chlorine treatments of oocysts resulted in minimal effective disinfection, with <0.1 log10 unit being inactivated. These results demonstrate the inability of MIOX to inactivate Cryptosporidium. The assay is a valuable tool for the evaluation of disinfection systems for drinking water and recycled water.

Kelley, S. T., U. Theisen, et al. (2004). "Molecular Analysis of Shower Curtain Biofilm Microbes." Appl. Envir. Microbiol. **70**(7): 4187-4192.

http://aem.asm.org/cgi/content/abstract/70/7/4187

Households provide environments that encourage the formation of microbial communities, often as biofilms. Such biofilms constitute potential reservoirs for pathogens, particularly for immune-compromised individuals. One household environment that potentially accumulates microbial biofilms is that provided by vinyl shower curtains. Over time, vinyl shower curtains accumulate films, commonly referred to as "soap scum," which microscopy reveals are constituted of lush microbial biofilms. To determine the kinds of microbes that constitute shower curtain biofilms and thereby to identify potential opportunistic pathogens, we conducted an analysis of rRNA genes obtained by PCR from four vinyl shower curtains from different households. Each of the shower curtain communities was highly complex. No sequence was identical to one in the databases, and no identical sequences were encountered in the different communities. However, the sequences generally represented similar phylogenetic kinds of organisms. Particularly abundant sequences represented members of the {alpha}-group of proteobacteria, mainly Sphingomonas spp. and

Methylobacterium spp. Both of these genera are known to include opportunistic pathogens, and several of the sequences obtained from the environmental DNA samples were closely related to known pathogens. Such organisms have also been linked to biofilm formation associated with water reservoirs and conduits. In addition, the study detected many other kinds of organisms at lower abundances. These results show that shower curtains are a potential source of opportunistic pathogens associated with biofilms. Frequent cleaning or disposal of shower curtains is indicated, particularly in households with immune-compromised individuals.

Kim, G.-B., C. M. Miyamoto, et al. (2004). "Cloning and Characterization of the Bile Salt Hydrolase Genes (bsh) from Bifidobacterium bifidum Strains." Appl. Envir. Microbiol. **70**(9): 5603-5612.

http://aem.asm.org/cgi/content/abstract/70/9/5603

Biochemical characterization of the purified bile salt hydrolase (BSH) from Bifidobacterium bifidum ATCC 11863 revealed some distinct characteristics not observed in other species of Bifidobacterium. The bsh gene was cloned from B. bifidum, and the DNA flanking the bsh gene was sequenced. Comparison of the deduced amino acid sequence of the cloned gene with previously known sequences revealed high homology with BSH enzymes from several microorganisms and penicillin V amidase (PVA) of Bacillus sphaericus. The proposed active sites of PVA were highly conserved, including that of the Cys-1 residue. The importance of the SH group in the N-terminal cysteine was confirmed by substitution of Cys with chemically and structurally similar residues, Ser or Thr, both of which resulted in an inactive enzyme. The transcriptional start point of the bsh gene has been determined by primer extension analysis. Unlike Bifidobacterium longum bsh, B. bifidum bsh was transcribed as a monocistronic unit, which was confirmed by Northern blot analysis. PCR amplification with the type-specific primer set revealed the high level of sequence homology in their bsh genes within the species of B. bifidum.

Klamer, M., M. S. Roberts, et al. (2002). "Influence of Elevated CO2 on the Fungal Community in a Coastal Scrub Oak Forest Soil Investigated with Terminal-Restriction Fragment Length Polymorphism Analysis." Appl. Envir. Microbiol. **68**(9): 4370-4376.

http://aem.asm.org/cgi/content/abstract/68/9/4370

Sixteen open-top chambers (diameter, 3.66 m) were established in a scrub oak habitat in central Florida where vegetation was removed in a planned burn prior to chamber installation. Eight control chambers have been continuously exposed to ambient air and eight have been continuously exposed to elevated CO2 at twice-ambient concentration ([~]700 ppm) for 5 years. Soil cores were collected from each chamber to examine the influence of elevated atmospheric CO2 on the fungal community in different soil fractions. Each soil sample was physically fractionated into bulk soil, rhizosphere soil, and roots for separate analyses. Changes in relative fungal biomass were estimated by the ergosterol technique. In the bulk soil and root fractions, a significantly increased level of ergosterol was detected in the elevated CO2 treatments relative to ambient controls. Fungal community composition was determined by terminal-restriction fragment length polymorphism (T-RFLP) analysis of the internal transcribed spacer (ITS) region. The specificities of different ITS primer sets were evaluated against plant and fungal species isolated from the experimental site. Changes in community composition were assessed by principal component analyses of T-RFLP profiles resolved by capillary electrophoresis. Fungal species richness, defined by the total number of terminal restriction fragments, was not significantly affected by either CO2 treatment or soil fraction.

Kobayashi, D. Y., R. M. Reedy, et al. (2002). "Characterization of a Chitinase Gene from Stenotrophomonas maltophilia Strain 34S1 and Its Involvement in Biological Control." Appl. Envir. Microbiol. **68**(3): 1047-1054.

http://aem.asm.org/cgi/content/abstract/68/3/1047

A chitinase gene was cloned on a 2.8-kb DNA fragment from Stenotrophomonas maltophilia strain 34S1 by heterologous expression in Burkholderia cepacia. Sequence analysis of this fragment identified an open reading frame encoding a deduced protein of 700 amino acids. Removal of the signal peptide sequence resulted in a predicted protein that was 68 kDa in size. Analysis of the sequence indicated that the chitinase contained a catalytic domain belonging to family 18 of glycosyl hydrolases. Three putative binding domains, a chitin binding domain, a novel polycystic kidney disease (PKD) domain, and a fibronectin type III domain, were also identified within the sequence. Pairwise comparisons of each domain to the most closely related sequences found in database searches clearly demonstrated variation in gene sources and the species from which related sequences originated. A 51-kDa protein with chitinolytic activity was purified from culture filtrates of S. maltophilia strain 34S1 by hydrophobic interaction chromatography. Although the protein was significantly smaller than the size predicted from the sequence, the N-terminal sequence verified that the first 15 amino acids were identical to the deduced sequence of the mature protein encoded by chiA. Marker exchange mutagenesis of chiA resulted in mutant strain C5, which was devoid of chitinolytic activity and lacked the 51-kDa protein in culture filtrates. Strain C5 was also reduced in the ability to suppress summer patch disease on Kentucky bluegrass, supporting a role for the enzyme in the biocontrol activity of S. maltophilia.

Koenig, G. L. (2003). "Viability of and Plasmid Retention in Frozen Recombinant Escherichia coli over Time: a Ten-Year Prospective Study." Appl. Envir. Microbiol. **69**(11): 6605-6609.

http://aem.asm.org/cgi/content/abstract/69/11/6605

The long-term viability and plasmid retention of recombinant Escherichia coli strains were investigated by real-time testing of master cell banks (MCBs) stored at the Roche Molecular Systems Culture Collection (RMSCC). MCBs at the RMSCC were cryogenically frozen and stored at -80{degrees}C for long-term preservation. At regular intervals during a period of 5 to more than 10 years, representative cryovials of each MCB were tested for viability and plasmid retention. Plasmid retention and viability for all 30 MCBs were stable over time. Twenty-seven MCBs maintained high levels of plasmid retention (at or near 100%), while three MCBs showed lower plasmid retention rates (ranging from 13.9 to 96.5%) that were consistent over time. New MCBs with high plasmid retention were created from two of the MCBs with lower plasmid retention by selective pressure with high levels of antibiotics. These new MCBs have shown stable viability and high plasmid retention over the first 5 months of storage. In conclusion, this study shows that properly selected, frozen and stored MCBs retain viability and maintain plasmid retention over time. Moreover, it is possible to recover cultures with high plasmid retention from MCBs with low plasmid retention by selecting clones grown in the presence of high levels of antibiotics.

Kralj, S., G. H. van Geel-Schutten, et al. (2002). "Molecular Characterization of a Novel Glucosyltransferase from Lactobacillus reuteri Strain 121 Synthesizing a Unique, Highly Branched Glucan with {alpha}-(1->4) and {alpha}-(1->6) Glucosidic Bonds." Appl. Envir. Microbiol. **68**(9): 4283-4291.

http://aem.asm.org/cgi/content/abstract/68/9/4283

Lactobacillus reuteri strain 121 produces a unique, highly branched, soluble glucan in which the majority of the linkages are of the {alpha}-(1[-&gt]4) glucosidic type. The glucan also contains {alpha}-(1[-&gt]6)-linked glucosyl units and 4,6-disubstituted {alpha}-glucosyl units at the branching points. Using degenerate primers, based on the amino acid sequences of conserved regions from known glucosyltransferase (gtf) genes from lactic acid bacteria, the L. reuteri strain 121 glucosyltransferase gene (gtfA) was isolated. The gtfA open reading frame (ORF) was 5,343 bp, and it encodes a protein of 1,781 amino acids with a deduced Mr of 198,637. The deduced amino acid sequence of GTFA revealed clear similarities with other glucosyltransferases. GTFA has a relatively large variable N-terminal domain (702 amino acids) with five unique repeats and a relatively short C-terminal domain (267 amino acids). The gtfA gene was expressed in Escherichia coli, yielding an active GTFA enzyme. With respect to binding type and size distribution, the recombinant GTFA enzyme and the L. reuteri strain 121 culture supernatants synthesized identical glucan polymers. Furthermore, the deduced amino acid sequence of the gtfA ORF and the N-terminal amino acid sequence of the glucosyltransferase isolated from culture supernatants of L. reuteri strain 121 were the same. GTFA is thus responsible for the synthesis of the unique glucan polymer in L. reuteri strain 121. This is the first report on the molecular characterization of a glucosyltransferase from a Lactobacillus strain.

Kuhn, R. C., C. M. Rock, et al. (2002). "Occurrence of Cryptosporidium and Giardia in Wild Ducks along the Rio Grande River Valley in Southern New Mexico." Appl. Envir. Microbiol. **68**(1): 161-165.

http://aem.asm.org/cgi/content/abstract/68/1/161

Fecal samples were taken from wild ducks on the lower Rio Grande River around Las Cruces, N. Mex., from September 2000 to January 2001. Giardia cysts and Cryptosporidium oocysts were purified from 69 samples by sucrose enrichment followed by cesium chloride (CsCl) gradient centrifugation and were viewed via fluorescent-antibody (FA) staining. For some samples, recovered cysts and oocysts were further screened via PCR to determine the presence of Giardia lamblia and Crytosporidium parvum. The results of this study indicate that 49% of the ducks were carriers of Cryptosporidium, and the Cryptosporidium oocyst concentrations ranged from 0 to 2,182 oocysts per g of feces (mean {+/-} standard deviation, 47.53 {+/-} 270.3 oocysts per g); also, 28% of the ducks were positive for Giardia, and the Giardia cyst concentrations ranged from 0 to 29,293 cysts per g of feces (mean {+/-} standard deviation, 436 {+/-} 3,525.4 cysts per g). Of the 69 samples, only 14 had (oo)cyst concentrations that were above the PCR detection limit. Samples did test positive for Cryptosporidium sp. However, C. parvum and G. lamblia were not detected in any of the 14 samples tested by PCR. Ducks on their southern migration through southern New Mexico were positive for Cryptosporidium and Giardia as determined by FA staining, but C. parvum and G. lamblia were not detected.

Kuske, C. R., L. O. Ticknor, et al. (2002). "Comparison of Soil Bacterial Communities in Rhizospheres of Three Plant Species and the Interspaces in an Arid Grassland." Appl. Envir. Microbiol. **68**(4): 1854-1863.

http://aem.asm.org/cgi/content/abstract/68/4/1854

Soil bacteria are important contributors to primary productivity and nutrient cycling in arid land ecosystems, and their populations may be greatly affected by changes in environmental conditions. In parallel studies, the composition of the total bacterial community and of members of the Acidobacterium division were assessed in arid grassland soils using terminal restriction fragment length polymorphism (TRF, also known as T-RFLP) analysis of 16S rRNA genes amplified from soil DNA. Bacterial communities associated with the rhizospheres of the native bunchgrasses Stipa hymenoides and Hilaria jamesii, the invading annual grass Bromus tectorum,

and the interspaces colonized by cyanobacterial soil crusts were compared at three depths. When used in a replicated field-scale study, TRF analysis was useful for identifying broad-scale, consistent differences in the bacterial communities in different soil locations, over the natural microscale heterogeneity of the soil. The compositions of the total bacterial community and Acidobacterium division in the soil crust interspaces were significantly different from those of the plant rhizospheres. Major differences were also observed in the rhizospheres of the three plant species and were most apparent with analysis of the Acidobacterium division. The total bacterial community and the Acidobacterium division bacteria were affected by soil depth in both the interspaces and plant rhizospheres. This study provides a baseline for monitoring bacterial community structure and dynamics with changes in plant cover and environmental conditions in the arid grasslands.

Latouche, G. N., M. Huynh, et al. (2003). "PCR-Restriction Fragment Length Polymorphism Analysis of the Phospholipase B (PLB1) Gene for Subtyping of Cryptococcus neoformans Isolates." Appl. Envir. Microbiol. **69**(4): 2080-2086.

http://aem.asm.org/cgi/content/abstract/69/4/2080

Cryptococcus neoformans is a pathogenic yeast that is currently divided into three varieties, five serotypes, and eight molecular types. The following report describes the use of PCR-restriction fragment length polymorphism (RFLP) analysis of the phospholipase B gene (PLB1) as a simple tool to differentiate between C. neoformans subgroups. A PLB1 fragment, 1,970 bp, was amplified and digested with either AvaI or HindIII. Both sets of profiles grouped the isolates into their respective varieties, but only the AvaI profiles allowed for the identification of the eight molecular types via the corresponding RFLP profiles A1 to A8. Digestion of the same fragments with HindIII resulted in RFLP profiles H1 to H5, which distinguished only between serotype A, AD, D, and B/C. Neither enzyme distinguished serotype B from serotype C. The serotype AD profile was a composite of the serotype A and D profiles. Further investigation showed that the serotype AD isolates used in this study are heterozygous, with one allele of PLB1 originating from a serotype A parent and the other from a serotype D parent.

LeChevallier, M. W., G. D. Di Giovanni, et al. (2003). "Comparison of Method 1623 and Cell Culture-PCR for Detection of Cryptosporidium spp. in Source Waters." Appl. Envir. Microbiol. **69**(2): 971-979.

http://aem.asm.org/cgi/content/abstract/69/2/971

Analysis of Cryptosporidium occurrence in six watersheds by method 1623 and the integrated cell culture-PCR (CC-PCR) technique provided an opportunity to evaluate these two methods. The average recovery efficiencies were 58.5% for the CC-PCR technique and 72% for method 1623, but the values were not significantly different (P = 0.06). Cryptosporidium oocysts were detected in 60 of 593 samples (10.1%) by method 1623. Infectious oocysts were detected in 22 of 560 samples (3.9%) by the CC-PCR technique. There was 87% agreement between the total numbers of samples positive as determined by method 1623 and CC-PCR for four of the sites. The other two sites had 16.3 and 24% correspondence between the methods. Infectious oocysts were detected in all of the watersheds. Overall, approximately 37% of the Cryptosporidium oocysts detected by the immunofluorescence method were viable and infectious. DNA sequence analysis of the Cryptosporidium parvum isolates detected by CC-PCR showed the presence of both the bovine and human genotypes. More than 90% of the C. parvum isolates were identified as having the bovine or bovine-like genotype. The estimates of the concentrations of infectious Cryptosporidium and the resulting daily and annual risks of infection compared well for the two methods. The results suggest that most surface water systems would require, on average, a 3-log reduction in source water Cryptosporidium levels to meet potable water goals.

Lin, M., D. A. Payne, et al. (2003). "Intraspecific Diversity of Vibrio vulnificus in Galveston Bay Water and Oysters as Determined by Randomly Amplified Polymorphic DNA PCR." Appl. Envir. Microbiol. **69**(6): 3170-3175.

http://aem.asm.org/cgi/content/abstract/69/6/3170

Randomly amplified polymorphic DNA (RAPD) PCR was used to analyze the temporal and spatial intraspecific diversity of 208 Vibrio vulnificus strains isolated from Galveston Bay water and oysters at five different sites between June 2000 and June 2001. V. vulnificus was not detected during the winter months (December through February). The densities of V. vulnificus in water and oysters were positively correlated with water temperature. Cluster analysis of RAPD PCR profiles of the 208 V. vulnificus isolates revealed a high level of intraspecific diversity among the strains. No correlation was found between the intraspecific diversity among the isolates and sampling site or source of isolation. After not being detected during the winter months, the genetic diversity of V. vulnificus strains first isolated in March was 0.9167. Beginning in April, a higher level of intraspecific diversity (0.9933) and a major shift in population structure were observed among V. vulnificus isolates. These results suggest that a great genetic diversity of V. vulnificus strains exists in Galveston Bay water and oysters and that the population structure of this species is linked to changes in environmental conditions, especially temperature.

Lofstrom, C., R. Knutsson, et al. (2004). "Rapid and Specific Detection of Salmonella spp. in Animal Feed Samples by PCR after Culture Enrichment." Appl. Envir. Microbiol. **70**(1): 69-75.

http://aem.asm.org/cgi/content/abstract/70/1/69

A PCR procedure has been developed for routine analysis of viable Salmonella spp. in feed samples. The objective was to develop a simple PCR-compatible enrichment procedure to enable DNA amplification without any sample pretreatment such as DNA extraction or cell lysis. PCR inhibition by 14 different feed samples and natural background flora was circumvented by the use of the DNA polymerase Tth. This DNA polymerase was found to exhibit a high level of resistance to PCR inhibitors present in these feed samples compared to DyNAzyme II, FastStart Taq, Platinum Taq, Pwo, rTth, Taq, and Tfl. The specificity of the Tth assay was confirmed by testing 101 Salmonella and 43 non-Salmonella strains isolated from feed and food samples. A sample preparation method based on culture enrichment in buffered peptone water and DNA amplification with Tth DNA polymerase was developed. The probability of detecting small numbers of salmonellae in feed, in the presence of natural background flora, was accurately determined and found to follow a logistic regression model. From this model, the probability of detecting 1 CFU per 25 g of feed in artificially contaminated soy samples was calculated and found to be 0.81. The PCR protocol was evaluated on 155 naturally contaminated feed samples and compared to an established culture-based method, NMKL-71. Eight percent of the samples were positive by PCR, compared with 3% with the conventional method. The reasons for the differences in sensitivity are discussed. Use of this method in the routine analysis of animal feed samples would improve safety in the food chain.

Lunde, M., J. M. Blatny, et al. (2003). "Use of Real-Time Quantitative PCR for the Analysis of {phi}LC3 Prophage Stability in Lactococci." Appl. Envir. Microbiol. **69**(1): 41-48.

http://aem.asm.org/cgi/content/abstract/69/1/41

Bacteriophages are a common and constant threat to proper milk fermentation. It has become evident that lysogeny is widespread in lactic acid bacteria, and in this work the temperate lactococcal bacteriophage {phi}LC3 was used as a model to study prophage stability in lactococci. The stability was analyzed in six {phi}LC3 lysogenic Lactococcus lactis subsp. cremoris host strains when they were growing at 15 and 30{degrees}C. In order to perform these analyses, a real-time PCR assay was developed. The stability of the {phi}LC3 prophage was found to vary with the growth phase of its host L. lactis IMN-C1814, in which the induction rate increased during the exponential growth phase and reached a maximum level when the strain was entering the stationary phase. The maximum spontaneous induction frequency of the {phi}LC3 prophage varied between 0.32 and 9.1% (28-fold) in the six lysogenic strains. No correlation was observed between growth rates of the host cells and the spontaneous prophage induction frequencies. Furthermore, the level of extrachromosomal phage DNA after induction of the prophage varied between the strains (1.9 to 390%), and the estimated burst sizes varied up to eightfold. These results show that the host cells have a significant impact on the lytic and lysogenic life styles of temperate bacteriophages. The present study shows the power of the real-time PCR technique in the analysis of temperate phage biology and will be useful in work to reveal the impact of temperate phages and lysogenic bacteria in various ecological fields.

Maidhof, H., B. Guerra, et al. (2002). "A Multiresistant Clone of Shiga Toxin-Producing Escherichia coli O118:[H16] Is Spread in Cattle and Humans over Different European Countries." Appl. Envir. Microbiol. **68**(12): 5834-5842.

http://aem.asm.org/cgi/content/abstract/68/12/5834

Multiresistant Shiga toxin-producing Escherichia coli (STEC) O118:H16 and O118 nonmotile strains (designated O118:[H16]) were detected by examination of 171 STEC isolates for their antimicrobial sensitivity. Of 48 STEC O118:[H16] strains, 98% were resistant to sulfonamide, 96% were resistant to streptomycin, 79% were resistant to kanamycin, 75% were resistant to tetracycline, 67% were resistant to ampicillin, 60% were resistant to chloramphenicol, 48% were resistant to trimethoprim, and 10% each were resistant to gentamicin and nalidixic acid. Nalidixic acid resistance and reduced susceptibility to ciprofloxacin were associated with the mutation gyrALEU-83. The STEC O118:[H16] strains were found to belong to a single genetic clone as investigated by multilocus enzyme electrophoresis and by multilocus sequence analysis of E. coli housekeeping genes. The STEC O118:[H16] strains originated from humans and cattle and were isolated in seven different countries of Europe between 1986 and 1999. Strains showing multiresistance to up to eight different antimicrobials predominated among the more recent STEC O118:[H16] strains. The genes in parentheses were associated with resistance to kanamycin (aphA1-Ia), chloramphenicol (catA1), tetracycline [tet(A)], and ampicillin (blaTEM-1). Class 1 integrons containing sulI (sulfonamide resistance), aadA1a (streptomycin resistance), or dfrA1 (trimethoprim resistance)-aadA1a gene cassettes were detected in 28 strains. The blaTEM-1b gene was present in 18 of 21 strains that were examined by nucleotide sequencing. Class 1 integrons and blaTEM genes were localized on plasmids and/or on the chromosome in different STEC O118:[H16] strains. Hybridization of XbaI-digested chromosomal DNA separated by pulsed-field gel electrophoresis revealed that blaTEM genes were integrated at different positions in the chromosome of STEC O118:[H16] strains that could have occurred by Tn2 insertion. Our data suggest that strains belonging to the STEC O118:[H16] clonal group have a characteristic propensity for acquisition and maintenance of resistance determinants, thus contrasting to STEC belonging to other serotypes.

Maluquer de Motes, C., P. Clemente-Casares, et al. (2004). "Detection of Bovine and Porcine Adenoviruses for Tracing the Source of Fecal Contamination." Appl. Envir. Microbiol. **70**(3): 1448-1454.

http://aem.asm.org/cgi/content/abstract/70/3/1448

In this study, a molecular procedure for the detection of adenoviruses of animal origin was developed to evaluate the level of excretion of these viruses by swine and cattle and to design a test to facilitate the tracing of specific sources of environmental viral contamination. Two sets of oligonucleotides were designed, one to detect porcine adenoviruses and the other to detect bovine and ovine adenoviruses. The specificity of the assays was assessed in 31 fecal samples and 12 sewage samples that were collected monthly during a 1-year period. The data also provided information on the environmental prevalence of animal adenoviruses. Porcine adenoviruses were detected in 17 of 24 (70%) pools of swine samples studied, with most isolates being closely related to serotype 3. Bovine adenoviruses were present in 6 of 8 (75%) pools studied, with strains belonging to the genera Mastadenovirus and Atadenovirus and being similar to bovine adenoviruses of types 2, 4, and 7. These sets of primers produced negative results in nested PCR assays when human adenovirus controls and urban-sewage samples were tested. Likewise, the sets of primers previously designed for detection of human adenovirus also produced negative results with animal adenoviruses. These results indicate the importance of further studies to evaluate the usefulness of these tests to trace the source of fecal contamination in water and food and for environmental studies.

Marciano-Cabral, F., R. MacLean, et al. (2003). "Identification of Naegleria fowleri in Domestic Water Sources by Nested PCR." Appl. Envir. Microbiol. **69**(10): 5864-5869.

http://aem.asm.org/cgi/content/abstract/69/10/5864

The free-living amoeboflagellate Naegleria fowleri is the causative agent of primary amoebic meningoencephalitis (PAM), a rapidly fatal disease of the central nervous system. In the United States, the disease is generally acquired while swimming and diving in freshwater lakes and ponds. In addition to swimming, exposure to N. fowleri and the associated disease can occur by total submersion in bathwater or small backyard wading pools. In the present study, swipe samples and residual pipe water from homes in Arizona were examined for N. fowleri by nested PCR due to the death of two previously healthy children from PAM. Since neither child had a history of swimming in a freshwater lake or pond prior to the onset of disease symptoms, the domestic water supply was the suspected source of infection. Of 19 samples collected from bathroom and kitchen pipes and sink traps, 17 samples were positive for N. fowleri by PCR. A sample from a Micro-Wynd II filter was obtained by passing water from bathtubs through the filter. Organisms attached to the filter also tested positive by PCR. The two samples that tested negative for N. fowleri were one that was obtained from a kitchen sink trap and a swipe sample from the garbage disposal of one home.

Marcille, F., A. Gomez, et al. (2002). "Distribution of Genes Encoding the Trypsin-Dependent Lantibiotic Ruminococcin A among Bacteria Isolated from Human Fecal Microbiota." Appl. Envir. Microbiol. **68**(7): 3424-3431.

http://aem.asm.org/cgi/content/abstract/68/7/3424

Fourteen bacterial strains capable of producing a trypsin-dependent antimicrobial substance active against Clostridium perfringens were isolated from human fecal samples of various origins (from healthy adults and children, as well as from adults with chronic pouchitis). Identification of these strains showed that they belonged to Ruminococcus gnavus, Clostridium nexile, and Ruminococcus hansenii species or to new operational taxonomic units, all from the Clostridium coccoides phylogenetic group. In hybridization experiments with a probe specific for the structural

gene encoding the trypsin-dependent lantibiotic ruminococcin A (RumA) produced by R. gnavus, seven strains gave a positive response. All of them harbored three highly conserved copies of rumA-like genes. The deduced peptide sequence was identical to or showed one amino acid difference from the hypothetical precursor of RumA. Our results indicate that the rumA-like genes have been disseminated among R. gnavus and phylogenetically related strains that can make up a significant part of the human fecal microbiota.

Matsui, G. Y., D. B. Ringelberg, et al. (2004). "Sulfate-Reducing Bacteria in Tubes Constructed by the Marine Infaunal Polychaete Diopatra cuprea." Appl. Envir. Microbiol. **70**(12): 7053-7065.

http://aem.asm.org/cgi/content/abstract/70/12/7053

Marine infaunal burrows and tubes greatly enhance solute transport between sediments and the overlying water column and are sites of elevated microbial activity. Biotic and abiotic controls of the compositions and activities of burrow and tube microbial communities are poorly understood. The microbial communities in tubes of the marine infaunal polychaete Diopatria cuprea collected from two different sediment habitats were examined. The bacterial communities in the tubes from a sandy sediment differed from those in the tubes from a muddy sediment. The difference in community structure also extended to the sulfate-reducing bacterial (SRB) assemblage, although it was not as pronounced for this functional group of species. PCR-amplified 16S rRNA gene sequences recovered from Diopatra tube SRB by clonal library construction and screening were all related to the family Desulfobacteriaceae. This finding was supported by phospholipid fatty acid analysis and by hybridization of 16S rRNA probes specific for members of the genera Desulfosarcina, Desulfobacter, Desulfobacterium, Desulfobotulus, Desulfococcus, and Desulfovibrio and some members of the genera Desulfomonas, Desulfuromonas, and Desulfomicrobium with 16S rRNA gene sequences resolved by denaturing gradient gel electrophoresis. Two of six SRB clones from the clone library were not detected in tubes from the sandy sediment. The habitat in which the D. cuprea tubes were constructed had a strong influence on the tube bacterial community as a whole, as well as on the SRB assemblage.

Mayer, Z., P. Farber, et al. (2003). "Monitoring the Production of Aflatoxin B1 in Wheat by Measuring the Concentration of nor-1 mRNA." Appl. Envir. Microbiol. **69**(2): 1154-1158.

http://aem.asm.org/cgi/content/abstract/69/2/1154

A real-time reverse transcription-PCR system has been used to monitor the expression of an aflatoxin biosynthetic gene of Aspergillus flavus in wheat. Therefore, total RNA was isolated from infected wheat samples, reverse transcribed and subjected to real-time PCR. In parallel all samples were analyzed by high-pressure liquid chromatography for aflatoxin B1 production. The primer-probe system of the real-time PCR was targeted against nor-1, a gene of the aflatoxin biosynthetic pathway. By application of this method the nor-1 transcription was quantified during the course of incubation. After 4 days of incubation nor-1 mRNA could be detected for the first time. The amount of nor-1 mRNA increased rapidly, and the maximum was achieved after 6 days. Then, starting very slowly, the mRNA was degraded until day 8, and this was followed by a very fast degradation, reaching nondetectable levels at days 9 and 10. First traces of aflatoxin B1could be detected between the 5th and 6th day of incubation. The aflatoxin concentration reached its maximum after 9 days of incubation and remained constant for the whole period of observation. To ensure that differences in the nor-1 mRNA concentration were due to different expression levels, the expression of the constitutively expressed {beta}-tubulin gene (benA56) has also been monitored. The expression of benA56 remained constant during the whole incubation time. As a parameter for fungal growth, the number of nor-1 gene copies was determined during the course of incubation. The numbers of nor-1 gene copies increased at the beginning of the incubation and

reached a plateau at day 5. They correlate well with the viable counts albeit at a higher level.

McMahon, K. D., M. A. Dojka, et al. (2002). "Polyphosphate Kinase from Activated Sludge Performing Enhanced Biological Phosphorus Removal." Appl. Envir. Microbiol. **68**(10): 4971-4978.

http://aem.asm.org/cgi/content/abstract/68/10/4971

A novel polyphosphate kinase (PPK) was retrieved from an uncultivated organism in activated sludge carrying out enhanced biological phosphorus removal (EBPR). Acetate-fed laboratory-scale sequencing batch reactors were used to maintain sludge with a high phosphorus content (approximately 11% of the biomass). PCR-based clone libraries of small subunit rRNA genes and fluorescent in situ hybridization (FISH) were used to verify that the sludge was enriched in Rhodocyclus-like {beta}-Proteobacteria known to be associated with sludges carrying out EBPR. These organisms comprised approximately 80% of total bacteria in the sludge, as assessed by FISH. Degenerate PCR primers were designed to retrieve fragments of putative ppk genes from a pure culture of Rhodocyclus tenuis and from organisms in the sludge. Four novel ppk homologs were found in the sludge, and two of these (types I and II) shared a high degree of amino acid similarity with R. tenuis PPK (86 and 87% similarity, respectively). Dot blot analysis of total RNA extracted from sludge demonstrated that the Type I ppk mRNA was present, indicating that this gene is expressed during EBPR. Inverse PCR was used to obtain the full Type I sequence from sludge DNA, and a full-length PPK was cloned, overexpressed, and purified to near homogeneity. The purified PPK has a specific activity comparable to that of other PPKs, has a requirement for Mg2+, and does not appear to operate in reverse. PPK activity was found mainly in the particulate fraction of lysed sludge microorganisms.

Meguro, N., Y. Kodama, et al. (2005). "Molecular Characterization of Resistance-Nodulation-Division Transporters from Solvent- and Drug-Resistant Bacteria in Petroleum-Contaminated Soil." Appl. Envir. Microbiol. **71**(1): 580-586.

http://aem.asm.org/cgi/content/abstract/71/1/580

PCR assays for analyzing resistance-nodulation-division transporters from solvent- and drug-resistant bacteria in soil were developed. Sequence analysis of amplicons showed that the PCR successfully retrieved transporter gene fragments from soil. Most of the genes retrieved from petroleum-contaminated soils formed a cluster (cluster PCS) that was distantly related to known transporter genes. Competitive PCR showed that the abundance of PCS genes is increased in petroleum-contaminated soil.

Moore, J. E., M. McCalmont, et al. (2002). "Asaia sp., an Unusual Spoilage Organism of Fruit-Flavored Bottled Water." Appl. Envir. Microbiol. **68**(8): 4130-4131.

http://aem.asm.org/cgi/content/abstract/68/8/4130

A gram-negative bacillus was isolated from a batch of fruit-flavored bottled water, which had spoiled as a result of bacterial overgrowth (>106 CFU/ml). The spoilage organism was extremely difficult to identify phenotypically and was poorly identified as Pasturella sp. (78.7% identification profile) employing the API 20NE identification scheme, which gave the profile 5040000. Molecular identification through PCR amplification of a partial region of the 16S rRNA gene followed by direct automated sequencing of the PCR amplicon allowed identification of the organism. Due to

the sequence identity (100%) between the spoilage organism and a reference strain in GenBank, the spoilage isolate was considered to be an Asaia sp., a recently described genus and member of the acetic acid bacteria. This is the first report of Asaia sp. causing spoilage of a foodstuff and highlights the benefits of molecular identification techniques based on 16S rRNA gene sequences in the identification of unusual spoilage organisms.

Myrmel, M., E. M. M. Berg, et al. (2004). "Detection of Enteric Viruses in Shellfish from the Norwegian Coast." Appl. Envir. Microbiol. **70**(5): 2678-2684.

http://aem.asm.org/cgi/content/abstract/70/5/2678

Common blue mussels (Mytilus edulis), horse mussels (Modiolus modiolus), and flat oysters (Ostrea edulis) obtained from various harvesting and commercial production sites along the Norwegian coast were screened for the presence of norovirus by a real-time reverse transcription (RT)-nested PCR assay and for possible indicators of fecal contamination, i.e., for F-specific RNA bacteriophages (F-RNA phages) by plaque assay and for human adenoviruses and human circoviruses by nested PCR assay. The aims were to obtain relevant information for assessing the risk of transmission of enteric viruses by shellfish and to investigate the potential of various indicator viruses in routine screening. Noroviruses were detected in 6.8% of the samples, and the indicators were detected in 23.8% (F-RNA phages), 18.6% (adenoviruses), and 8.0% (circoviruses) of the samples. A seasonal variation was observed, with the exception of circoviruses, with more positive samples in the winter. A positive correlation was found between F-RNA phages and noroviruses. However, F-RNA phages were present in only 43% of the norovirus-positive samples. The results show that mussels from the Norwegian coast can constitute a risk of infection with enteric viruses and that routine testing of samples may be justified. Advantages and disadvantages of various options for screening are discussed.

Nuanualsuwan, S. and D. O. Cliver (2003). "Capsid Functions of Inactivated Human Picornaviruses and Feline Calicivirus." Appl. Envir. Microbiol. **69**(1): 350-357.

http://aem.asm.org/cgi/content/abstract/69/1/350

The exceptional stability of enteric viruses probably resides in their capsids. The capsid functions of inactivated human picornaviruses and feline calicivirus (FCV) were determined. Viruses were inactivated by UV, hypochlorite, high temperature (72{degrees}C), and physiological temperature (37{degrees}C), all of which are pertinent to transmission via food and water. Poliovirus (PV) and hepatitis A virus (HAV) are transmissible via water and food, and FCV is the best available surrogate for the Norwalk-like viruses, which are leading causes of food-borne and waterborne disease in the United States. The capsids of all 37{degrees}C-inactivated viruses still protected the viral RNA against RNase, even in the presence of proteinase K, which contrasted with findings with viruses inactivated at 72{degrees}C. The loss of ability of the virus to attach to homologous cell receptors was universal, regardless of virus type and inactivation method, except for UV-inactivated HAV, and so virus inactivation was almost always accompanied by the loss of virus attachment. Inactivated HAV and FCV were captured by homologous antibodies. However, inactivated PV type 1 (PV-1) was not captured by homologous antibody and 37{degrees}C-inactivated PV-1 was only partially captured. The epitopes on the capsids of HAV and FCV are evidently discrete from the receptor attachment sites, unlike those of PV-1. These findings indicate that the primary target of UV, hypochlorite, and 72{degrees}C inactivation is the capsid and that the target of thermal inactivation (37{degrees}C versus 72{degrees}C) is temperature dependent.

Panicker, G., D. R. Call, et al. (2004). "Detection of Pathogenic Vibrio spp. in Shellfish by Using Multiplex PCR and DNA Microarrays." Appl. Envir. Microbiol. **70**(12): 7436-7444.

http://aem.asm.org/cgi/content/abstract/70/12/7436

This study describes the development of a gene-specific DNA microarray coupled with multiplex PCR for the comprehensive detection of pathogenic vibrios that are natural inhabitants of warm coastal waters and shellfish. Multiplex PCR with vvh and viuB for Vibrio vulnificus, with ompU, toxR, tcpI, and hlyA for V. cholerae, and with tlh, tdh, trh, and open reading frame 8 for V. parahaemolyticus helped to ensure that total and pathogenic strains, including subtypes of the three Vibrio spp., could be detected and discriminated. For DNA microarrays, oligonucleotide probes for these targeted genes were deposited onto epoxysilane-derivatized, 12-well, Teflon-masked slides by using a MicroGrid II arrayer. Amplified PCR products were hybridized to arrays at 50{degrees}C and detected by using tyramide signal amplification with Alexa Fluor 546 fluorescent dye. Slides were imaged by using an arrayWoRx scanner. The detection sensitivity for pure cultures without enrichment was 102 to 103 CFU/ml, and the specificity was 100%. However, 5 h of sample enrichment followed by DNA extraction with Instagene matrix and multiplex PCR with microarray hybridization resulted in the detection of 1 CFU in 1 g of oyster tissue homogenate. Thus, enrichment of the bacterial pathogens permitted higher sensitivity in compliance with the Interstate Shellfish Sanitation Conference guideline. Application of the DNA microarray methodology to natural oysters revealed the presence of V. vulnificus (100%) and V. parahaemolyticus (83%). However, V. cholerae was not detected in natural oysters. An assay involving a combination of multiplex PCR and DNA microarray hybridization would help to ensure rapid and accurate detection of pathogenic vibrios in shellfish, thereby improving the microbiological safety of shellfish for consumers.

Panicker, G., M. L. Myers, et al. (2004). "Rapid Detection of Vibrio vulnificus in Shellfish and Gulf of Mexico Water by Real-Time PCR." Appl. Envir. Microbiol. **70**(1): 498-507.

http://aem.asm.org/cgi/content/abstract/70/1/498

In this paper we describe optimization of SYBR Green I-based real-time PCR parameters and testing of a large number of microbial species with vvh-specific oligonucleotide primers to establish a rapid, specific, and sensitive method for detection of Vibrio vulnificus in oyster tissue homogenate and Gulf of Mexico water (gulf water). Selected oligonucleotide primers for the vvh gene were tested for PCR amplification of a 205-bp DNA fragment with a melting temperature of approximately 87{degrees}C for 84 clinical and environmental strains of V. vulnificus. No amplification was observed with other vibrios or nonvibrio strains with these primers. The minimum level of detection by the real-time PCR method was 1 pg of purified genomic DNA or 102 V. vulnificus cells in 1 g of unenriched oyster tissue homogenate or 10 ml of gulf water. It was possible to improve the level of detection to one V. vulnificus cell in samples that were enriched for 5 h. The standard curves prepared from the real-time PCR cycle threshold values revealed that there was a strong correlation between the number of cells in unenriched samples and the number of cells in enriched samples. Detection of a single cell of V. vulnificus in 1 g of enriched oyster tissue homogenate is in compliance with the recent Interstate Shellfish Sanitation Conference guidelines. The entire detection method, including sample processing, enrichment, and real-time PCR amplification, was completed within 8 h, making it a rapid single-day assay. Rapid and sensitive detection of V. vulnificus would ensure a steady supply of postharvest treated oysters to consumers, which should help decrease the number of illnesses or outbreaks caused by this pathogen.

Park, J.-H. and C. A. Batt (2004). "Restoration of a Defective Lactococcus lactis Xylose Isomerase." Appl. Envir. Microbiol. **70**(7): 4318-4325.

http://aem.asm.org/cgi/content/abstract/70/7/4318

The genes (xylA) encoding xylose isomerase (XI) from two Lactococcus lactis subsp. lactis strains, 210 (Xyl-) and IO-1 (Xyl+), were cloned, and the activities of their expressed proteins in recombinant strains of Escherichia coli were investigated. The nucleotide and amino acid sequence homologies between the xylA genes were 98.4 and 98.6%, respectively, and only six amino acid residues differed between the two XIs. The purified IO-1 XI was soluble with Km and kcat being 2.25 mM and 184/s, respectively, while the 210 XI was insoluble and inactive. Site-directed mutagenesis on 210 xylA showed that a triple mutant possessing R202M/Y218D/V275A mutations regained XI activity and was soluble. The Km and kcat of this mutant were 4.15 mM and 141/s, respectively. One of the IO-1 XI mutants, S388T, was insoluble and showed negligible activity similar to that of 210 XI. The introduction of a K407E mutation to the IO-1 S388T XI mutant restored its activity and solubility. The dissolution of XI activity in L. lactis subsp. lactis involves a series of mutations that collectively eliminate enzyme activity by reducing the solubility of the enzyme.

Parke, D. and L. N. Ornston (2003). "Hydroxycinnamate (hca) Catabolic Genes from Acinetobacter sp. Strain ADP1 Are Repressed by HcaR and Are Induced by Hydroxycinnamoyl-Coenzyme A Thioesters." Appl. Envir. Microbiol. **69**(9): 5398-5409.

http://aem.asm.org/cgi/content/abstract/69/9/5398

Hydroxycinnamates are plant products catabolized through the diphenol protocatechuate in the naturally transformable bacterium Acinetobacter sp. strain ADP1. Genes for protocatechuate catabolism are central to the dca-pca-qui-pob-hca chromosomal island, for which gene designations corresponding to catabolic function are dca (dicarboxylic acid), pca (protocatechuate), qui (quinate), pob (p-hydroxybenzoate), and hca (hydroxycinnamate). Acinetobacter hcaC had been cloned and shown to encode a hydroxycinnamate:coenzyme A (CoA) SH ligase that acts upon caffeate, p-coumarate, and ferulate, but genes for conversion of hydroxycinnamoyl-CoA to protocatechuate had not been characterized. In this investigation, DNA from pobS to an XbaI site 5.3 kb beyond hcaC was captured in the plasmid pZR8200 by a strategy that involved in vivo integration of a cloning vector near the hca region of the chromosome. pZR8200 enabled Escherichia coli to convert p-coumarate to protocatechuate in vivo. Sequence analysis of the newly cloned DNA identified five open reading frames designated hcaA, hcaB, hcaK, hcaR, and ORF1. An Acinetobacter strain with a knockout of HcaA, a homolog of hydroxycinnamoyl-CoA hydratase/lyases, was unable to grow at the expense of hydroxycinnamates, whereas a strain mutated in HcaB, homologous to aldehyde dehydrogenases, grew poorly with ferulate and caffeate but well with p-coumarate. A chromosomal fusion of lacZ to the hcaE gene was used to monitor expression of the hcaABCDE promoter. LacZ was induced over 100-fold by growth in the presence of caffeate, p-coumarate, or ferulate. The protein deduced to be encoded by hcaR shares 28% identity with the aligned E. coli repressor, MarR. A knockout of hcaR produced a constitutive phenotype, as assessed in the hcaE:lacZ-Kmr genetic background, revealing HcaR to be a repressor as well. Expression of hcaE:lacZ in strains with knockouts in hcaA, hcaB, or hcaC revealed unambiguously that hydroxycinnamoyl-CoA thioesters relieve repression of the hcaABCDE genes by HcaR.

Parke, D. and L. N. Ornston (2004). "Toxicity Caused by Hydroxycinnamoyl-Coenzyme A Thioester Accumulation in Mutants of Acinetobacter sp. Strain ADP1." Appl. Envir. Microbiol. **70**(5): 2974-2983.

http://aem.asm.org/cgi/content/abstract/70/5/2974

Hydroxycinnamates, aromatic compounds that play diverse roles in plants, are dissimilated by enzymes encoded by the hca genes in the nutritionally versatile, naturally transformable bacterium Acinetobacter sp. strain ADP1. A key step in the hca-encoded pathway is activation of the natural substrates caffeate, p-coumarate, and ferulate by an acyl:coenzyme A (acyl:CoA) ligase encoded by hcaC. As described in this paper, Acinetobacter cells with a knockout of the next enzyme in the pathway, hydroxycinnamoyl-CoA hydratase/lyase (HcaA), are extremely sensitive to the presence of the three natural hydroxycinnamate substrates; Escherichia coli cells carrying a subclone with the hcaC gene are hydroxycinnamate sensitive as well. When the hcaA mutation was combined with a mutation in the repressor HcaR, exposure of the doubly mutated Acinetobacter cells to caffeate, p-coumarate, or ferulate at 10-6 M totally inhibited the growth of cells. The toxicity of p-coumarate and ferulate to a {Delta}hcaA strain was found to be a bacteriostatic effect. Although not toxic to wild-type cells initially, the diphenolic caffeate was itself converted to a toxin over time in the absence of cells; the converted toxin was bactericidal. In an Acinetobacter strain blocked in hcaA, a secondary mutation in the ligase (HcaC) suppresses the toxic effect. Analysis of suppression due to the mutation of hcaC led to the development of a positive-selection strategy that targets mutations blocking HcaC. An hcaC mutation from one isolate was characterized and was found to result in the substitution of an amino acid that is conserved in a functionally characterized homolog of HcaC.

Parshionikar, S. U., S. Willian-True, et al. (2003). "Waterborne Outbreak of Gastroenteritis Associated with a Norovirus." Appl. Envir. Microbiol. **69**(9): 5263-5268.

http://aem.asm.org/cgi/content/abstract/69/9/5263

The Wyoming Department of Health investigated an outbreak of acute gastroenteritis among persons who dined at a tourist saloon in central Wyoming during October 2001. Human caliciviruses (HuCVs) were suspected as the etiological agent of the outbreak based on the incubation period, duration of illness, and symptoms observed in ill patrons. A retrospective cohort study demonstrated that ill patrons were 4.5 times more likely to have exposure to drinking water and/or ice than nonill patrons. No food items were associated with illness. An environmental investigation gave evidence that the saloon's groundwater was contaminated with sewage. Water from the saloon's only well was processed for viruses. The processed water sample and stool samples collected from three ill patrons were analyzed by reverse transcription-PCR (RT-PCR) for the presence of HuCV. All positive RT-PCR results were confirmed by sequence and phylogenetic analyses of cloned RT-PCR products. A genogroup I, subtype 3, HuCV stain was found to be present in the well water sample and two stool samples. In addition, a genogroup II, subtype 6, strain was detected in one stool sample. The identification of the same HuCV strain in both the well water and stool samples strongly suggests a link between exposure to well water and the outbreak of gastroenteritis. The presence of a genogroup II, subtype 6, strain in one of the stool samples suggests that multiple HuCV strains may have been involved in this outbreak. The laboratory isolation of HuCV strains from outbreak-associated drinking water is relatively novel in the United States. This investigation outlines the procedure for virus isolation and illustrates the utility of RT-PCR for the identification of HuCV in large volumes of water and stool samples obtained during outbreaks of acute nonbacterial gastroenteritis.

Pena, J. A., S. Y. Li, et al. (2004). "Genotypic and Phenotypic Studies of Murine Intestinal Lactobacilli: Species Differences in Mice with and without Colitis." Appl. Envir. Microbiol. **70**(1): 558-568.

http://aem.asm.org/cgi/content/abstract/70/1/558

Lactobacilli represent components of the commensal mammalian gastrointestinal microbiota and are useful as probiotics, functional foods, and dairy products. This study includes systematic polyphasic analyses of murine intestinal Lactobacillus isolates and correlation of taxonomic findings with data from cytokine production assays. Lactobacilli were recovered from mice with microbiota-dependent colitis (interleukin-10 [IL-10]-deficient C57BL/6 mice) and from mice without colitis (Swiss Webster and inducible nitric oxide synthetase-deficient C57BL/6 mice). Polyphasic analyses were performed to elucidate taxonomic relationships among 88 reference and murine gastrointestinal lactobacilli. Genotypic tests included single-locus analyses (16S ribosomal DNA sequencing and 16S-23S rRNA intergenic spacer region PCR) and genomic DNA profiling (repetitive DNA element-based PCR), and phenotypic analyses encompassed more than 50 tests for carbohydrate utilization, enzyme production, and antimicrobial resistance. From 20 mice without colitis, six Lactobacillus species were recovered; the majority of the mice were colonized with L. reuteri or L. murinus (72% of isolates). In contrast, only, L. johnsonii was isolated from 14 IL-10-deficient mice. Using an in vitro assay, we screened murine isolates for their ability to inhibit tumor necrosis factor alpha (TNF-{alpha}) secretion by lipopolysaccharide-activated macrophages. Interestingly, a subpopulation of lactobacilli recovered from mice without colitis displayed TNF-{alpha} inhibitory properties, whereas none of the L. johnsonii isolates from IL-10-deficient mice exhibited this effect. We propose that differences among intestinal Lactobacillus populations in mammals, combined with host genetic susceptibilities, may account partly for variations in host mucosal responses.

Pereira, D. I. A. and G. R. Gibson (2002). "Cholesterol Assimilation by Lactic Acid Bacteria and Bifidobacteria Isolated from the Human Gut." Appl. Envir. Microbiol. **68**(9): 4689-4693.

http://aem.asm.org/cgi/content/abstract/68/9/4689

The objective of this study was to evaluate the effect of human gut-derived lactic acid bacteria and bifidobacteria on cholesterol levels in vitro. Continuous cultures inoculated with fecal material from healthy human volunteers with media supplemented with cholesterol and bile acids were used to enrich for potential cholesterol assimilators among the indigenous bacterial populations. Seven potential probiotics were found: Lactobacillus fermentum strains F53 and KC5b, Bifidobacterium infantis ATCC 15697, Streptococcus bovis ATCC 43143, Enterococcus durans DSM 20633, Enterococcus gallinarum, and Enterococcus faecalis. A comparative evaluation regarding the in vitro cholesterol reduction abilities of these strains along with commercial probiotics was undertaken. The degree of acid and bile tolerance of strains was also evaluated. The human isolate L. fermentum KC5b was able to maintain viability for 2 h at pH 2 and to grow in a medium with 4,000 mg of bile acids per liter. This strain was also able to remove a maximum of 14.8 mg of cholesterol per g (dry weight) of cells from the culture medium and therefore was regarded as a candidate probiotic.

Phister, T. G. and D. A. Mills (2003). "Real-Time PCR Assay for Detection and Enumeration of Dekkera bruxellensis in Wine." Appl. Envir. Microbiol. **69**(12): 7430-7434.

http://aem.asm.org/cgi/content/abstract/69/12/7430

Traditional methods to detect the spoilage yeast Dekkera bruxellensis from wine involve lengthy enrichments. To overcome this difficulty, we developed a quantitative real-time PCR method to directly detect and enumerate D. bruxellensis in wine. Specific PCR primers to D. bruxellensis were designed to the 26S rRNA gene, and nontarget yeast and bacteria common to the winery environment were not amplified. The assay was linear over a range of cell concentrations (6 log units) and could detect as little as 1 cell per ml in wine. The addition of large amounts of nontarget yeasts did not impact the efficiency of the assay. This method will be helpful to identify

possible routes of D. bruxellensis infection in winery environments. Moreover, the time involved in performing the assay (3 h) should enable winemakers to more quickly make wine processing decisions in order to reduce the threat of spoilage by D. bruxellensis.

Pickup, R. W., G. Rhodes, et al. (2005). "Mycobacterium avium subsp. paratuberculosis in the Catchment Area and Water of the River Taff in South Wales, United Kingdom, and Its Potential Relationship to Clustering of Crohn's Disease Cases in the City of Cardiff." Appl. Envir. Microbiol. **71**(4): 2130-2139.

http://aem.asm.org/cgi/content/abstract/71/4/2130

In South Wales, United Kingdom, a populated coastal region lies beneath hill pastures grazed by livestock in which Mycobacterium avium subsp. paratuberculosis is endemic. The Taff is a spate river running off the hills and through the principal city of Cardiff. We sampled Taff water above Cardiff twice weekly from November 2001 to November 2002. M. avium subsp. paratuberculosis was detected by IS900 PCR and culture. Thirty-one of 96 daily samples (32.3%) were IS900 PCR positive, and 12 grew M. avium subsp. paratuberculosis bovine strains. Amplicon sequences from colonies were identical to the sequence with GenBank accession no. X16293, whereas 16 of 19 sequences from river water DNA extracts had a single-nucleotide polymorphism at position 214. This is consistent with a different strain of M. avium subsp. paratuberculosis in the river, which is unculturable by the methods we used. Parallel studies showed that M. avium subsp. paratuberculosis remained culturable in lake water microcosms for 632 days and persisted to 841 days. Of four reservoirs controlling the catchment area of the Taff, M. avium subsp. paratuberculosis was present in surface sediments from three and in sediment cores from two, consistent with deposition over at least 50 years. Previous epidemiological research in Cardiff demonstrated a highly significant increase of Crohn's disease in 11 districts. These bordered the river except for a gap on the windward side. A topographical relief map shows that this gap is directly opposite a valley open to the prevailing southwesterly winds. This would influence the distribution of aerosols carrying M. avium subsp. paratuberculosis from the river.

Provencher, C., G. LaPointe, et al. (2003). "Consensus-Degenerate Hybrid Oligonucleotide Primers for Amplification of Priming Glycosyltransferase Genes of the Exopolysaccharide Locus in Strains of the Lactobacillus casei Group." Appl. Envir. Microbiol. **69**(6): 3299-3307.

http://aem.asm.org/cgi/content/abstract/69/6/3299

A primer design strategy named CODEHOP (consensus-degenerate hybrid oligonucleotide primer) for amplification of distantly related sequences was used to detect the priming glycosyltransferase (GT) gene in strains of the Lactobacillus casei group. Each hybrid primer consisted of a short 3' degenerate core based on four highly conserved amino acids and a longer 5' consensus clamp region based on six sequences of the priming GT gene products from exopolysaccharide (EPS)-producing bacteria. The hybrid primers were used to detect the priming GT gene of 44 commercial isolates and reference strains of Lactobacillus rhamnosus, L. casei, Lactobacillus zeae, and Streptococcus thermophilus. The priming GT gene was detected in the genome of both non-EPS-producing (EPS-) and EPS-producing (EPS+) strains of L. rhamnosus. The sequences of the cloned PCR products were similar to those of the priming GT gene of various gram-negative and gram-positive EPS+ bacteria. Specific primers designed from the L. rhamnosus RW-9595M GT gene were used to sequence the end of the priming GT gene in selected EPS+ strains of L. rhamnosus. Phylogenetic analysis revealed that Lactobacillus spp. form a distinctive group apart from other lactic acid bacteria for which GT genes have been characterized to date. Moreover, the sequences show a divergence existing among strains of L. rhamnosus with respect to the terminal region of the priming GT gene. Thus, the PCR approach

with consensus-degenerate hybrid primers designed with CODEHOP is a practical approach for the detection of similar genes containing conserved motifs in different bacterial genomes.

Reed, D. W., Y. Fujita, et al. (2002). "Microbial Communities from Methane Hydrate-Bearing Deep Marine Sediments in a Forearc Basin." Appl. Envir. Microbiol. **68**(8): 3759-3770.

http://aem.asm.org/cgi/content/abstract/68/8/3759

Microbial communities in cores obtained from methane hydrate-bearing deep marine sediments (down to more than 300 m below the seafloor) in the forearc basin of the Nankai Trough near Japan were characterized with cultivation-dependent and -independent techniques. Acridine orange direct count data indicated that cell numbers generally decreased with sediment depth. Lipid biomarker analyses indicated the presence of viable biomass at concentrations greater than previously reported for terrestrial subsurface environments at similar depths. Archaeal lipids were more abundant than bacterial lipids. Methane was produced from both acetate and hydrogen in enrichments inoculated with sediment from all depths evaluated, at both 10 and 35{degrees}C. Characterization of 16S rRNA genes amplified from the sediments indicated that archaeal clones could be discretely grouped within the Euryarchaeota and Crenarchaeota domains. The bacterial clones exhibited greater overall diversity than the archaeal clones, with sequences related to the Bacteroidetes, Planctomycetes, Actinobacteria, Proteobacteria, and green nonsulfur groups. The majority of the bacterial clones were either members of a novel lineage or most closely related to uncultured clones. The results of these analyses suggest that the microbial community in this environment is distinct from those in previously characterized methane hydrate-bearing sediments.

Requena, T., J. Burton, et al. (2002). "Identification, Detection, and Enumeration of Human Bifidobacterium Species by PCR Targeting the Transaldolase Gene." Appl. Envir. Microbiol. **68**(5): 2420-2427.

http://aem.asm.org/cgi/content/abstract/68/5/2420

Methods that enabled the identification, detection, and enumeration of Bifidobacterium species by PCR targeting the transaldolase gene were tested. Bifidobacterial species isolated from the feces of human adults and babies were identified by PCR amplification of a 301-bp transaldolase gene sequence and comparison of the relative migrations of the DNA fragments in denaturing gradient gel electrophoresis (DGGE). Two subtypes of Bifidobacterium longum, five subtypes of Bifidobacterium adolescentis, and two subtypes of Bifidobacterium pseudocatenulatum could be differentiated using PCR-DGGE. Bifidobacterium angulatum and B. catenulatum type cultures could not be differentiated from each other. Bifidobacterial species were also detected directly in fecal samples by this combination of PCR and DGGE. The number of species detected was less than that detected by PCR using species-specific primers targeting 16S ribosomal DNA (rDNA). Real-time quantitative PCR targeting a 110-bp transaldolase gene sequence was used to enumerate bifidobacteria in fecal samples. Real-time quantitative PCR measurements of bifidobacteria in fecal samples from adults correlated well with results obtained by culture when either a 16S rDNA sequence or the transaldolase gene sequence was targeted. In the case of samples from infants, 16S rDNA-targeted PCR was superior to PCR targeting the transaldolase gene for the quantification of bifidobacterial populations.

Rich, J. J., R. S. Heichen, et al. (2003). "Community Composition and Functioning of Denitrifying Bacteria

from Adjacent Meadow and Forest Soils." Appl. Envir. Microbiol. **69**(10): 5974-5982.

http://aem.asm.org/cgi/content/abstract/69/10/5974

We investigated communities of denitrifying bacteria from adjacent meadow and forest soils. Our objectives were to explore spatial gradients in denitrifier communities from meadow to forest, examine whether community composition was related to ecological properties (such as vegetation type and process rates), and determine phylogenetic relationships among denitrifiers. nosZ, a key gene in the denitrification pathway for nitrous oxide reductase, served as a marker for denitrifying bacteria. Denitrifying enzyme activity (DEA) was measured as a proxy for function. Other variables, such as nitrification potential and soil C/N ratio, were also measured. Soil samples were taken along transects that spanned meadow-forest boundaries at two sites in the H. J. Andrews Experimental Forest in the Western Cascade Mountains of Oregon. Results indicated strong functional and structural community differences between the meadow and forest soils. Levels of DEA were an order of magnitude higher in the meadow soils. Denitrifying community composition was related to process rates and vegetation type as determined on the basis of multivariate analyses of nosZ terminal restriction fragment length polymorphism profiles. Denitrifier communities formed distinct groups according to vegetation type and site. Screening 225 nosZ clones yielded 47 unique denitrifying genotypes; the most dominant genotype occurred 31 times, and half the genotypes occurred once. Several dominant and less-dominant denitrifying genotypes were more characteristic of either meadow or forest soils. The majority of nosZ fragments sequenced from meadow or forest soils were most similar to nosZ from the Rhizobiaceae group in {alpha}-Proteobacteria species. Denitrifying community composition, as well as environmental factors, may contribute to the variability of denitrification rates in these systems.

Romanova, N., S. Favrin, et al. (2002). "Sensitivity of Listeria monocytogenes to Sanitizers Used in the Meat Processing Industry." Appl. Envir. Microbiol. **68**(12): 6405-6409.

http://aem.asm.org/cgi/content/abstract/68/12/6405

Nineteen Listeria monocytogenes strains were characterized by automated ribotyping, pulsed-field gel electrophoresis, and plasmid profiling to determine the relationship between genotype and sanitizer resistance. Isolates within a ribogroup had a consistent sensitivity or resistance phenotype except for ribogroup C isolates. All isolates with resistance phenotypes harbored two plasmids. The sensitivity of L. monocytogenes strains to quaternary ammonium compounds (QACs) was correlated with sensitivity to sanitizers and antibiotics with other modes of action. All isolates tested contained the mdrL gene, which encodes an efflux pump that confers resistance to QACs and is both chromosome and plasmid borne.

Rudi, K., H. K. Hoidal, et al. (2004). "Direct Real-Time PCR Quantification of Campylobacter jejuni in Chicken Fecal and Cecal Samples by Integrated Cell Concentration and DNA Purification." Appl. Envir. Microbiol. **70**(2): 790-797.

http://aem.asm.org/cgi/content/abstract/70/2/790

Campylobacter jejuni is a major cause of diarrheal disease and food-borne gastroenteritis. The main reservoir of C. jejuni in poultry is the cecum, with an estimated content of 6 to 8 log10 CFU/g. If a flock is infected with C. jejuni, the majority of the birds in that flock will harbor the bacterium. Diagnostics at the flock level could thus be an important control point. The aim of the work presented here was to develop a complete quantitative PCR-based detection assay for C.

jejuni obtained directly from cecal contents and fecal samples. We applied an approach in which the same paramagnetic beads were used both for cell isolation and for DNA purification. This integrated approach enabled both fully automated and quantitative sample preparation and a DNA extraction method. We developed a complete quantitative diagnostic assay through the combination of the sample preparation approach and real-time 5'-nuclease PCR. The assay was evaluated both by spiking the samples with C. jejuni and through the detection of C. jejuni in naturally colonized chickens. Detection limits between 2 and 25 CFU per PCR and a quantitative range of >4 log10 were obtained for spiked fecal and cecal samples. Thirty-one different poultry flocks were screened for naturally colonized chickens. A total of 262 (204 fecal and 58 cecal) samples were analyzed. Nineteen of the flocks were Campylobacter positive, whereas 12 were negative. Two of the flocks contained Campylobacter species other than C. jejuni. There was a large difference in the C. jejuni content, ranging from 4 to 8 log10 CFU/g of fecal or cecal material, for the different flocks tested. Some issues that have not yet promoted much attention are the prequantitative differences in the ability of C. jejuni to colonize poultry and the importance of these differences for causing human disease through food contamination. Understanding the colonization kinetics in poultry is therefore of great importance for controlling human infections by this bacterium.

Rudi, K., B. Moen, et al. (2005). "Use of Ethidium Monoazide and PCR in Combination for Quantification of Viable and Dead Cells in Complex Samples." Appl. Envir. Microbiol. **71**(2): 1018-1024.

http://aem.asm.org/cgi/content/abstract/71/2/1018

The distinction between viable and dead cells is a major issue in many aspects of biological research. The current technologies for determining viable versus dead cells cannot readily be used for quantitative differentiation of specific cells in mixed populations. This is a serious limitation. We have solved this problem by developing a new concept with the viable/dead stain ethidium monoazide (EMA) in combination with real-time PCR (EMA-PCR). A dynamic range of approximately 4 log10 was obtained for the EMA-PCR viable/dead assay. Viable/dead differentiation is obtained by covalent binding of EMA to DNA in dead cells by photoactivation. EMA penetrates only dead cells with compromised membrane/cell wall systems. DNA covalently bound to EMA cannot be PCR amplified. Thus, only DNA from viable cells can be detected. We evaluated EMA-PCR with the major food-borne bacterium Campylobacter jejuni as an example. Traditional diagnosis of this bacterium is very difficult due to its specific growth requirements and because it may enter a state where it is viable but not cultivable. The conditions analyzed included detection in mixed and natural samples, survival in food, and survival after disinfection or antibiotic treatment. We obtained reliable viable/dead quantifications for all conditions tested. Comparison with standard fluorescence-based viable/dead techniques showed that the EMA-PCR has a broader dynamic range and enables quantification in mixed and complex samples. In conclusion, EMA-PCR offers a novel real-time PCR method for quantitative distinction between viable and dead cells with potentially very wide application.

Sails, A. D., F. J. Bolton, et al. (2002). "Detection of Campylobacter jejuni and Campylobacter coli in Environmental Waters by PCR Enzyme-Linked Immunosorbent Assay." Appl. Envir. Microbiol. **68**(3): 1319-1324.

http://aem.asm.org/cgi/content/abstract/68/3/1319

A PCR enzyme-linked immunosorbent assay (ELISA) assay was applied to the detection of Campylobacter jejuni and Campylobacter coli in environmental water samples after enrichment culture. Bacterial cells were concentrated from 69 environmental water samples by using filtration, and the filtrates were cultured in Campylobacter blood-free broth. After enrichment

culture, DNA was extracted from the samples by using a rapid-boiling method, and the DNA extracts were used as a template in a PCR ELISA assay. A total of 51 samples were positive by either PCR ELISA or culture; of these, 43 were found to be positive by PCR ELISA and 43 were found to be positive by culture. Overall, including positive and negative results, 59 samples were concordant in both methods. Several samples were positive in the PCR ELISA assay but were culture negative; therefore, this assay may be able to detect sublethally damaged or viable nonculturable forms of campylobacters. The method is rapid and sensitive, and it significantly reduces the time needed for the detection of these important pathogens by 2 to 3 days.

Salles, J. F., F. A. De Souza, et al. (2002). "Molecular Method To Assess the Diversity of Burkholderia Species in Environmental Samples." Appl. Envir. Microbiol. **68**(4): 1595-1603.

http://aem.asm.org/cgi/content/abstract/68/4/1595

In spite of the importance of many members of the genus Burkholderia in the soil microbial community, no direct method to assess the diversity of this genus has been developed so far. The aim of this work was the development of soil DNA-based PCR-denaturing gradient gel electrophoresis (DGGE), a powerful tool for studying the diversity of microbial communities, for detection and analysis of the Burkholderia diversity in soil samples. Primers specific for the genus Burkholderia were developed based on the 16S rRNA gene sequence and were evaluated in PCRs performed with genomic DNAs from Burkholderia and non-Burkholderia species as the templates. The primer system used exhibited good specificity and sensitivity for the majority of established species of the genus Burkholderia. DGGE analyses of the PCR products obtained showed that there were sufficient differences in migration behavior to distinguish the majority of the 14 Burkholderia species tested. Sequence analysis of amplicons generated with soil DNA exclusively revealed sequences affiliated with sequences of Burkholderia species, demonstrating that the PCR-DGGE method is suitable for studying the diversity of this genus in natural settings. A PCR-DGGE analysis of the Burkholderia communities in two grassland plots revealed differences in diversity mainly between bulk and rhizosphere soil samples; the communities in the latter samples produced more complex patterns.

Sarma-Rupavtarm, R. B., Z. Ge, et al. (2004). "Spatial Distribution and Stability of the Eight Microbial Species of the Altered Schaedler Flora in the Mouse Gastrointestinal Tract." Appl. Envir. Microbiol. **70**(5): 2791-2800.

http://aem.asm.org/cgi/content/abstract/70/5/2791

The overall complexity of the microbial communities in the gastrointestinal (GI) tracts of mammals has hindered observations of dynamics and interactions of individual bacterial populations. However, such information is crucial for understanding the diverse disease-causing and protective roles that gut microbiota play in their hosts. Here, we determine the spatial distribution, interanimal variation, and persistence of bacteria in the most complex defined-flora (gnotobiotic) model system to date, viz., mice colonized with the eight strains of the altered Schaedler flora (ASF). Quantitative PCR protocols based on the 16S rRNA sequence of each ASF strain were developed and optimized to specifically detect as few as 10 copies of each target. Total numbers of the ASF strains were determined in the different regions of the GI tracts of three C.B-17 SCID mice. Individual strain abundance was dependent on oxygen sensitivity, with microaerotolerant Lactobacillus murinus ASF361 present at 105 to 107 cells/g of tissue in the upper GI tract and obligate anaerobic ASF strains being predominant in the cecal and colonic flora at 108 to 1010 cells/g of tissue. The variation between the three mice was small for most ASF strains, except for Clostridium sp. strain ASF502 and Bacteroides sp. strain ASF519 in the cecum. A comparison of the relative distribution of the ASF strains in feces and the colon indicated large differences,

suggesting that fecal bacterial levels may provide a poor approximation of colonic bacterial levels. All ASF strains were detected by PCR in the feces of C57BL/6 restricted flora mice, which had been maintained in an isolator without sterile food, water, or bedding for several generations, providing evidence for the stability of these strains in the face of potential competition by bacteria introduced into the gut.

Schonfeld, J., H. Heuer, et al. (2003). "Specific and Sensitive Detection of Ralstonia solanacearum in Soil on the Basis of PCR Amplification of fliC Fragments." Appl. Envir. Microbiol. **69**(12): 7248-7256.

http://aem.asm.org/cgi/content/abstract/69/12/7248

Ralstonia solanacearum is the causative agent of bacterial wilt in many important crops. A specific and sensitive PCR detection method that uses primers targeting the gene coding for the flagella subunit, fliC, was established. Based on the first fliC gene sequence of R. solanacearum strain K60 available at GenBank, the Ral_fliC PCR primer system was designed; this system yielded a single 724-bp product with the DNAs of all of the R. solanacearum strains tested. However, R. pickettii and four environmental Ralstonia isolates also yielded amplicons. The Ral_fliC PCR products obtained with 12 strains (R. solanacearum, R. pickettii, and environmental isolates) were sequenced. By sequence alignment, Rsol_fliC primers specific for R. solanacearum were designed. With this primer system, a specific 400-bp PCR product was obtained from all 82 strains of R. solanacearum tested. Six strains of R. pickettii and several closely related environmental isolates yielded no PCR product; however, a product was obtained with one Pseudomonas syzygii strain. A GC-clamped 400-bp fliC product could be separated in denaturing gradient gels and allowed us to distinguish P. syzygii from R. solanacearum. The Rsol_fliC PCR system was applied to detect R. solanacearum in soil. PCR amplification, followed by Southern blot hybridization, allowed us to detect about one target DNA molecule per PCR, which is equivalent to 103 CFU g of bulk soil-1. The system was applied to survey soils from different geographic origins for the presence of R. solanacearum.

Schwab, K. J. and J. J. McDevitt (2003). "Development of a PCR-Enzyme Immunoassay Oligoprobe Detection Method for Toxoplasma gondii Oocysts, Incorporating PCR Controls." Appl. Envir. Microbiol. **69**(10): 5819-5825.

http://aem.asm.org/cgi/content/abstract/69/10/5819

Infections caused by Toxoplasma gondii are widely prevalent in animals and humans throughout the world. In the United States, an estimated 23% of adolescents and adults have laboratory evidence of T. gondii infection. T. gondii has been identified as a major opportunistic pathogen in immunocompromised individuals, in whom it can cause life-threatening disease. Water contaminated with feces from domestic cats or other felids may be an important source of human exposure to T. gondii oocysts. Because of the lack of information regarding the prevalence of T. gondii in surface waters, there is a clear need for a rapid, sensitive method to detect T. gondii from water. Currently available animal models and cell culture methods are time-consuming, expensive, and labor-intensive, requiring days or weeks for results to be obtained. Detection of T. gondii nucleic acid by PCR has become the preferred method. We have developed a PCR amplification and detection method for T. gondii oocyst nucleic acid that incorporates the use of hot-start amplification to reduce nonspecific primer annealing, uracil-N-glycosylase to prevent false-positive results due to carryover contamination, an internal standard control to identify false-negative results due to inadequate removal of sample inhibition, and PCR product oligoprobe confirmation using a nonradioactive DNA hybridization immunoassay. This method can provide positive, confirmed results in less than 1 day. Fewer than 50 oocysts can be detected following recovery of oocyst DNA. Development of a T. gondii oocyst PCR detection method will provide a

useful technique to estimate the levels of T. gondii oocysts present in surface waters.

Sheehan, K. B., J. M. Henson, et al. (2005). "Legionella Species Diversity in an Acidic Biofilm Community in Yellowstone National Park." Appl. Envir. Microbiol. **71**(1): 507-511.

http://aem.asm.org/cgi/content/abstract/71/1/507

Legionella species are frequently detected in aquatic environments, but their occurrence in extreme, acidic, geothermal habitats has not been explored with cultivation-independent methods. We investigated a predominately eukaryotic algal mat community in a pH 2.7 geothermal stream in Yellowstone National Park for the presence of Legionella and potential host amoebae. Our analyses, using PCR amplification with Legionella-specific primers targeting 16S rRNA genes, detected four known Legionella species, as well as Legionella sequences from species that are not represented in sequence databases, in mat samples and cultivated isolates. The nonrandom occurrence of sequences detected at lower (30{degrees}C) and higher (35 to 38{degrees}C) temperatures suggests that natural thermal gradients in the stream influence Legionella species distributions in this mat community. We detected only one sequence, Legionella micdadei, from cultivated isolates. We cultured and sequenced partial 18S rRNA gene regions from two potential hosts, Acanthamoeba and Euglena species.

Shields, J. M. and B. H. Olson (2003). "PCR-Restriction Fragment Length Polymorphism Method for Detection of Cyclospora cayetanensis in Environmental Waters without Microscopic Confirmation." Appl. Envir. Microbiol. **69**(8): 4662-4669.

http://aem.asm.org/cgi/content/abstract/69/8/4662

We developed an alternative nested-PCR-restriction fragment length polymorphism (RFLP) protocol for the detection of Cyclospora cayetanensis in environmental samples that obviates the need for microscopic examination. The RFLP method, with the restriction enzyme AluI, differentiates the amplified target sequence from C. cayetanensis from those that may cross-react. This new protocol was used to reexamine a subset (121 of 180) of surface water samples. Samples previously positive when the CYCF3E and CYCR4B primers (33) and RFLP with MnlI (20) were used were also PCR positive with the new primers; however, they were RFLP negative. We verified, by sequencing these amplicons, that while two were most likely other Cyclospora species, they were not C. cayetanensis. We can detect as few as one oocyst seeded into an autoclaved pellet flocculated from 10 liters of surface water. This new protocol should be of great use for environmental microbiologists and public health laboratories.

Shigematsu, T., Y. Tang, et al. (2004). "Effect of Dilution Rate on Metabolic Pathway Shift between Aceticlastic and Nonaceticlastic Methanogenesis in Chemostat Cultivation." Appl. Envir. Microbiol. **70**(7): 4048-4052.

http://aem.asm.org/cgi/content/abstract/70/7/4048

Acetate conversion pathways of methanogenic consortia in acetate-fed chemostats at dilution rates of 0.025 and 0.6 day-1 were investigated by using 13C-labeled acetates, followed by gas chromatography-mass spectrometry (GC-MS) analysis of the CH4 and CO2 produced. Nonaceticlastic syntrophic oxidation by acetate-oxidizing syntrophs and hydrogenotrophic methanogens was suggested to occupy a primary pathway (approximately 62 to 90%) in total

methanogenesis at the low dilution rate. In contrast, aceticlastic cleavage of acetate by aceticlastic methanogens was suggested to occupy a primary pathway (approximately 95 to 99%) in total methanogenesis at the high dilution rate. Phylogenetic analyses of transcripts of the methyl coenzyme M reductase gene (mcrA) confirmed that a significant number of transcripts of the genera Methanoculleus (hydrogenotrophic methanogens) and Methanosarcina (aceticlastic methanogens) were present in the chemostats at the low and high dilution rates, respectively. The mcrA transcripts of the genus Methanosaeta (aceticlastic methanogens), which dominated the population in a previous study (T. Shigematsu, Y. Tang, H. Kawaguchi, K. Ninomiya, J. Kijima, T. Kobayashi, S. Morimura, and K. Kida, J. Biosci. Bioeng. 96:547-558, 2003), were poorly detected at both dilution rates due to the limited coverage of the primers used. These results demonstrated that the dilution rate could cause a shift in the primary pathway of acetate conversion to methane in acetate-fed chemostats.

Short, C. M. and C. A. Suttle (2005). "Nearly Identical Bacteriophage Structural Gene Sequences Are Widely Distributed in both Marine and Freshwater Environments." Appl. Envir. Microbiol. **71**(1): 480-486.

http://aem.asm.org/cgi/content/abstract/71/1/480

Primers were designed to amplify a 592-bp region within a conserved structural gene (g20) found in some cyanophages. The goal was to use this gene as a proxy to infer genetic richness in natural cyanophage communities and to determine if sequences were more similar in similar environments. Gene products were amplified from samples from the Gulf of Mexico, the Arctic, Southern, and Northeast and Southeast Pacific Oceans, an Arctic cyanobacterial mat, a catfish production pond, lakes in Canada and Germany, and a depth of ca. 3,246 m in the Chuckchi Sea. Amplicons were separated by denaturing gradient gel electrophoresis, and selected bands were sequenced. Phylogenetic analysis revealed four previously unknown groups of g20 clusters, two of which were entirely found in freshwater. Also, sequences with >99% identities were recovered from environments that differed greatly in temperature and salinity. For example, nearly identical sequences were recovered from the Gulf of Mexico, the Southern Pacific Ocean, an Arctic freshwater cyanobacterial mat, and Lake Constance, Germany. These results imply that closely related hosts and the viruses infecting them are distributed widely across environments or that horizontal gene exchange occurs among phage communities from very different environments. Moreover, the amplification of g20 products from deep in the cyanobacterium-sparse Chuckchi Sea suggests that this primer set targets bacteriophages other than those infecting cyanobacteria.

Short, S. M. and C. A. Suttle (2002). "Sequence Analysis of Marine Virus Communities Reveals that Groups of Related Algal Viruses Are Widely Distributed in Nature." Appl. Envir. Microbiol. **68**(3): 1290-1296.

http://aem.asm.org/cgi/content/abstract/68/3/1290

Algal-virus-specific PCR primers were used to amplify DNA polymerase (pol) gene fragments from geographically isolated natural virus communities. Natural algal virus communities were obtained from coastal sites in the Pacific Ocean in British Columbia, Canada, and the Southern Ocean near the Antarctic peninsula. Genetic fingerprints of algal virus communities were generated using denaturing gradient gel electrophoresis (DGGE). Sequencing efforts recovered 33 sequences from the gradient gel. Of the 33 sequences examined, 25 encoded a conserved amino acid motif indicating that the sequences were pol gene fragments. Furthermore, the 25 pol sequences were related to pol gene fragments from known algal viruses. In addition, similar virus sequences (>98% sequence identity) were recovered from British Columbia and Antarctica.

Results from this study demonstrate that DGGE with degenerate primers can be used to qualitatively fingerprint and assess genetic diversity in specific subsets of natural virus communities and that closely related viruses occur in distant geographic locations. DGGE is a powerful tool for genetically fingerprinting natural virus communities and may be used to examine how specific components of virus communities respond to experimental manipulations.

Singh, B. K., A. Walker, et al. (2003). "Effects of Soil pH on the Biodegradation of Chlorpyrifos and Isolation of a Chlorpyrifos-Degrading Bacterium." Appl. Envir. Microbiol. **69**(9): 5198-5206.

http://aem.asm.org/cgi/content/abstract/69/9/5198

We examined the role of microorganisms in the degradation of the organophosphate insecticide chlorpyrifos in soils from the United Kingdom and Australia. The kinetics of degradation in five United Kingdom soils varying in pH from 4.7 to 8.4 suggested that dissipation of chlorpyrifos was mediated by the cometabolic activities of the soil microorganisms. Repeated application of chlorpyrifos to these soils did not result in the development of a microbial population with an enhanced ability to degrade the pesticide. A robust bacterial population that utilized chlorpyrifos as a source of carbon was detected in an Australian soil. The enhanced ability to degrade chlorpyrifos in the Australian soil was successfully transferred to the five United Kingdom soils. Only soils with a pH of [IMG]=" BORDER="0">6.7 were able to maintain this degrading ability 90 days after inoculation. Transfer and proliferation of degrading microorganisms from the Australian soil to the United Kingdom soils was monitored by molecular fingerprinting of bacterial 16S rRNA genes by PCR-denaturing gradient gel electrophoresis (DGGE). Two bands were found to be associated with enhanced degradation of chlorpyrifos. Band 1 had sequence similarity to enterics and their relatives, while band 2 had sequence similarity to strains of Pseudomonas. Liquid enrichment culture using the Australian soil as the source of the inoculum led to the isolation of a chlorpyrifos-degrading bacterium. This strain had a 16S rRNA gene with a sequence identical to that of band 1 in the DGGE profile of the Australian soil. DNA probing indicated that genes similar to known organophosphate-degrading (opd) genes were present in the United Kingdom soils. However, no DNA hybridization signal was detected for the Australian soil or the isolated degrader. This indicates that unrelated genes were present in both the Australian soil and the chlorpyrifos-degrading isolate. These results are consistent with our observations that degradation of chlorpyrifos in these systems was unusual, as it was growth linked and involved complete mineralization. As the 16S rRNA gene of the isolate matched a visible DGGE band from the Australian soil, the isolate is likely to be both prominent and involved in the degradation of chlorpyrifos in this soil.

Song, J. K., B. Chung, et al. (2002). "Construction of DNA-Shuffled and Incrementally Truncated Libraries by a Mutagenic and Unidirectional Reassembly Method: Changing from a Substrate Specificity of Phospholipase to That of Lipase." Appl. Envir. Microbiol. **68**(12): 6146-6151.

http://aem.asm.org/cgi/content/abstract/68/12/6146

A method of mutagenic and unidirectional reassembly (MURA) that can generate libraries of DNA-shuffled and randomly truncated proteins was developed. The method involved fragmenting the template gene(s) randomly by DNase I and reassembling the small fragments with a unidirectional primer by PCR. The MURA products were treated with T4 DNA polymerase and subsequently with a restriction enzyme whose site was located on the region of the MURA primer. The N-terminal-truncated and DNA-shuffled library of a Serratia sp. phospholipase A1 prepared by this method had an essentially random variation of truncated size and also showed point mutations associated with DNA shuffling. After high-throughput screening on triglyceride-emulsified plates, several mutants exhibiting absolute lipase activity (NPL variants) were

obtained. The sequence analysis and the lipase activity assay on the NPL variants revealed that N-terminal truncations at a region beginning with amino acids 61 to 71, together with amino acid substitutions, resulted in the change of substrate specificity from a phospholipase to a lipase. We therefore suggest that the MURA method, which combines incremental truncation with DNA shuffling, can contribute to expanding the searchable sequence space in directed evolution experiments.

Stanton, T. B. and S. B. Humphrey (2003). "Isolation of Tetracycline-Resistant Megasphaera elsdenii Strains with Novel Mosaic Gene Combinations of tet(O) and tet(W) from Swine." Appl. Envir. Microbiol. **69**(7): 3874-3882.

http://aem.asm.org/cgi/content/abstract/69/7/3874

Anaerobic bacteria insensitive to chlortetracycline (64 to 256 {micro}g/ml) were isolated from cecal contents and cecal tissues of swine fed or not fed chlortetracycline. A nutritionally complex, rumen fluid-based medium was used for culturing the bacteria. Eight of 84 isolates from seven different animals were identified as Megasphaera elsdenii strains based on their large-coccus morphology, rapid growth on lactate, and 16S ribosomal DNA sequence similarities with M. elsdenii LC-1T. All eight strains had tetracycline MICs of between 128 and 256 {micro}g/ml. Based on PCR assays differentiating 14 tet classes, the strains gave a positive reaction for the tet(O) gene. By contrast, three ruminant M. elsdenii strains recovered from 30-year-old culture stocks had tetracycline MICs of 4 {micro}g/ml and did not contain tet genes. The tet genes of two tetracycline-resistant M. elsdenii strains were amplified and cloned. Both genes bestowed tetracycline resistance (MIC = 32 to 64 {micro}g/ml) on recombinant Escherichia coli strains. Sequence analysis revealed that the M. elsdenii genes represent two different mosaic genes formed by interclass (double-crossover) recombination events involving tet(O) and tet(W). One or the other genotype was present in each of the eight tetracycline-resistant M. elsdenii strains isolated in these studies. These findings suggest a role for commensal bacteria not only in the preservation and dissemination of antibiotic resistance in the intestinal tract but also in the evolution of resistance.

Suzuki, Y., S. D. Kelly, et al. (2003). "Microbial Populations Stimulated for Hexavalent Uranium Reduction in Uranium Mine Sediment." Appl. Envir. Microbiol. **69**(3): 1337-1346.

http://aem.asm.org/cgi/content/abstract/69/3/1337

Uranium-contaminated sediment and water collected from an inactive uranium mine were incubated anaerobically with organic substrates. Stimulated microbial populations removed U almost entirely from solution within 1 month. X-ray absorption near-edge structure analysis showed that U(VI) was reduced to U(IV) during the incubation. Observations by transmission electron microscopy, selected area diffraction pattern analysis, and energy-dispersive X-ray spectroscopic analysis showed two distinct types of prokaryotic cells that precipitated only a U(IV) mineral uraninite (UO2) or both uraninite and metal sulfides. Prokaryotic cells associated with uraninite and metal sulfides were inferred to be sulfate-reducing bacteria. Phylogenetic analysis of 16S ribosomal DNA obtained from the original and incubated sediments revealed that microbial populations were changed from microaerophilic Proteobacteria to anaerobic low-G+C gram-positive sporeforming bacteria by the incubation. Forty-two out of 94 clones from the incubated sediment were related to sulfate-reducing Desulfosporosinus spp., and 23 were related to fermentative Clostridium spp. The results suggest that, if in situ bioremediation were attempted in the uranium mine ponds, Desulfosporosinus spp. would be a major contributor to U(VI) and sulfate reduction and Clostridium spp. to U(VI) reduction.

Trujillo, M. E., A. Willems, et al. (2005). "Nodulation of Lupinus albus by Strains of Ochrobactrum lupini sp. nov." Appl. Envir. Microbiol. **71**(3): 1318-1327.

http://aem.asm.org/cgi/content/abstract/71/3/1318

The nodulation of legumes has for more than a century been considered an exclusive capacity of a group of microorganisms commonly known as rhizobia and belonging to the {alpha}-Proteobacteria. However, in the last 3 years four nonrhizobial species, belonging to {alpha} and {beta} subclasses of the Proteobacteria, have been described as legume-nodulating bacteria. In the present study, two fast-growing strains, LUP21 and LUP23, were isolated from nodules of Lupinus honoratus. The phylogenetic analysis based on the 16S and 23S rRNA gene sequences showed that the isolates belong to the genus Ochrobactrum. The strains were able to reinfect Lupinus plants. A plasmid profile analysis showed the presence of three plasmids. The nodD and nifH genes were located on these plasmids, and their sequences were obtained. These sequences showed a close resemblance to the nodD and nifH genes of rhizobial species, suggesting that the nodD and nifH genes carried by strain LUP21T were acquired by horizontal gene transfer. A polyphasic study including phenotypic, chemotaxonomic, and molecular features of the strains isolated in this study showed that they belong to a new species of the genus Ochrobactrum for which we propose the name Ochrobactrum lupini sp. nov. Strain LUP21T (LMG 20667T) is the type strain.

Umeno, D. and F. H. Arnold (2003). "A C35 Carotenoid Biosynthetic Pathway." Appl. Envir. Microbiol. **69**(6): 3573-3579.

http://aem.asm.org/cgi/content/abstract/69/6/3573

Upon coexpression with Erwinia geranylgeranyldiphosphate (GGDP) synthase in Escherichia coli, C30 carotenoid synthase CrtM from Staphylococcus aureus produces novel carotenoids with the asymmetrical C35 backbone. The products of condensation of farnesyldiphosphate and GDP, C35 structures comprise 40 to 60% of total carotenoid accumulated. Carotene desaturases and carotene cyclases from C40 or C30 pathways accepted and converted the C35 substrate, thus creating a C35 carotenoid biosynthetic pathway in E. coli. Directed evolution to modulate desaturase step number, together with combinatorial expression of the desaturase variants with lycopene cyclases, allowed us to produce at least 10 compounds not previously described. This result highlights the plastic and expansible nature of carotenoid pathways and illustrates how combinatorial biosynthesis coupled with directed evolution can rapidly access diverse chemical structures.

Valinsky, L., G. Della Vedova, et al. (2002). "Oligonucleotide Fingerprinting of rRNA Genes for Analysis of Fungal Community Composition." Appl. Envir. Microbiol. **68**(12): 5999-6004.

http://aem.asm.org/cgi/content/abstract/68/12/5999

Thorough assessments of fungal diversity are currently hindered by technological limitations. Here we describe a new method for identifying fungi, oligonucleotide fingerprinting of rRNA genes (OFRG). ORFG sorts arrayed rRNA gene (ribosomal DNA [rDNA]) clones into taxonomic clusters through a series of hybridization experiments, each using a single oligonucleotide probe. A simulated annealing algorithm was used to design an OFRG probe set for fungal rDNA. Analysis of 1,536 fungal rDNA clones derived from soil generated 455 clusters. A pairwise sequence

analysis showed that clones with average sequence identities of 99.2% were grouped into the same cluster. To examine the accuracy of the taxonomic identities produced by this OFRG experiment, we determined the nucleotide sequences for 117 clones distributed throughout the tree. For all but two of these clones, the taxonomic identities generated by this OFRG experiment were consistent with those generated by a nucleotide sequence analysis. Eighty-eight percent of the clones were affiliated with Ascomycota, while 12% belonged to Basidiomycota. A large fraction of the clones were affiliated with the genera Fusarium (404 clones) and Raciborskiomyces (176 clones). Smaller assemblages of clones had high sequence identities to the Alternaria, Ascobolus, Chaetomium, Cryptococcus, and Rhizoctonia clades.

Waldenstrom, J., T. Broman, et al. (2002). "Prevalence of Campylobacter jejuni, Campylobacter lari, and Campylobacter coli in Different Ecological Guilds and Taxa of Migrating Birds." Appl. Envir. Microbiol. **68**(12): 5911-5917.

http://aem.asm.org/cgi/content/abstract/68/12/5911

A total of 1,794 migrating birds trapped at a coastal site in southern Sweden were sampled for detection of Campylobacter spp. All isolates phenotypically identified as Campylobacter jejuni and a subset of those identified as non-C. jejuni were identified to the species level by PCR-based techniques. C. jejuni was found in 5.0% of the birds, Campylobacter lari was found in 5.6%, and Campylobacter coli was found in 0.9%. An additional 10.7% of the tested birds were infected with hippurate hydrolysis-negative Campylobacter spp. that were not identified to the species level. The prevalence of Campylobacter spp. differed significantly between ecological guilds of birds. Shoreline-foraging birds feeding on invertebrates and opportunistic feeders were most commonly infected (76.8 and 50.0%, respectively). High prevalence was also shown in other ground-foraging guilds, i.e., ground-foraging invertebrate feeders (11.0%), ground-foraging insectivores (20.3%), and plant-eating species (18.8%). Almost no Campylobacter spp. were found in ground-foraging granivores (2.3%), arboreal insectivores (0.6%), aerial insectivores (0%), or reed- and herbaceous plant-foraging insectivores (3.5%). During the autumn migration, a high proportion of samples from juveniles were positive (7.1% in passerines, 55.0% in shorebirds), indicating transmission on the breeding grounds or during the early part of migration. Prevalence of Campylobacter spp. was associated with increasing body mass among passerine bird species. Furthermore, prevalence was higher in short-distance migrants wintering in Europe than in long-distance migrants wintering in Africa, the Middle East, or Asia. Among ground-foraging birds of the Muscicapidae, those of the subfamily Turdinae (i.e., Turdus spp.) showed a high prevalence of Campylobacter spp., while the organism was not isolated in any member of the subfamily Muscicapinae (i.e., Erithacus and Luscinia). The prevalence of Campylobacter infection in wild birds thus seems to be linked to various ecological and phylogenetic factors, with great variations in carriership between different taxa and guilds.

Watanabe, K., Y. Kodama, et al. (2002). "Diversity, Abundance, and Activity of Archaeal Populations in Oil-Contaminated Groundwater Accumulated at the Bottom of an Underground Crude Oil Storage Cavity." Appl. Envir. Microbiol. **68**(8): 3899-3907.

http://aem.asm.org/cgi/content/abstract/68/8/3899

Fluorescence in situ hybridization has shown that cells labeled with an Archaea-specific probe (ARCH915) accounted for approximately 10% of the total cell count in oil-contaminated groundwater accumulated at the bottom of an underground crude oil storage cavity. Although chemical analyses have revealed vigorous consumption of nitrate in cavity groundwater, the present study found that the methane production rate was higher than the nitrate consumption rate. To characterize the likely archaeal populations responsible for methane production in this

system, fragments of 16S ribosomal DNA (rDNA) were amplified by PCR using eight different combinations of universal and Archaea-specific primers. Sequence analysis of 324 clones produced 23 different archaeal sequence types, all of which were affiliated with the kingdom Euryarchaeota. Among them, five sequence types (KuA1, KuA6, KuA12, KuA16, and KuA22) were obtained in abundance. KuA1 and KuA6 were closely related to the known methanogens Methanosaeta concilii (99% identical) and Methanomethylovorans hollandica (98%), respectively. Although no closely related organism was found for KuA12, it could be affiliated with the family Methanomicrobiaceae. KuA16 and KuA22 showed substantial homology only to some environmental clones. Both of these branched deeply in the Euryarchaeota, and may represent novel orders. Quantitative competitive PCR showed that KuA12 was the most abundant, accounting for [~]50% of the total archaeal rDNA copies detected. KuA1 and KuA16 also constituted significant proportions of the total archaeal rDNA copies (7 and 17%, respectively). These results suggest that limited species of novel archaea were enriched in the oil storage cavity. An estimate of specific methane production rates suggests that they were active methanogens.

Wawrik, B., J. H. Paul, et al. (2002). "Real-Time PCR Quantification of rbcL (Ribulose-1,5-Bisphosphate Carboxylase/Oxygenase) mRNA in Diatoms and Pelagophytes." Appl. Envir. Microbiol. **68**(8): 3771-3779.

http://aem.asm.org/cgi/content/abstract/68/8/3771

Transcriptional activity is often used as a surrogate for gene expression in environmental microbial communities. We developed a real-time PCR assay in which the ABI-Prism (PE Applied Biosystems) detection system is used for quantification of large-subunit ribulose-1,5-bisphosphate caboxylase/oxygenase (rbcL) mRNA in diatoms and pelagophytes both in cultures and from natural phytoplankton communities. Plasmid DNA containing rbcL inserts, as well as in vitro transcribed mRNA of the plasmids, was used to generate standard curves with a dynamic range of more than 6 orders of magnitude with high accuracy and precision (R2 = 0.998). Expression levels in a cultured diatom (Phaeodactylum tricornutum) were quantified through one light-dark cycle by using traditional 35S-labeled oligonucleotide hybridization and real-time PCR. The mRNA levels detected by the two techniques were similar and correlated well (R2 = 0.95; slope = 1.2). The quantities obtained by hybridization were slightly, yet significantly, larger (t = 5.29; P = 0.0011) than the quantities obtained by real-time PCR. This was most likely because partially degraded transcripts were not detected by real-time PCR. rbcL mRNA detection by real-time PCR was 3 orders of magnitude more sensitive than rbcL mRNA detection by hybridization. Diatom and pelagophyte rbcL mRNAs were also quantified in a profile from an oligotrophic site in the Gulf of Mexico. We detected the smallest amount of diatom rbcL expression in the surface water and maximum expression at a depth that coincided with the depth of the subsurface chlorophyll maximum. These results indicate that real-time PCR may be utilized for quantification of microbial gene expression in the environment.

Wiens, G. D., R. Pascho, et al. (2002). "A Single Ala139-to-Glu Substitution in the Renibacterium salmoninarum Virulence-Associated Protein p57 Results in Antigenic Variation and Is Associated with Enhanced p57 Binding to Chinook Salmon Leukocytes." Appl. Envir. Microbiol. **68**(8): 3969-3977.

http://aem.asm.org/cgi/content/abstract/68/8/3969

The gram-positive bacterium Renibacterium salmoninarum produces relatively large amounts of a 57-kDa protein (p57) implicated in the pathogenesis of salmonid bacterial kidney disease. Antigenic variation in p57 was identified by using monoclonal antibody 4C11, which exhibited

severely decreased binding to R. salmoninarum strain 684 p57 and bound robustly to the p57 proteins of seven other R. salmoninarum strains. This difference in binding was not due to alterations in p57 synthesis, secretion, or bacterial cell association. The molecular basis of the 4C11 epitope loss was determined by amplifying and sequencing the two identical genes encoding p57, msa1 and msa2. The 5' and coding sequences of the 684 msa1 and msa2 genes were identical to those of the ATCC 33209 msa1 and msa2 genes except for a single C-to-A nucleotide mutation. This mutation was identified in both the msa1 and msa2 genes of strain 684 and resulted in an Ala139-to-Glu substitution in the amino-terminal region of p57. We examined whether this mutation in p57 altered salmonid leukocyte and rabbit erythrocyte binding activities. R. salmoninarum strain 684 extracellular protein exhibited a twofold increase in agglutinating activity for chinook salmon leukocytes and rabbit erythrocytes compared to the activity of the ATCC 33209 extracellular protein. A specific and quantitative p57 binding assay confirmed the increased binding activity of 684 p57. Monoclonal antibody 4C11 blocked the agglutinating activity of the ATCC 33209 extracellular protein but not the agglutinating activity of the 684 extracellular protein. These results indicate that the Ala139-to-Glu substitution altered immune recognition and was associated with enhanced biological activity of R. salmoninarum 684 p57.

Yang, T. H., J. G. Pan, et al. (2004). "Use of Pseudomonas putida EstA as an Anchoring Motif for Display of a Periplasmic Enzyme on the Surface of Escherichia coli." Appl. Envir. Microbiol. **70**(12): 6968-6976.

http://aem.asm.org/cgi/content/abstract/70/12/6968

The functional expression of proteins on the surface of bacteria has proven important for numerous biotechnological applications. In this report, we investigated the N-terminal fusion display of the periplasmic enzyme {beta}-lactamase (Bla) on the surface of Escherichia coli by using the translocator domain of the Pseudomonas putida outer membrane esterase (EstA), which is a member of the lipolytic autotransporter enzymes. To find out the transport function of a C-terminal domain of EstA, we generated a set of Bla-EstA fusion proteins containing N-terminally truncated derivatives of the EstA C-terminal domain. The surface exposure of the Bla moiety was verified by whole-cell immunoblots, protease accessibility, and fluorescence-activated cell sorting. The investigation of growth kinetics and host cell viability showed that the presence of the EstA translocator domain in the outer membrane neither inhibits cell growth nor affects cell viability. Furthermore, the surface-exposed Bla moiety was shown to be enzymatically active. These results demonstrate for the first time that the translocator domain of a lipolytic autotransporter enzyme is an effective anchoring motif for the functional display of heterologous passenger protein on the surface of E. coli. This investigation also provides a possible topological model of the EstA translocator domain, which might serve as a basis for the construction of fusion proteins containing heterologous passenger domains.

Yeager, C. M., J. L. Kornosky, et al. (2004). "Diazotrophic Community Structure and Function in Two Successional Stages of Biological Soil Crusts from the Colorado Plateau and Chihuahuan Desert." Appl. Envir. Microbiol. **70**(2): 973-983.

http://aem.asm.org/cgi/content/abstract/70/2/973

The objective of this study was to characterize the community structure and activity of N2-fixing microorganisms in mature and poorly developed biological soil crusts from both the Colorado Plateau and Chihuahuan Desert. Nitrogenase activity was approximately 10 and 2.5 times higher in mature crusts than in poorly developed crusts at the Colorado Plateau site and Chihuahuan Desert site, respectively. Analysis of nifH sequences by clone sequencing and the terminal restriction fragment length polymorphism technique indicated that the crust diazotrophic

community was 80 to 90% heterocystous cyanobacteria most closely related to Nostoc spp. and that the composition of N2-fixing species did not vary significantly between the poorly developed and mature crusts at either site. In contrast, the abundance of nifH sequences was approximately 7.5 times greater (per microgram of total DNA) in mature crusts than in poorly developed crusts at a given site as measured by quantitative PCR. 16S rRNA gene clone sequencing and microscopic analysis of the cyanobacterial community within both crust types demonstrated a transition from a Microcoleus vaginatus-dominated, poorly developed crust to mature crusts harboring a greater percentage of Nostoc and Scytonema spp. We hypothesize that ecological factors, such as soil instability and water stress, may constrain the growth of N2-fixing microorganisms at our study sites and that the transition to a mature, nitrogen-producing crust initially requires bioengineering of the surface microenvironment by Microcoleus vaginatus.

Yeager, C. M., D. E. Northup, et al. (2005). "Changes in Nitrogen-Fixing and Ammonia-Oxidizing Bacterial Communities in Soil of a Mixed Conifer Forest after Wildfire." Appl. Envir. Microbiol. **71**(5): 2713-2722.

http://aem.asm.org/cgi/content/abstract/71/5/2713

This study was undertaken to examine the effects of forest fire on two important groups of N-cycling bacteria in soil, the nitrogen-fixing and ammonia-oxidizing bacteria. Sequence and terminal restriction fragment length polymorphism (T-RFLP) analysis of nifH and amoA PCR amplicons was performed on DNA samples from unburned, moderately burned, and severely burned soils of a mixed conifer forest. PCR results indicated that the soil biomass and proportion of nitrogen-fixing and ammonia-oxidizing species was less in soil from the fire-impacted sites than from the unburned sites. The number of dominant nifH sequence types was greater in fire-impacted soils, and nifH sequences that were most closely related to those from the spore-forming taxa Clostridium and Paenibacillus were more abundant in the burned soils. In T-RFLP patterns of the ammonia-oxidizing community, terminal restriction fragments (TRFs) representing amoA cluster 1, 2, or 4 Nitrosospira spp. were dominant (80 to 90%) in unburned soils, while TRFs representing amoA cluster 3A Nitrosospira spp. dominated (65 to 95%) in fire-impacted soils. The dominance of amoA cluster 3A Nitrosospira spp. sequence types was positively correlated with soil pH (5.6 to 7.5) and NH3-N levels (0.002 to 0.976 ppm), both of which were higher in burned soils. The decreased microbial biomass and shift in nitrogen-fixing and ammonia-oxidizing communities were still evident in fire-impacted soils collected 14 months after the fire.

Zuniga, M., M. d. C. Miralles, et al. (2002). "The Product of arcR, the Sixth Gene of the arc Operon of Lactobacillus sakei, Is Essential for Expression of the Arginine Deiminase Pathway." Appl. Envir. Microbiol. **68**(12): 6051-6058.

http://aem.asm.org/cgi/content/abstract/68/12/6051

Lactobacillus sakei is a lactic acid bacterium commonly used as a starter culture for dry sausage production and can utilize arginine via the arginine deiminase pathway. The arcABCTD cluster of L. sakei has been characterized, and transcriptional studies have shown that its expression is subject to carbon catabolite repression and induction by arginine. Downstream of arcD an additional gene has been found; this gene, arcR, codes for a putative regulatory protein of the Crp/Fnr family. Transcriptional studies have shown that arcR is coordinately transcribed with the remaining arc genes, and therefore, these genes constitute the arcABCTDR operon. Northern analysis also showed a complex pattern of transcripts, suggesting that processing and partial termination may play a role in regulation of the expression of individual genes of the operon. Inactivation of arcR led to arrest of transcription of the operon, indicating that the ArcR protein is

essential for expression of the arc genes. The availability of this mutant made it possible to study whether the ability to utilize arginine affects the growth of L. sakei in meat fermentations. Under our experimental conditions, expression of arginine deiminase does not confer an obvious advantage to L. sakei, since the wild type and an arcR mutant strain displayed similar dynamics of growth.

Applied and Environmental Microbiology (1)

Qiu, X., L. Wu, et al. (2001). "Evaluation of PCR-Generated Chimeras, Mutations, and Heteroduplexes with 16S rRNA Gene-Based Cloning." Applied and Environmental Microbiology **67**(2): 880.

http://aem.asm.org/cgi/content/abstract/67/2/880

To evaluate PCR-generated artifacts (i.e., chimeras, mutations, and heteroduplexes) with the 16S ribosomal DNA (rDNA)-based cloning approach, a model community of four species was constructed from alpha, beta, and gamma subdivisions of the division Proteobacteria as well as gram-positive bacterium, all of which could be distinguished by HhaI restriction digestion patterns. The overall PCR artifacts were significantly different among the three Taq DNA polymerases examined: 20% for Z-Taq, with the highest processitivity; 15% for LA-Taq, with the highest fidelity and intermediate processitivity; and 7% for the conventionally used DNA polymerase, AmpliTaq. In contrast to the theoretical prediction, the frequency of chimeras for both Z-Taq (8.7%) and LA-Taq (6.2%) was higher than that for AmpliTaq (2.5%). The frequencies of chimeras and of heteroduplexes for Z-Taq were almost three times higher than those of AmpliTaq. The total PCR artifacts increased as PCR cycles and template concentrations increased and decreased as elongation time increased. Generally the frequency of chimeras was lower than that of mutations but higher than that of heteroduplexes. The total PCR artifacts as well as the frequency of heteroduplexes increased as the species diversity increased. PCR artifacts were significantly reduced by using AmpliTaq and fewer PCR cycles (fewer than 20 cycles), and the heteroduplexes could be effectively removed from PCR products prior to cloning by polyacrylamide gel purification or T7 endonuclease I digestion. Based upon these results, an optimal approach is proposed to minimize PCR artifacts in 16S rDNA-based microbial community studies.

Applied Soil Ecology (2)

Kozdroj, J. and J. D. van Elsas (2001). "Structural diversity of microbial communities in arable soils of a heavily industrialised area determined by PCR-DGGE fingerprinting and FAME profiling." Applied Soil Ecology **17**(1): 31.

http://www.sciencedirect.com/science/article/B6T4B-42M1F7S-3/2/26b0f0ef82018b25d662cb771b5386f1

Microbial community structure in soil sampled from sites contaminated with different levels of

heavy metals was assessed by PCR-DGGE analysis of 16S rDNA fragments and MIDI-FAME profiling of total cell fatty acids. Total community DNA was extracted from these soils by three methods to compare their usefulness for generation of representative pools of bacterial community 16S rRNA genes. Crude DNA extracts were purified and then amplified using eubacterial primers. PCR products were analysed by DGGE to obtain bacterial community patterns. Culturable fractions of fast growing bacteria separated from soil colloids by blending and differential centrifugation were also analysed by profiling of cellular fatty acids. PCR-DGGE analysis showed significant differences in microbial community structure between the soils studied, which were related to the contamination levels. Polluted soils could be characterised by a community differing in 'richness' and structure of dominating bacterial populations from those of a pristine soil. The differences in the bacterial community structure were still visible after 10-fold dilution of the target DNA, indicating that even less dominant populations were affected by heavy metals. However, organic matter content, soil type and crop cultivation could also affect the bacterial populations that established in these soils. The direct methods for DNA extraction from soil generated information about the microbial community composition different from that of the indirect method. The latter method was less efficient than both direct methods with respect to the generation of representative pools of bacterial community 16S rRNA genes. The structure of the culturable bacterial community was not dependent on the concentrations of heavy metals in soil, as determined by MIDI-FAME profiling. It is possible that this fraction of soil bacteria was less diverse (dominated by gram-positive bacteria) than the total community analysed at the DNA level without prior cultivation.

Tamimi, S. M. and J. P. W. Young (2004). "Rhizobium etli is the dominant common bean nodulating rhizobia in cultivated soils from different locations in Jordan." Applied Soil Ecology **26**(3): 193.

http://www.sciencedirect.com/science/article/B6T4B-4BWYHR4-2/2/bdf9c63a3996256803e3e9d6d99b7329

Thirty rhizobial isolates that nodulate common bean (Phaseolus vulgaris L.) plants were obtained from a range of cultivated soils covering 16 geographical sites in Jordan. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis for nodA, nifH and glnII genes and sequencing methods showed that the rhizobial isolates formed two main groups. The first, which makes up 80% of isolates, were identified as Rhizobium etli while the second group comprising the remaining 20% were related to Rhizobium tropici. These findings indicate that R. etli is the predominant rhizobium that nodulates common bean in Jordan.

Aquaculture (28)

Apines-Amar, M. J. S., S. Satoh, et al. (2004). "Amino acid-chelate: a better source of Zn, Mn and Cu for rainbow trout, Oncorhynchus mykiss." Aquaculture **240**(1-4): 345.

http://www.sciencedirect.com/science/article/B6T4D-4C04W1B-1/2/f66be13807fc527854f32f33f134e472

This study aimed to evaluate the amino acid-chelated trace elements as dietary supplement to rainbow trout. Three diets were formulated containing trace elements either from the inorganic salt (SF) or amino acid-chelate (AM). Diets 1 (SF) and 2 (AM) contained the same amount of

trace elements from inorganic and amino acid-chelates, respectively. Diet 3 (AM-Hf) was added with trace elements from amino acid-chelatex at one-half of their levels in Diets 1 and 2. Each diet was fed for 15 weeks to three groups of 30 fish each, with an average weight of 1.52+/-0.21 g. Growth of fish was not affected by the treatment (P>0.05). However, bone (PPPPPP<0.05) than the inorganic salt. Half supplementation of those fed the elements from AM was at par with the full provision from the inorganic source tested.

Bum Jeong, J., K. Hyun Park, et al. (2004). "Multiplex PCR for the diagnosis of red sea bream iridoviruses isolated in Korea." Aquaculture **235**(1-4): 139.

http://www.sciencedirect.com/science/article/B6T4D-4C5PVWC-2/2/399589e1075fa6e7ef0455d59ec2783a

Amplification by the polymerase chain reaction (PCR) was done to determine the presence of red sea bream iridovirus (RSIV) in sea perch (Lateolabrax sp.) imported from China, targeting four genomic regions, the ribonucleotide reductase small subunit (RNRS) gene, the adenosine triphosphatase (ATPase) gene, the DNA polymerase (DPOL) gene, and the Pst I restriction fragment, which have been considered to be potential target regions for the diagnosis of RSIV infection. In contrast to two other RSIVs, RSIV Sachun and RSIV Namhae, which were isolated in Korea, the newly isolated RSIV CH-1 was not detected by PCR with one reported primer set specific for the Pst I restriction fragment. We cloned full-length Pst I restriction fragments from the genomic DNA of three different RSIVs after PCR with primers derived from regions just outside the Pst I restriction fragment using previously reported sequences (4436 bp long and designated as the K1 region), and sequenced the resulting cloned DNA. Two locations of sequence variation, around positions 24-41 and 425-446 in the Pst I restriction fragment, were found in closely related viruses. Nucleotide differences at the first position in RSIV CH-1 prevented the binding of the sense primer derived from the sequence of the reference strain (RSIV Ehime-1) and appeared to cause a negative result in PCR amplification of the targeted Pst I restriction fragment. For differentiation of these three different RSIVs, two primers, NF and CR, specific to RSIV Namhae and CH-1, respectively, were strategically designed by taking advantage of the nucleotide substitutions and a deletion of three successive nucleotides in the two variable positions of the Pst I restriction fragment. The specificity of these primers in general PCR was confirmed by using viral genomic DNAs and plasmids containing target genes of the different RSIVs as templates. In multiplex PCR with all four primers derived from two variable and two conserved positions in the Pst I restriction fragment of RSIV, it was possible to distinguish the three different RSIVs, RSIV Namhae, Sachun, and CH-1, from one another depending upon the different sizes of the PCR amplicons. Thus, the multiplex PCR developed in this study using a minimum number of strategically designed primers provides a basis for rapid and simple differentiation of RSIVs from different hosts or countries merely by the observation of the predicted amplicons and without the necessity of making nucleotide sequence comparisons.

Douglas, S. E., S. Mandla, et al. (2000). "Molecular analysis of the amylase gene and its expression during development in the winter flounder, Pleuronectes americanus." Aquaculture **190**(3-4): 247.

http://www.sciencedirect.com/science/article/B6T4D-40YYFJB-N/2/28830942d86b900823b943b4eede8c96

Determination of the onset of amylase production in marine fish larvae is difficult due to their small size and the possible presence of exogenous amylases from prey organisms in the diet or from the gut flora. In order to develop a sensitive PCR-based assay for the detection of fish-specific amylase in larvae, a complete cDNA and partial genomic sequence, the first reported from a teleost fish, were determined from winter flounder.The complete cDNA for alpha amylase

is 1539 bp and the deduced polypeptide sequence is 512 amino acids, including a putative 15 amino acid signal peptide. The molecular weight of the mature protein is 55,769 Da and the predicted isoelectric point is 6.76. Southern hybridisation analysis showed that the winter flounder amylase cDNA could be used to detect homologs in other species, particularly flatfish, and that there are likely two copies of the gene in the winter flounder genome. The winter flounder genomic sequence corresponding to amino acids 194-404 (including three introns) was amplified by the polymerase chain reaction (PCR) and the sequence used to design primers for PCR-based assays for amylase gene expression in larval and adult fish.The levels of expression of the amylase gene from larvae sampled at 5, 13, 20, 27 and 41 days post-hatch (dph) were determined using the housekeeping gene, actin, as a control. Amylase transcripts were first detected at 5 dph, peaked at 20 dph and then decreased during metamorphosis. The amylase gene is highly expressed in adult winter flounder. This sensitive assay will be useful for investigating amylase gene expression under different feeding conditions and help in the development of optimal diets.

Durand, S. V., R. M. Redman, et al. (2003). "Qualitative and quantitative studies on the relative virus load of tails and heads of shrimp acutely infected with WSSV." Aquaculture **216**(1-4): 9.

http://www.sciencedirect.com/science/article/B6T4D-478GXNS-2/2/ff2ef4875cbbaf249dac51c49fa3c834

There is currently concern and controversy in the shrimp industries of the Americas about the risk posed by the importation and reprocessing of shrimp infected with white spot syndrome virus (WSSV) and yellow head virus (YHV). To further understand the risk, more knowledge concerning the quantitative virus load of infected shrimp is needed. The present study was carried out to better define, using qualitative and quantitative methods, the relative virus load of shrimp heads and tails. For these studies, specific pathogen-free (SPF) Penaeus vannamei were infected with WSSV. Emergency harvest of these shrimp was simulated by collecting the infected shrimp at the onset of postinfection mortalities and determining the relative virus loads of the head and tails by quantitative real-time PCR and histology methods. Routine histology and in situ hybridization assay with a WSSV-specific DNA probe demonstrated qualitatively similar levels of WSSV infection in the heads and tails of experimental infected shrimp. The novel real-time PCR method demonstrated quantitatively that the head had a slightly higher WSSV load than did the tail. However, since the tail represents 58% of the total body weight, the total virus load on a per weight basis turns out to be similar in the head (49%) and tail (51%) of the same shrimp with acute phase WSSV infections. In proportion to the total tail weight, the virus load of the peeled shell represents 55% of the total viral load in tail.

Ford, S. E., Z. Xu, et al. (2001). "Use of particle filtration and UV irradiation to prevent infection by Haplosporidium nelsoni (MSX) and Perkinsus marinus (Dermo) in hatchery-reared larval and juvenile oysters." Aquaculture **194**(1-2): 37.

http://www.sciencedirect.com/science/article/B6T4D-420SK5S-4/2/fd37b30f4678fbed9c746d2961447f25

Means to control infection by pathogenic organisms are needed to help ensure that aquaculture is not a source for the spread of infectious diseases in wild and cultured stocks. Questions often arise as to whether larval or juvenile stages become infected in the hatchery or nursery phase of production, and if so, how they might be protected. To help answer these questions, we utilized both traditional and molecular diagnostic methods to detect two eastern oyster, Crassostrea virginica, pathogens (Haplosporidium nelsoni, cause of MSX disease and Perkinsus marinus, cause of Dermo disease) in larval and juvenile oysters reared at a hatchery/on-shore nursery

receiving water in which both parasites are enzootic. Our study indicated that filtration of water to 1 [mu]m using a cartridge filter followed by exposure to 30,000 [mu]W s-1 cm-2 ultraviolet (UV) irradiation was an effective means of preventing infections of the larvae and post-set. Once the juveniles were moved from the highly treated hatchery water to an upweller nursery receiving only roughly (150 [mu]m) bag-filtered water, however, they became infected by both parasites.

Gomez-Requeni, P., M. Mingarro, et al. (2004). "Protein growth performance, amino acid utilisation and somatotropic axis responsiveness to fish meal replacement by plant protein sources in gilthead sea bream (Sparus aurata)." Aquaculture **232**(1-4): 493.

http://www.sciencedirect.com/science/article/B6T4D-49M6RDP-5/2/07d168dcd17f689b33c3ee9b5b5230a2

Partial or total replacement of fish meal by a mixture of plant protein sources (corn gluten meal, wheat gluten, extruded peas, rapeseed meal) balanced with indispensable amino acids (IAA) was examined in juvenile gilthead sea bream (Sparus aurata) over the course of a 12-week growth trial. A diet with fish meal (FM) as the sole protein source was compared to diets with 50%, 75% and 100% of replacement (PP50, PP75, PP100). Protein retention was improved with more plant protein supply, and just a slight decrease in the final weight gain was found in fish fed PP50 and PP75 diets. However, in the PP100 group, weight gain was depressed up to 30% mainly as the result of a marked reduction of feed intake. These fish also showed a lower fat gain along with a marked hypocholesterolemic effect. Dietary treatment did not alter the hepatic activity of amino acid catabolising enzymes (alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), glutamate dehydrogenase (GDH)), although the size of the total muscle free amino acid (FAA) pool was increased by more plant protein supply. The activity of the somatotropic axis also varied among experimental groups, and the up-regulation of circulating growth hormone (GH) levels with a high plant protein supply followed the decrease in growth rates, plasma levels of insulin-like growth factor-I (IGF-I), and liver mRNA transcripts of IGF-I and GH receptors. This catabolic feature evidenced a liver desensitisation to the anabolic action of GH in the PP100 group, and to a lesser extent in the PP75 group. Taken together all these findings, up to 50-75% of fish meal replacement seems to be feasible with IAA supplementation, but further research is needed to fully identify the responsible factors for the depressed feed intake in order to achieve a full replacement in a fish species having high dietary protein requirements.

Hara, M. and M. Sekino (2003). "Efficient detection of parentage in a cultured Japanese flounder Paralichthys olivaceus using microsatellite DNA marker." Aquaculture **217**(1-4): 107.

http://www.sciencedirect.com/science/article/B6T4D-45FGXDX-4/2/19a15d0be283aa57d52826f57194914d

Parentage of offspring in a stock of a cultured Japanese flounder Paralichthys olivaceus was determined using four hyper-variable microsatellite DNA loci with many unique alleles. It was found that only 57% of the 14 broodstock parents actually contributed to the production of offspring in this experiment. The number of alleles per locus in the offspring was reduced 29% compared to the broodstock. The average heterozygosity of offspring (He=0.883) was significantly lower ($P<0.01$) than that of their parents (He=0.943). In this study, use of hyper-variable microsatellite markers with many rare alleles was effective for unambiguous parentage determination and estimation of genetic diversity in hatchery and natural populations of Japanese flounder. And it was shown using parentage determination that the difference between the number of parents contributing to reproduction and the number of fish stocked caused loss of genetic variability.

Huang, T.-s., L. Cerenius, et al. (1994). "Analysis of genetic diversity in the crayfish plague fungus, Aphanomyces astaci, by random amplification of polymorphic DNA." Aquaculture **126**(1-2): 1.

http://www.sciencedirect.com/science/article/B6T4D-49NXBDR-167/2/8c25d3d4e7c0e0d2c4f6baf8351a158a

Arbitrary primers and the DNA polymerase chain reaction (PCR) technique were applied to study genetic variation between different strains of the crayfish plague fungus, Aphanomyces astaci. Eight different primers among 15 tested were chosen for a comparative analysis of the different A. astaci strains. On average, each primer gave rise to 5-8 bands and a majority of the bands was polymorphic for at least some strains. Two main groups among the different Swedish isolates were clearly discernible. One group included isolates from the indigenous crayfish species Astacus astacus and one isolate from As. leptodactylus originating from Turkey. The other main group included fungal isolates from both As. astacus and the introduced North American crayfish Pacifastacus leniusculus. In this latter case it seems likely that the introduced American signal crayfish has served as a vector and transmitted the pathogen to the indigenous crayfish.

Jeong, J. B., L. J. Jun, et al. (2003). "Characterization of the DNA nucleotide sequences in the genome of red sea bream iridoviruses isolated in Korea." Aquaculture **220**(1-4): 119.

http://www.sciencedirect.com/science/article/B6T4D-47BX9GB-N/2/30b1ea4e78fef37159a67f57add37072

The nucleotide sequences of DNA fragments amplified by polymerase chain reaction (PCR) from four different genomic regions of nine red sea bream iridoviruses (RSIVs) isolated from different species of fish, different areas and in different years in Korea were compared with the reported reference sequences. One isolate, RSIV Namhae, showed 100% homology to the reference sequences, while the other eight isolates, which appeared to contain identical nucleotide sequences, showed 96.6-98.9% homology with reference sequences depending upon the target regions of PCR gene amplification. However, differences in nucleotide sequences were not apparent between the RSIVs isolated in different locations, in different years or in different host species. We also cloned and sequenced the 3' end flanking region (K1) of the DNA polymerase (DPOL) gene using the cassette ligation-mediated PCR method. This sequence was 4436-bp long and possessed two open reading frames (ORF-1 and ORF-2) oriented in opposite directions. The putative proteins encoded by these two ORFs could not be characterized by comparison with the proteins of other species in the data banks. The presence of the ribonucleotide reductase small subunit (RNRS) gene at the 3' end of the K1 region allowed us to determine that these two genes, RNRS and DPOL, are separated 5508 bp and oriented in the same direction in the genome of RSIV. Moreover, it is of interest that a PstI-restriction fragment, of which the sequence but not the location within the RSIV genome had previously been reported, is located at nucleotide positions from 1096 to 2054, extending from within the ORF-1 region, spanning the intervening sequence between ORF-1 and ORF-2, and extending into the ORF-2 region. Various repeating sequences up to 86 bp were present at the 3' ends of ORFs, especially within the nucleotide sequences at the 3' terminus of ORF-2. No similarities were detected when the DNA sequences of the K1 region were compared to the DNA sequences of a repetitive element in the genome of other iridoviruses.

Jin Jun, L., J. Bum Jeong, et al. (2004). "Detection of tetracycline-resistance determinants by multiplex polymerase chain reaction in Edwardsiella tarda isolated from fish farms in Korea." Aquaculture

240(1-4): 89.

http://www.sciencedirect.com/science/article/B6T4D-4D98XYS-4/2/a2d78e0f24a06d3a4597fb0e7c7b6d13

To determine and discriminate the types of tetracycline (Tc)-resistance determinants (tet), we developed a multiplex polymerase chain reaction (PCR). With minimized numbers of primers derived from the variable and conserved regions of six different types of tet genes, tet(A)-(E) and (G), the multiplex PCR was sensitive and specific enough to discriminate the various types of tet genes, even multiple tet genes in an individual resistant isolate, by the different sizes of the resulting PCR products. Each of 20 Tc-resistant Edwardsiella tarda (Ed. tarda) isolates from diseased fish from aquatic farms in Korea carrying either one or two tet genes of types (A), (D), (B), or (G) gave PCR products of the appropriate lengths. Among the four types of tet genes found in Ed. tarda, two types, tet(A) and (D), were always present on mobile plasmids, and the other two types, tet(B) and (G), were located on the nonmobile nucleic acids. This is the first time that either of these two genes, type (B) or (G), have been found in Ed. tarda isolates. The two most common types of Tc-resistance determinant were, tet(A) and (D), occurring in 55% and 45% of total Tc-resistant Ed. tarda isolates, respectively.

Kim, M. S. and H. D. Jeong (2001). "Development of 16S rRNA targeted PCR methods for the detection and differentiation of Vibrio vulnificus in marine environments." Aquaculture **193**(3-4): 199.

http://www.sciencedirect.com/science/article/B6T4D-41XV7CM-1/2/58cd3871252c03f0384fc2bd5ad4ddc9

A PCR method for the detection and differentiation of Vibrio vulnificus strains was developed as an alternative to culture methods by using combined primers directed against the variable regions of 16S rRNA. Primers designed from two variable regions of the Vibrionaceae 16S rRNA (corresponding to nucleotide numbers 1006 to 1023 and 1278 to 1258 in Escherichia coli 16S rRNA) was found to be species-specific for V. vulnificus by PCR. Additionally, tri-primer PCR of 16S rRNA was evaluated for the differentiation of V. vulnificus strains. Although the third primer, which was derived from the variable region, positions 454 to 473, cannot discriminate V. vulnificus from other bacteria, it was used to avoid the detection of type B 16S rRNA of this organism in PCR. The resulting 825 bp fragment in the presence of the 273 bp fragment, which is specific to V. vulnificus, in tri-primer PCR clearly differentiated type A 16S rRNA strains from type B. Enumeration of V. vulnificus in the samples of oyster and environmental samples was done by most probable numbers' (MPN) method of five preenrichment tubes of alkaline peptone water supplemented with polymyxin B following up the confirmation of positive tubes by streaking the samples onto mCPC agar or by 16S rRNA gene amplification. Higher numbers of presumptive V. vulnificus confirmed by selective media compared with those confirmed by PCR method in MPN method suggested that there would be some bacteria that cannot be discriminated from V. vulnificus on mCPC agar in environmental samples. In the biotyping of the V. vulnificus isolates in oyster samples, the majority of the strains (92.5%) belonged to biotype 1, and 7.5% of the strains belonged to biotype 2. However, strains of 16S rRNA of V. vulnificus isolates in the marine environment determined by tri-primer PCR appeared to be 35% type A and 65% type B. These results implied that the marine environment can serve as reservoir of both V. vulnificus biotypes 1 and 2, and strains of 16S rRNA type B were more frequent than strains of type A in that environment.

Li, Q., C. Park, et al. (2004). "Loss of genetic variation at microsatellite loci in hatchery strains of the Pacific abalone (Haliotis discus hannai)." Aquaculture **235**(1-4): 207.

http://www.sciencedirect.com/science/article/B6T4D-4BMTJ11-6/2/0c4c007f65527bd41383b2ec02890651

In order to assess the utility of microsatellite DNA markers for detecting changes of genetic diversity in hatchery strains and for estimating their genetic relationships, we used six microsatellite markers to estimate the level of genetic diversity within three hatchery strains and two wild populations of Pacific abalone, and compared the degree of genetic differentiation between them. High polymorphism at the microsatellite loci was found within both hatchery and wild abalone populations. Compared to wild populations, all the hatchery strains showed less genetic variation as revealed in lower number of alleles and lower expected heterozygosity, indicating that bottleneck effects occurred when each strain was founded. Significant differentiation was found between the hatchery strains, and between the hatchery strains and wild populations (Fst range: 0.059-0.427; Rst range: 0.056-0.351), and no obvious difference was detected between the wild populations (Fst=0.004; Rst=0.007). According to the neighbor-joining tree topology constructed on the basis of genetic distances among individuals, almost all individuals from each hatchery strain were closely clustered, demonstrating the feasibility of microsatellite analysis for discrimination between hatchery strains. The results obtained in this study indicate that it is necessary to genetically characterize the abalone strains that are being released every year in order to monitor the effect on the genetic diversity of wild populations.

Lilley, J. H., L. Cerenius, et al. (1997). "RAPD evidence for the origin of crayfish plague outbreaks in Britain." Aquaculture **157**(3-4): 181.

http://www.sciencedirect.com/science/article/B6T4D-3S12526-J/2/b71ad64c7f20b0ace61e575654c64fce

Two isolates of Aphanomyces astaci obtained from diseased white-clawed crayfish (Austropotamobius pallipes) in Herefordshire, England were compared against representative isolates of three groups of the fungus found in Sweden and one from Spain by means of random amplification of polymorphic DNA (RAPD). The English isolates proved to be very similar to a Swedish strain which is considered to have been introduced from North America with shipments of the signal crayfish (Pacifastacus leniusculus) from 1970 onwards, and has since spread to indigenous populations of noble crayfish (Astacus astacus). This strain has not been found to be involved in recent incidences of crayfish plague in Turkey and Spain. It is therefore most likely that at least some of the outbreaks of crayfish plague in England resulted from imports of P. leniusculus from northern Europe after 1970, or directly from North America.

Nunan, L. M., B. T. Poulos, et al. (1998). "The detection of White Spot Syndrome Virus (WSSV) and Yellow Head Virus (YHV) in imported commodity shrimp." Aquaculture **160**(1-2): 19.

http://www.sciencedirect.com/science/article/B6T4D-3TCVY0T-2/2/e4d6c9be52c8b252e6d7d2220b4124d4

Transmission of exotic pathogens occurs through a variety of means, including migration with humans and animals, rapid transit by land, sea or air or through the shipment of infected frozen food products. White Spot Syndrome Virus (WSSV) and Yellow Head Virus (YHV) have caused mass mortalities of cultured shrimp in Asia beginning in 1992. In 1995, these viruses appeared for the first time in the Western Hemisphere causing high mortalities in farm reared shrimp in Texas, USA. The purpose of this study was to determine if WSSV and YHV are present in frozen shrimp products imported into the United States from Asia. Infectivity assays, transmission electron microscopy (TEM), and polymerase chain reaction (PCR) showed these viruses were detectable

and infectious in frozen shrimp imports. Frozen shrimp were used to infect indicator shrimp (Penaeus stylirostris) which resulted in mortalities. The cause of these mortalities was determined by histology and TEM to be by YHV. PCR confirmed the presence of WSSV in the frozen, purchased products. The results from this study indicate that exotic shrimp pathogens can be transmitted via imported frozen products.

Nunan, L. M., K. Tang-Nelson, et al. (2004). "Real-time RT-PCR determination of viral copy number in Penaeus vannamei experimentally infected with Taura syndrome virus." Aquaculture **229**(1-4): 1.

http://www.sciencedirect.com/science/article/B6T4D-48S4NGB-4/2/d60e91fd8dd071d5c5df19ba18ac670f

This study examined the viral copy number as determined by real-time RT-PCR, in different tissue samples from Penaeus vannamei exposed to Taura syndrome virus (TSV). Two routes of exposure, injection and per os, were investigated. Six different body parts from each shrimp were assessed for viral copy numbers. Eight shrimp were analyzed per treatment. In addition, eight specific pathogen free (SPF) P. vannamei were analyzed and served as a negative control. The tissue samples examined included: whole tail muscle, tail muscle (shell removed), gills, pleopods, head (cephalothorax with the hepatopancreas included) and tail fan. The results from these experiments showed a significant level of difference between the SPF and the injection treatments. As was expected, there was also a significant difference between the negative control and the per os treatment groups. There was no significant difference between the viral copy number contained in different body parts from the injection experiment. These results contrasted to the per os results, in which there was a statistically significant difference between tail/gills, tail/head, tail/tail fan, whole tail/tail fan and pleopods/tail fan. The tail samples had the lower viral copy numbers, as did the whole tail and pleopods when compared to the tail fan. The mean viral copy number per nanogram of total RNA (x cn/ng tRNA) extracted in the injection study ranged from 1.4 x 105 in the gills to 2.3 x 105 in the whole tail. In the per os experiment, the x cn/ng of extracted tRNA ranged from 2.5 x 104 in the tail muscle to 4.3 x 105 in the head. When these values are converted to mean viral copy number per gram (x cn/g) of tissue, the values increased in range. In the injection study, the x cn/g of tissue ranged from 1.2 x 109 in the tail fan to 7.4 x 109 in the head. These values contrast to the x cn/g of tissue in the per os study in that the lowest value of 1.7 x 108 was in the tail muscle and the highest x, 1.7 x 1010, was in the head. Overall, all body parts from infected shrimp, regardless of treatment type, quantitatively analyzed by real-time RT-PCR, determined the presence of TSV.

Overturf, K., M. T. Casten, et al. (2003). "Comparison of growth performance, immunological response and genetic diversity of five strains of rainbow trout (Oncorhynchus mykiss)." Aquaculture **217**(1-4): 93.

http://www.sciencedirect.com/science/article/B6T4D-452F9RK-2/2/1877208da0e9562c343f10f643dab869

The domestication of rainbow trout, Oncorhynchus mykiss, has led to the development of distinguishable isolated populations. In this study, five different strains of rainbow trout from the Northwest, USA were examined for variability in growth, immunological response, and genetic diversity. Growth rates for the different strains were monitored and compared for 28 weeks, with the animals fed at a fixed rate or to apparent satiation. Feed conversion ratios (FCR), specific growth rates (SGR), and thermal-unit growth coefficients (TGC) for the entire period were calculated for each of the strains. The different strains were also evaluated immunologically with infectious hematopoietic necrosis virus (IHNV), and their post-immunization antibody neutralization titers were monitored for a period of 12 weeks. Using microsatellites, the genetic

variability between the strains was examined from a representational sample of the population of each strain. Fastest growing strains grew to a set weight of 350 g more rapidly regardless of whether they were fed at a fixed rate or to apparent satiation. These faster growing strains also exhibited a lower FCR and higher SGR and TGC values, and higher percentage protein retention. The IHNV neutralization titers for the strains varied considerably with one high humoral response group and one low humoral response group. The remaining three strains clustered approximately midway between the other two. Genetically, the strains exhibited a pattern of wide divergence, with only 9 common alleles out of a total of 89 different alleles between the five strains. As expected, commercial aquaculture strains reared locally were genetically more similar, and strains that have undergone intense selection tended to have a strong correlation between reduced genetic variability, FCR, and SGR as compared to noncommercial strains.

Palsson, S. and E. Arnason (1994). "Sequence variation for cytochrome b genes of three salmonid species from Iceland." Aquaculture **128**(1-2): 29.

http://www.sciencedirect.com/science/article/B6T4D-49NY4SG-H/2/ceee388be644f98b8ae8206bb19cf7a2

Sequence variation was detected by polymerase chain reaction (PCR) and direct sequencing of a 295-nucleotide region of the mitochondrial cytochrome b gene of Arctic charr (Salvelinus alpinus), Atlantic salmon (Salmo salar) and brown trout (Salmo trutta) sampled around Iceland. Four haplotypes were found within Atlantic salmon, no variation was found within Arctic charr or brown trout. Synonymous substitution rates among the species were according to a molecular clock. There was a significant transition/transversion bias but apparent transitional bias among purines and pyrimidines was not significant, given permissible mutations which would not alter the amino acid sequence. The amino acid sequences were identical among the species where intraspecific variation did not exist.

Parnes, S., E. Mills, et al. (2004). "Reproductive readiness of the shrimp Litopenaeus vannamei grown in a brackish water system." Aquaculture **236**(1-4): 593.

http://www.sciencedirect.com/science/article/B6T4D-4C2NMG3-1/2/50caf9be93d44ed93fb9ed3a381a974d

The reproductive readiness of the marine shrimp Litopenaeus vannamei (formerly Penaeus vannamei) cultured in brackish water was characterized by applying morphological, physiological, and molecular tools. The shrimp were cultured on two commercial shrimp farms in brackish water that was pumped from artesian wells that tap into a geothermal aquifer. The shrimp populations exhibited a bimodal growth curve with the females being significantly bigger than the males at the end of the growout period. Some male shrimp started to develop spermatophores about 6 months after the first post-larval (PL0) stage, and some with developed, normal-looking, white spermatophores were observed 8 months after PL0. The sperm count in these males was 10.1 x 106+/-5.8 x 106 cells per compound spermatophore, and 81.6+/-19.8% of the cells were spiked. Melanization of the males, which eventually affected about a third of the male population, first became evident before the appearance of white spermatophores. Female ovaries were transparent and appeared to be arrested in a previtellogenic stage. However, beyond a weight/age threshold of 20 g/8 months, some of the ovaries had become opaque and the vitellogenin gene was found to be expressed in the ovary--but not in the hepatopancreas--of 7 out of 10 females. A unique case of a 46.8 g female with a fully developed ovary that was found in the brackish-water-held broodstock is reported.

Perez-Casanova, J. C., H. M. Murray, et al. (2004). "Bile salt-activated lipase expression during larval development in the haddock (Melanogrammus aeglefinus)." Aquaculture **235**(1-4): 601.

http://www.sciencedirect.com/science/article/B6T4D-4C52NYK-2/2/f9770452e6f904b344e20605e548b8e7

In the present work, we report the characterization of the partial cDNA sequence of a bile salt-activated lipase (BAL) cDNA from haddock. The predicted polypeptide encoded by the cDNA sequence contains the bile salt-binding site characteristic of all BALs at amino acid positions 36-45, and the lipid-binding site thus far only reported in fish BALs starting at position 345. Other features of BAL are also present including: the active site serine motif at positions 111-117, the catalytic triad formed by the residues S114, D239 and H358, and an N-glycosylation site at position 107. The relative levels of BAL gene expression were determined in haddock larvae during ontogeny by reverse transcription-polymerase chain reaction (RT-PCR) with the earliest detectable transcript levels identified at hatch. Using in situ hybridization, the BAL transcripts were localized consistently in the pancreas of haddock larvae from mouth opening until 401 degree days (DD). Using biochemical techniques, the specific activity of BAL was found to decline significantly over time. Our results also suggest that haddock larvae are capable of digesting lipids at the time of mouth opening.

Qian, P.-Y., C. Y. Wu, et al. (1996). "Integrated cultivation of the red alga Kappaphycus alvarezii and the pearl oyster Pinctada martensi." Aquaculture **147**(1-2): 21.

http://www.sciencedirect.com/science/article/B6T4D-3W2XKYC-G/2/a533a428639946a21f0dea50846ab32a

The effect of simultaneously cultivating the pearl oyster Pinctada martensi and the red alga Kappaphycus alvarezii on growth rates of both species was investigated in laboratory and field studies conducted from December 1993 to June 1995. The two study sites were in subtidal areas 100 km apart off the east coast of Hainan Island, China. Pearl oysters were cultivated in the center of an algal farm and red alga was cultivated in the center of the pearl oyster farm. These field experiments showed higher growth rates of both P. martensi and K. alvarezii in a co-culture system than in a monospecies culture system. Laboratory studies showed that the algae removed nitrogenous wastes released by pearl oysters. Algae treated with pearl oyster wastes grew much faster than those without oyster wastes. Algae treated with the seawater to which NH4Cl, NaNO3 and NaNO2 were added grew at the same rate as those treated with natural seawater containing oyster nitrogenous wastes, suggesting that enhanced growth of algae in the co-culture system was largely due to nitrogenous metabolites of the pearl oysters. In the co-culture, growth of pearl oysters was positively influenced by the presence of rapidly growing algae but when seawater temperature decreased below 20 [deg]C, the algae grew slowly and there was no measurable benefit of mixed culture to either algae or pearl oyster.

Riquelme, C., R. Araya, et al. (2000). "Selective incorporation of bacteria by Argopecten purpuratus larvae: implications for the use of probiotics in culturing systems of the Chilean scallop." Aquaculture **181**(1-2): 25.

http://www.sciencedirect.com/science/article/B6T4D-3Y51T0X-3/2/790aa0c84f5b63a3c6f5478f1f3173c7

Experiments on ingestion rates, colonization and impact of inhibitory producer substances bacteria (IPB) on larvae of Argopecten purpuratus, were carried out to evaluate potential use of bacteria as probiotics in cultures of this Chilean scallop. Three selected strains, named as 11, C33 and 77, obtained from larval cultures of A. purpuratus were tested at different concentrations and incubation times. After 6 h of incubation at a concentration of 106 cells ml-1, A. purpuratus larvae ingested cells of strains 11 and 77, but not those of C33. When comparing bacterial incorporation among these strains, the 77 became the dominant bacteria of the larval microflora, causing no differences in larval survival at different bacterial concentrations. Our results suggest that strain 77 appears as a potential probiotic for scallop larvae and hence, as a promising method to control and prevent infections in hatcheries systems.

Sekino, M., M. Hara, et al. (2002). "Loss of microsatellite and mitochondrial DNA variation in hatchery strains of Japanese flounder Paralichthys olivaceus." Aquaculture **213**(1-4): 101.

http://www.sciencedirect.com/science/article/B6T4D-44TV9D6-1/2/aacd283ed0d7ebc97a37428a81c65f3c

Genetic divergence within and between hatchery strains and wild populations of Japanese flounder Paralichthys olivaceus was assessed by means of microsatellite and mitochondrial DNA (mtDNA) sequencing analysis. All of the 11 microsatellite loci screened in this study showed marked polymorphisms. Sequences of the mtDNA control region of Japanese flounder were also highly variable: of approximately 443 base pairs (bp) sequenced, 132 variable sites comprised of 149 base-substitutions were found among the 490 individuals. Marked reductions of genetic variability in the hatchery strains compared with the wild populations were observed in terms of number of both microsatellite alleles and mtDNA haplotypes, and mtDNA haplotype diversity. Both molecular markers yielded high values of FST ([Phi]ST) between the hatchery strains, and between the hatchery strains and wild populations. We conclude that, based on the reduced genetic variability observed in all the hatchery strains examined, bottleneck effects occurred when each strain was founded.

Sekino, M., K. Saitoh, et al. (2003). "Microsatellite-based pedigree tracing in a Japanese flounder Paralichthys olivaceus hatchery strain: implications for hatchery management related to stock enhancement program." Aquaculture **221**(1-4): 255.

http://www.sciencedirect.com/science/article/B6T4D-483STD5-1/2/6b4750624727fef716adea015456810a

Effective population size in captive populations is affected by several factors such as the number of contributing broodstock to the next generation, sex ratio of parents, and variations in family size, which can be accurately evaluated by examination of the pedigree structure in the populations of interest. Here we present an example of microsatellite-based pedigree tracing in a hatchery strain of Japanese flounder Paralichthys olivaceus to be stocked into natural sea areas. We also detail the potential effect of selective operations (size selection) on the pedigree structure. The hatchery strain we screened was founded by using 18 wild captives (6 males and 12 females) through the use of the mesocosm spawning method, and the pedigree of the offspring including 113 individual larvae collected within 24 h after hatching, 216 individuals of 1 month old, and 407 individuals of 4 month old was unambiguously identified. The contribution of candidate broodstock to the next generation was highly skewed as the contribution to almost all of the offspring was monopolized by a single male, and a half of the females did not produce any offspring. The contribution of one family to the released fish selected for larger size (total length) was significantly high, while those of other two families were low ($P<0.008$).

Spanggaard, B., I. Huber, et al. (2000). "The microflora of rainbow trout intestine: a comparison of traditional and molecular identification." Aquaculture **182**(1-2): 1.

http://www.sciencedirect.com/science/article/B6T4D-3Y9G6WN-1/2/22607991f79785bbd98348f76f87b6fd

The culturability of the intestinal microflora of 48 rainbow trout was detected by comparing direct microscopic counts (4',6-diamidino-2-phenylindole, DAPI) with plate counts (tryptone soya agar, TSA). In general, a high percentage (average 50%) of the microflora could be cultured. The counts of the intestinal microflora varied 3-5 log units between fish within the same sampling point. A total of 504 bacteria were identified by physiologic criteria and 153 strains also by partial sequencing of the 16S rRNA gene. High agreement was found between classical and molecular identification. The dominant intestinal microflora was identified as bacteria belonging to the gamma subclass of Proteobacteria (of the genera Citrobacter, Aeromonas and Pseudomonas), to the Gram-positive bacteria with low G+C-content (of the genus Carnobacterium) and as bacteria belonging to the beta subclass of Proteobacteria. However, the composition of the intestinal microflora showed high variation among three investigated fish farms and also at different time points within one fish farm.

Thongpan, A., M. Mingmuang, et al. (1997). "Genomic identification of catfish species by polymerase chain reaction and restriction enzyme analysis of the gene encoding the immunoglobulin M heavy chain constant region." Aquaculture **156**(1-2): 129.

http://www.sciencedirect.com/science/article/B6T4D-3RXXXTD-C/2/55b187eb8ad1ba8f2683d3a312f0d9dc

Nuclear DNA was isolated from the blood cells of catfish representing three families (clariidae, pangasiidae and ictaluridae) for analysis by polymerase chain reaction (PCR) and restriction enzymes. Primers specific for the CH4 exon of the gene encoding the immunoglobulin M heavy chain of channel catfish (Ictalurus punctatus) were used. Nuclear DNA amplified with these primers yielded a single band of about 300 base pairs (bp) for Clarias macrocephalus, Pangasius gigas, Pangasius hypophthalmus and the hybrid of P. gigas X P. hypophthalmus. However, the same primers yielded two DNA bands of about 300 and 340 bp in Clarias gariepinus and in the hybrid of C. macrocephalus X C. gariepinus. Nucleotide sequences of the amplified DNA were determined for I. punctatus, C. macrocephalus, P. gigas and P. hypophthalmus. Based on the DNA sequence data, the restriction enzyme HpaI was used to further characterize the PCR products of P. gigas, P. hypophthalmus and their hybrid. Digestion with this restriction enzyme yielded one DNA band (300 bp) for P. gigas, two bands (100 and 200 bp) for P. hypophthalmus and three bands (100, 200 and 300 bp) for the hybrid. These findings would aid in identifying genetic contributions in hybrid, androgenetic, gynogenetic and polyploid catfish.

Van Eenennaam, A. L., J. P. Van Eenennaam, et al. (1996). "Rapid verification of meiotic gynogenesis and polyploidy in white sturgeon (Acipenser transmontanus Richardson)." Aquaculture **147**(3-4): 177.

http://www.sciencedirect.com/science/article/B6T4D-3W31BC5-4/2/04cf67b7dba00bc3d921d3c7816f6e38

We describe a novel random amplified polymorphic DNA (RAPD) based technique to rapidly

assess the overall success of treatments designed to induce gynogenesis. To test this technique we produced white sturgeon (Acipenser transmontanus) meiotic gynogens. A total of 108 putative gynogens of known parentage from four different experimental treatments were screened using RAPD primers which were known to generate sire-specific markers. Only two individuals showed amplification of sire-specific markers indicating that they had received some paternal inheritance and were not true gynogens. This simple RAPD-based technique could be generally applicable for the verification of gynogenesis or androgenesis in other species, especially those which lack suitable phenotypic markers to trace the transmission of parental inheritance. We also determined the ploidy of 2469 diploid, triploid, tetraploid and mosaic sturgeon by using a Coulter Counter to analyze erythrocyte nuclei size and verified the results with flow cytometry. The Coulter Counter proved to be a rapid and accurate technique for ploidy analysis in sturgeon.

Woods, L. C., B. Ely, et al. (1995). "Evidence for genetic purity of captive and domestic striped bass broodstocks." Aquaculture **137**(1-4): 41.

http://www.sciencedirect.com/science/article/B6T4D-3Y45FPW-5/2/582f01374640c0a3ebd2ed0e8cd77a3b

The culture of striped bass or its hybrids is currently one of the fastest growing segments of aquaculture in the United States. Although this industry is still in the early stages of development, it is already estimated that cultured striped bass and hybrids exceed that of the wild harvest. One major problem limiting the growth of the industry is the dependency on wild brood stock for seed supply. The Crane Aquaculture Facility (CAF) maintains the largest Chesapeake Bay (Maryland, USA) population of captive (F1) and domestic (F2 or greater) striped bass. These striped bass originated from wild populations of Chesapeake Bay where hybrids of Morone exist sympatrically, and where evidence of introgressive hybridization among Morone has occurred. Given this evidence, we felt it was imperative to screen all of the CAF stock for genetic purity before selective breeding efforts were initiated. We utilized genomic DNA techniques to validate genetic purity because of the ease of sampling and the high level of sensitivity to introgessive hybridization. No white bass alleles were found among the samples tested. Thus, white bass alleles if present at all are extremely rare in the CAF striped bass stocks.

Yoo, M. H., M.-D. Huh, et al. (2003). "Characterization of chloramphenicol acetyltransferase gene by multiplex polymerase chain reaction in multidrug-resistant strains isolated from aquatic environments." Aquaculture **217**(1-4): 11.

http://www.sciencedirect.com/science/article/B6T4D-45TTPPW-5/2/a654e103b28f63f102be3ebd7b50b154

Multidrug-resistant isolates carrying the chloramphenicol resistance gene were obtained from aquatic farms in various locations in Korea and studied to determine the distribution and origin of the cat gene. Out of the 134 isolates examined, 10 showed multidrug resistance to several different antibiotics including chloramphenicol (CP). One of these 10 multidrug-resistant bacteria, Vibrio damsela JE1 (V. damsela JE1) contained a transferable R plasmid encoding CP and tetracycline (TC) resistance genes. The presence of the R plasmid was confirmed by conjugation using the chromocult medium (CC) as a selective and differential medium for transconjugants with identification based on the growth or colors of the colonies. To determine the types of chloramphenicol acetyltransferase genes (cat), polymerase chain reaction (PCR) with primers derived from the variable and conserved regions of different types cat genes, appeared to be a very specific and sensitive method. Additionally, we developed the multiplex PCR that allowed us to determine the types of cat genes by the different sizes of the resulting PCR products in a single reaction tube. With the PCR method, we determined that all multidrug-resistant isolates of Vibrio

from the farms of the South and East Sea that contained the cat gene carried type IV gene in an R plasmid or other nucleic acids, and the remaining isolates including Edwardsiella tarda, Aeromonas hydrophila and Shewanella sp. carried the type II gene. This result suggested that the type of cat gene in the multidrug-resistant bacteria originated from a very limited environmental or biological source and was not dependent on the location of the area of isolation in Korea.

Aquatic Botany (2)

Jewett-Smith, J., C. McMillan, et al. (1997). "Flowering and genetic banding patterns of Halophila johnsonii and conspecifics." Aquatic Botany **59**(3-4): 323.

http://www.sciencedirect.com/science/article/B6T4F-3S5BM03-B/2/1d808499cc943c3498d505872f18165e

Halophila johnsonii Eiseman is a seagrass that is restricted in distribution to the Indian River of Florida from Sebastian Inlet on the north to Biscayne Bay on the south, and currently is being considered for listing as a rare/endangered species by the National Marine Fisheries Service (NMFS). At the time it was described as a new species in 1980, no staminate flowers had been reported. After numerous searches in the Indian River in the late 1980s and early 1990s and after culture in the laboratory, only pistillate flowers are known. DNA testing indicates that H. johnsonii is distinct from H. decipiens Ostenfeld. Isozymes and sulfated flavonoids had suggested earlier that H. johnsonii may be closely related to the small-leafed plants in H. minor (Zoll.) den Hartog complex that are widely distributed in the Indo-Pacific. H. johnsonii may represent the vegetative development of a single pistillate clone.

Madeira, P. T., T. K. Van, et al. (1997). "Random amplified polymorphic DNA analysis of the phenetic relationships among world-wide accessions of Hydrilla verticillata." Aquatic Botany **59**(3-4): 217.

http://www.sciencedirect.com/science/article/B6T4F-3S5BM03-3/2/734c7359d67b4c9a5c3ccf72140753cb

The phenetic relationships among forty-four accessions of Hydrilla verticillata from various regions of the world were determined using random amplified polymorphic DNA (RAPD) analysis of bulked genomic samples. Five primers were used producing a total of 85 resolvable, polymorphic bands. The accessions were compared using Gower and Dice metrics, clustered using unweighted pair-group arithmetic average clustering (UPGMA) and consensus algorithms, and factored using principal coordinate analysis. Four major clusters (Asian, Australian, Indonesian, monoecious U.S.) and one minor outlier cluster (Japan/Poland) were identified. The U.S. dioecious accessions formed a group closest to an accession from Bangalore, India, possibly lending credence to historical reports that it was imported from Sri Lanka. The U.S. monoecious plants cluster with an accession from Seoul, Korea. Accessions from Taiwan, Burundi, and Panama join the Asian cluster late. The New Zealand accessions cluster loosely with those from Australia. The use of band intensity in combination with the Gower similarity coefficient generated a cophenetic correlation coefficient (similarity matrix vs. UPGMA matrix) of r = 0.92, superior to that for the corresponding Dice metric (r = 0.85).

Aquatic Toxicology (6)

Chen, W.-Y., J. A. C. John, et al. (2004). "Expression of metallothionein gene during embryonic and early larval development in zebrafish." Aquatic Toxicology **69**(3): 215.

http://www.sciencedirect.com/science/article/B6T4G-4CXKG2J-2/2/e272df2116afa7a30ef88a26702eabda

Metallothionein (Mt) has been considered as a molecular marker of metal pollution in aquatic ecosystems. Less is known about the expression of mt gene during embryogenesis. Here, we report the cloning, sequencing, and the expression pattern of mt gene during developmental stages in zebrafish. The zebrafish embryogenesis when takes place in a medium containing a dosage of 1000 [mu]M zinc resulted in high mortality, indicating the deleterious effect of zinc on development. The zebrafish mt gene consists of three exons encoding 60 amino acids with 20 conserved cysteine residues. RT-PCR result indicates the maternal contribution of Mt transcripts. Using digoxigenin (DIG)-labeled anti-sense RNA probe, whole-mount in situ hybridization was performed to observe the expression pattern of zebrafish mt gene during embryonic and early larval stages. Stronger as well as ubiquitous expression of mt gene during early embryonic stages narrowed to specific expression after hatching. The mt promoter region contains seven copies of putative metal-responsive elements (MREs), which are shown to be important for the high level activity by deletion analysis. The expression of mt gene during embryogenesis implies its significant role on development.

Hestermann, E. V., J. J. Stegeman, et al. (2002). "Relationships among the cell cycle, cell proliferation, and aryl hydrocarbon receptor expression in PLHC-1 cells." Aquatic Toxicology **58**(3-4): 201.

http://www.sciencedirect.com/science/article/B6T4G-45S3JM5-7/2/8fe007e10a978a6f8d701535a25e7a9e

Aryl hydrocarbon receptor (AHR) ligands such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) cause altered cell proliferation in many tissues in vivo and cell types in vitro, and the AHR has been suggested to play a role in cell cycle regulation in mammalian systems. However, the mechanisms underlying these effects are poorly understood. The overall objective of the present work was to investigate possible interactions between cell proliferation, the cell cycle, and AHR signal transduction in a piscine system, the PLHC-1 cell line, which is being used increasingly in aquatic toxicological research. The specific objectives were to characterize proliferation rates and the cell cycle in these cells, to measure effects of TCDD on cell proliferation, and to determine if expression of the AHR varies during the cell cycle. The doubling time of PLHC-1 cells was determined to be 22 h, and the durations of the G1, S and G2/M stages of the cell cycle were 13, 3, and 6 h, respectively. A minimum seeding density of 1.2 x 105 cells/cm2 in medium with 10% calf serum and 0.3 x 105 cells/cm2 in 10% fetal bovine serum was found to be required for subsequent proliferation. Of several cell cycle inhibitors tested, only aphidicolin and nocodazole were effective for obtaining synchronous cell populations. TCDD was found to inhibit PLHC-1 cell proliferation in a time- and dose-dependent manner in multiple passages of one sub-clone, but not in several other sub-clones. Neither AHR mRNA nor protein expression varied during the cell cycle, as measured by RT-PCR and specific binding of [3H]TCDD in synchronous PLHC-1 cells. This work establishes techniques for identifying and characterizing possible interactions between the cell cycle and AHR signal transduction in PLHC-1 cells. Taken together, the results indicate that PLHC-1 cells are amenable to analysis of AHR-cell cycle interactions, but that heterogeneity

of sub-clones may complicate their use for investigating AHR-mediated changes in proliferation.

Islinger, M., D. Willimski, et al. (2003). "Effects of 17a-ethinylestradiol on the expression of three estrogen-responsive genes and cellular ultrastructure of liver and testes in male zebrafish." Aquatic Toxicology **62**(2): 85.

http://www.sciencedirect.com/science/article/B6T4G-45SH8MV-1/2/c75f29ce849159502176d569dd0867d0

In order to monitor the influence of estrogenic compounds on the reproductive physiology of fish, molecular markers for zebrafish vitellogenin, estrogen receptor and ZP2 were developed. For this purpose, sequence information about the zebrafish estrogen receptor and vitellogenin had to be obtained. By means of RT-PCR, a sequence fragment of the zebrafish estrogen receptor [alpha] was cloned and sequenced. Continuous cDNAs of two zebrafish vitellogenin-like gene products (zfvg1 and zfvg3) were constructed by the help of expressed sequence tags of zebrafish and completely sequenced. The sequences of the estrogen receptor and of the vitellogenins showed significant similarities to corresponding cDNAs of other fish species. Expression of these gene products was measured following exposure to 17[alpha]-ethinylestradiol and compared with histological endpoints. RT-PCR was used as a semiquantitative technique to record gene expression in adult male zebrafish, which were exposed to 17[alpha]-ethinylestradiol in time-and dose-response experiments. As for time-dependent expression, all hepatic genes investigated were expressed at considerable amounts from 24 h after onset of exposure to 50 ng/l 17[alpha]-ethinylestradiol to the end of experiment (17 days). In testes, expression of the estrogen receptor- as well as ZP2-mRNA remained unchanged for the entire experiment, except for the individuals exposed for 17 days, which displayed elevated expression levels of ZP2. In the dose-response experiment, male zebrafish were exposed to 17[alpha]-ethinylestradiol in concentrations from 0.25-85 ng/l for 4 and 21 days. LOECs for vitellogenin as well as estrogen receptor [alpha] expression were found to be 2.5 ng/l already after 4 d of exposure. Extension of the exposure time to 21 days resulted in enhanced transcription of vitellogenin-mRNAs at 2.5 ng/l 17[alpha]-ethinylestradiol, whereas the detection limit could not be lowered. In contrast, in testes no induction of both ZP2 as well as estrogen receptor expression was detected at any concentration tested. To examine estrogen-caused alterations at the ultrastructural level, liver and testes of males exposed to 25 ng/l 17[alpha]-ethinylestradiol were analysed. Male livers responded with a feminisation reflected by the proliferation of rough endoplasmatic reticulum and Golgi apparatus typical of female hepatocytes during vitellogenesis. However, in testes no signs of feminisation were detectable; rather, destructive phenomena like phagocytosis of sperm cells by Sertoli cells were observed. Thus, in sexually differentiated males no reorganisation of the gonadal tissue towards an ovary could be definitely detected at any level investigated.

Kwak, H.-I., M.-H. Lee, et al. (2000). "Interrelationship of apoptosis, mutation, and cell proliferation in N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)-induced medaka carcinogenesis model." Aquatic Toxicology **50**(4): 317.

http://www.sciencedirect.com/science/article/B6T4G-41306X9-3/2/786cf1ad36e62713f06046f743007f8a

The present study examined the interrelationship of GSH depletion, apoptosis, mutation, and cell proliferation following carcinogen exposure. Medaka (Oryzias latipes) were investigated following a 28 day, three times/week pulse exposure to N-methyl-N'-nitro-N-nitrosoguanidine (MNNG). Fish (5 weeks old) were exposed to MNNG at concentrations of 0, 0.5, and 1 mg l-1 and reared for 3, 5 and 7 more months after the last day of exposure. GSH levels were decreased in the higher concentration groups and longer-reared groups. Flow cytometric analysis revealed that fish from

the groups reared 3 and 5 months showed active apoptotic changes in the dose- and time-dependent manner, but the group reared 7 months had fewer apoptotic, rather showed more necrotic and carcinogenic alterations. Mutational responses were detected by an arbitrarily primed polymerase chain reaction (AP-PCR) fingerprinting method using whole body DNA samples as templates and pBR primer. A mutational change was expressed by a loss or gain of a band. There was a time-dependent mutational change, but no distinctive concentration-dependent one. A band from normal fish sample that disappeared after treatment of MNNG was excised and sequenced. The band had an 869 base pair-long sequence, however, there was no putative protein-coding region based on an analysis by DNAsis. Spindle cell sarcomas invading muscle were detected on the whole body sections from three of ten fish examined, and immunohistochemical analysis with PCNA showed that tumor cells were actively proliferating. However, terminal deoxynucleotidyl transferase (TdT) assay showed that tumored fish still had active apoptotic cell changes in the tissues without tumor. This study shows not only the interrelationship of GSH depletion, apoptosis, mutation and cell proliferation, but also indicates that medaka is appropriate as a fish model for research on the passage of carcinogenesis.

Lin, C.-H., J. A. C. John, et al. (2004). "Cloning and characterization of metallothionein gene in ayu Plecoglossus altivelis." Aquatic Toxicology **66**(2): 111.

http://www.sciencedirect.com/science/article/B6T4G-4BRBFGM-2/2/5bbab68165d69b7827042a1b9f8c3604

Metallothionein (MT) has been used widely as a potential molecular marker to detect the deleterious effects of heavy metals in aquatic ecosystem. Here we exposed ayu, Plecoglossus altivelis, to zinc (Zn) and tested the distribution as well as the induction of MT in various tissues such as liver, kidney, intestine and stomach. MT induction was significant in liver tissue, followed by kidney and intestine, whereas no induction was detected in stomach. The gene encoding ayu MT was successfully cloned and characterized. Complete nucleotide sequencing and analysis of the 4.5 kb DNA fragment containing the ayu MT gene revealed that the gene has three exons interrupted by two introns, a 5'-flanking region of about 2.5 kb and about 1.6 kb of 3'-flanking region. In grouper heart and kidney cells, the 2.5 kb promoter containing eight metal responsive elements (MREs), two hepatic nuclear factor 5 responsive elements (HNF5REs) and one cAMP responsive element (CRE) had the highest reporter activity.

Willett, K. L., C. Wilson, et al. (2000). "Evidence for and against the presence of polynuclear aromatic hydrocarbon and 2,3,7,8-tetrachloro-p-dioxin binding proteins in the marine mussels, Bathymodiolus and Modiolus modiolus." Aquatic Toxicology **48**(1): 51.

http://www.sciencedirect.com/science/article/B6T4G-3Y2F9FF-5/2/dd170d4f683f3fd2cf6993918d00a475

Chemosynthetic mussels were collected in the vicinity of gas and petroleum seeps in the Gulf of Mexico. Aryl hydrocarbon hydroxylase (AHH) and glutathione S-transferase (GST) activities in the hepatopancreas and gill, respectively, were elevated in mussels collected at the site more highly contaminated with polynuclear aromatic hydrocarbons (PAHs). The aryl hydrocarbon receptor (AhR) and the 4S PAH binding protein (PBP) are ligand activated transcription factors which regulate expression of various genes including cytochrome P450 1A. The presence of these proteins was investigated in PAH-exposed mussels. RT-PCR analysis revealed only a 45.2% nucleotide similarity between the AhR2 from Fundulus heteroclitus and from mussel mRNA transcripts containing a putative member of the PAS gene family. Furthermore, in gel electrophoretic mobility shift and protein cross-linking assays utilizing mussel cytosol which was incubated with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), no specifically-bound retarded band

with a [32P]dioxin responsive element was clearly indicated. Likewise, sucrose density gradient analysis of cytosol incubated with [3H]TCDD did not give a specifically-bound 8-10S peak associated with the AhR complex. In contrast, incubation of mussel cytosol with [3H]benzo(a)pyrene (BaP) gave a 4S peak which was not displaced by TCDD but was nonspecifically decreased by excess BaP or benzo(ghi)perylene. The potential role of the 4S PAH binding protein in the induction of CYP1A-dependent activity in these species is currently unclear. Mussels collected from the North Sea were treated with BaP (5 mg/kg) or TCDD (20 [mu]g/kg) for 48 h to investigate induction of enzyme activities in mussels from a pristine location. Western blot analysis indicated the presence of a 33 kDa protein when a PAH binding protein antibody was used, but no detectable induction of AHH or GST activity was observed in the treated mussels.

Behav. Ecol. (2)

Johnson, L. S., B. G. Hicks, et al. (2002). "Increased cuckoldry as a cost of breeding late for male house wrens (Troglodytes aedon)." Behav. Ecol. **13**(5): 670-675.

http://beheco.oupjournals.org/cgi/content/abstract/13/5/670

One factor hypothesized to influence the reproductive behavior of individuals is the degree to which reproductive efforts are synchronized with others in the population. We asked whether the timing of a pair's breeding cycle, relative to cycles of pairs on neighboring territories, affected rates of extrapair mating over 2 years in a Wyoming population of house wrens (Troglodytes aedon). Extrapair young (identified using 5 microsatellite loci) occurred in 74% of nests of 19 pairs whose cycles began later than cycles of one or more neighbors compared to only 26% of nests of 27 pairs whose cycles began earlier than, or simultaneously with, cycles of all neighbors. Extrapair offspring occurred in 65% of 17 nests belonging to males who initially settled and began nesting early relative to neighbors but who were forced to renest late after we removed their first mates. Rates of cuckoldry were not significantly different for forced-late and naturally late males. Our experimental approach controlled for possible effects of male quality, clearly demonstrating an effect of timing of breeding on extrapair mating activity.

Utami, S. S., B. Goossens, et al. (2002). "Male bimaturism and reproductive success in Sumatran orang-utans." Behav. Ecol. **13**(5): 643-652.

http://beheco.oupjournals.org/cgi/content/abstract/13/5/643

Although orang-utans live solitary lives most of the time, they have a complex social structure and are characterized by extreme sexual dimorphism. However, whereas some adult male orang-utans develop full secondary sexual characteristics, such as cheek flanges, others may stay in an "arrested" unflanged condition for up to 20 years after reaching sexual maturity. The result is a marked bimaturism among adult males. Flanged males allow females to overlap with their home range and often tolerate the presence of unflanged males. However, wherever possible flanged males actively prevent unflanged males from copulating with females. Two competing hypotheses, previously untested, have been advanced to explain male reproductive behavior and bimaturism in orang-utans: (1) the "range-guardian" hypothesis, which asserts that the flanged males are postreproductive and defend a range in which they tolerate sexually active, unflanged

male relatives; and (2) the "female choice" hypothesis, which asserts that flanged males tolerate unflanged males in their range because they rely on female preference to favor flanged males. We investigated these hypotheses and a third hypothesis that the two male morphs represent co-existing alternative male reproductive strategies ("sitting, calling, and waiting" for flanged males versus "going, searching, and finding" for unflanged males). Fecal samples were collected from a well-studied population in Indonesia, and eight human microsatellites were analyzed for 30 individuals that have been behaviorally monitored for up to 27 years. By carrying out paternity analysis on 11 offspring born over 15 years, we found that unflanged males fathered about half (6) of the offspring. Relatedness between successful unflanged males and resident dominant males was significantly lower than 0.5, and for some unflanged/flanged male pairs, relatedness values were negative, indicating that unflanged males are not offspring of the flanged males.

Biochemical Education (1)

Howarth, J., H. Waters, et al. (1996). "The role of molecular biology in characterisation of [alpha] thalassaemia." Biochemical Education **24**(1): 59.

http://www.sciencedirect.com/science/article/B6T4N-45BMCDF-Y/2/5571ec7bbd93c6321ad212fb7ceeafe8

Biochemical Systematics and Ecology (6)

Foltz, D. W., S. K. Sarver, et al. (1995). "Genetic structure of brackish water clams (Rangia spp.)." Biochemical Systematics and Ecology **23**(3): 223.

http://www.sciencedirect.com/science/article/B6T4R-3YCMKSW-28/2/7b9bcdc715818c040e3fa48be11f5483

Two congeneric species of brackish water clams, Rangia cuneata (Sowerby, 1831) and Rangia flexuosa (Conrad, 1839), that are sympatric in the northern Gulf of Mexico, were analyzed for electrophoretic a variation at 19 allozyme loci. Rangia cuneata also was analyzed for restriction-site and (partial) nucleotide sequence variation for a mitochondrial gene, cytochrome oxidase I (COI). Rangia cuneata has greatly increased its abundance along the mid-Atlantic coast of the U.S.A. within the last 100 years, due either to colonization from southern populations or to expansion of indigenous populations. Rangia cuneata populations from Virginia to southern Florida formed a discrete and homogeneous group based on allozyme allele frequencies, as did populations from Mississippi to Texas (Nei's unbiased genetic distance between the two groups was 0.03). Atlantic coast populations of R. cuneata also had uniformly lower expected heterozygosities and lower numbers of alleles per locus than Gulf populations. The COI gene was largely monomorphic and mostly uninformative about possible genetic differences between Atlantic and Gulf coast R. cuneata, but a polymorphic MboII restriction site in the Gulf was monomorphic in all examined Atlantic populations. These data are more consistent with the indigenous-expansion model than the southern-expansion model. Nei's genetic distance between

R. cuneata and R. flexuosa was large (1.45), but there was no evidence for the existence of a complex of sibling or semi-species within R. cuneata.

Han, H.-Y. and B. A. McPheron (1994). "Phylogenetic study of selected tephritid flies (Insecta: Diptera: Tephritidae) using partial sequences of the nuclear 18S ribosomal DNA." Biochemical Systematics and Ecology **22**(5): 447.

http://www.sciencedirect.com/science/article/B6T4R-47S10PH-3/2/45001087a12b8ae78341c03a554fa7da

We analyzed sequence data from 107 base pairs within the nuclear 18S ribosomal RNA gene from 26 tephritid species and four out-group taxa. A total of 45 sites were variable within the Tephritidae, and 28 sites were considered informative for parsimony analysis. Phylogenetic information was extracted from this data set using maximum parsimony and neighbor joining methods and compared to a phylogenetic hypothesis proposed from the morphological literature. Our molecular data suggest: (1) monophyly of the subfamily Tephritinae: (2) membership of Gymnocarena; (3) the possible existence of a large monophyletic group including the subfamily Dacinae and a large part of the Trypetinae. We propose that molecular approaches show great promise for helping to create a phylogenetically-based higher classification for the Tephritidae.

Li, X., M. H. Ralphs, et al. (2002). "Genetic variation within and among 22 accessions of three tall larkspur species (Delphinium spp.) based on RAPD markers." Biochemical Systematics and Ecology **30**(2): 91.

http://www.sciencedirect.com/science/article/B6T4R-447NKGM-5/2/699e8ecbada9b31283b7a5ac8cb92c4e

Random amplified polymorphic DNA (RAPD) markers were used to assess genetic diversity in three species of toxic larkspurs (Delphinium spp). A total of 184 plants from 22 accessions in five western states were analyzed by 23 RAPD primers that amplified 188 reproducible bands. There were 144 polymorphic bands; 10 shared by Delphinium glaucum and Delphinium occidentale, eight shared by Delphinium barbeyi and D. glaucum, and 18 shared by D. occidentale and D. barbeyi. Thirteen bands were specific for D. occidentale, 18 for D. glaucum and 19 for D. barbeyi. There were 58 bands that were specific for individual accessions and 44 bands that were common to all three species. Some of the species-specific bands were cloned and tested in Southern hybridization. Based on the presence or absence of the 144 polymorhpic RAPD bands in individuals, a dendrogram was generated to assess the genetic similarity among the samples. The cophenetic values were 0.64 between D. occidentale and D. barbeyi, and 0.55 between the cluster of these two species and D. glaucum. These relationships are congruent with those based on morphological characters and support the contention that these are separate species. Understanding the genetic relationships among these three tall larkspur species will provide basic knowledge useful in developing strategies to reduce livestock losses by these poisonous plants.

Spalter, R. A., D. Walsh, et al. (1997). "Sequence heterogeneity of the ribosomal RNA intergenic region Alexandrium species." Biochemical Systematics and Ecology **25**(3): 231.

http://www.sciencedirect.com/science/article/B6T4R-3RH095Y-4/2/cefd3dcdd68731408e129627fac5536e

We report on the identification of unique sequences within the highly variable rDNA ITS regions and more conserved 5.8S regions which provide data for the design of species- and genus-specific probes, and we examine some aspects of the phylogeny of Alexandrium isolates from New Zealand waters.

Tyrrell, J. V., P. R. Bergquist, et al. (1996). "Phylogeny of the Raphidophytes Heterosigma carterae and Chattonella antiqua Using 'V4' Domain SSU rDNA Sequences." Biochemical Systematics and Ecology **24**(3): 221.

http://www.sciencedirect.com/science/article/B6T4R-3W4930J-6/2/4937f24e4e0d62055a191c4a07e848c4

The systematic relationships of the Class Raphidophyceae within the chromophyte algae are uncertain. The 'V4' domain small-subunit rDNA gene ('V4' domain SSU rDNA gene) was sequenced for the taxa Heterosigma carterae and Chattonella antiqua to provide characters for phylogenetic analyses. These analyses showed that the Classes Raphidophyceae and Eustigamatophyceae form a sister clade. A close systematic relationship for these two classes has not been shown before. Numerous phylogentic analyses in this study have failed to provide resolution of the deep branches of the five major evolutionary assemblages--Classes Raphidophyceae and Eustigamatophyceae; Classes Synurophyceae and Chrysophyceae; Classes Phaeophyceae and Xanthophyceae; Class Bacillariophyceae; Class Prymnesiophyceae. Transition frequency analysis shows that there is a major loss of phylogenetic signal within the chromophyte algae'V4' domain SSU rDNA gene sequences.

Wiley, E. O., G. David Johnson, et al. (2000). "The interrelationships of Acanthomorph fishes: A total evidence approach using molecular and morphological data." Biochemical Systematics and Ecology **28**(4): 319.

http://www.sciencedirect.com/science/article/B6T4R-3YVDB0Y-4/2/958a6a2dcd2fc9c48c4295dafde6412e

DNA sequence and morphological data were analyzed for specimens of twenty-five species of acanthomorph fishes and two specimens representing the outgroups Aulopiformes and Myctophiformes. A 572 base-pair (bp) segment of the 12S ribosomal mitochondrial gene, 1112 bp from three regions of the 28S ribosomal nuclear gene, and 38 morphological transformation series were analyzed under the criterion of maximum parsimony. The total evidence analysis resulted in a set of four most parsimonious trees. Relationships common to all trees are largely congruent with the hypothesis articulated by Johnson and Patterson (1993. Bull. Mar. Sci. 52, 554-626).

Biol. Bull. (1)

Belda-Baillie, C. A., B. K. Baillie, et al. (2002). "Specificity of a Model Cnidarian-Dinoflagellate Symbiosis." Biol. Bull. **202**(1): 74-85.

http://www.biolbull.org/cgi/content/abstract/202/1/74

To understand the flexibility of symbiotic associations in coral reefs, we investigated the specificity of the Aiptasia (cf. insignis)-Symbiodinium association in the laboratory by rendering the anemones aposymbiotic and inoculating them with different isolates of Symbiodinium. Infective algal symbionts were monitored over 3 months by re-isolation and identification using denaturing-gradient gel electrophoresis and sequence comparison of their amplified 18S rRNA hypervariable V1 + V2 gene region. Despite similarity in their external morphology, the algal isolates differed in their infectivity towards the host. Within days of single-isolate inoculation, aposymbiotic anemones formed associations with fresh or cultured isolates (clade B) from the anemones Aiptasia sp. or A. tagetes, respectively. They associated to a limited extent with cultured isolates (clade A) from the tridacnids Tridacna crocea or Hippopus hippopus, and not at all with a cultured isolate (clade C) from the stony coral Montipora verrucosa, nor with a free-living isolate (clade A) from subtidal sands. Aposymbiotic anemones inoculated with a mixture of all isolates had only the anemone taxon as their detectable symbionts. Re-inoculation of induced symbioses with a mixture of all isolates and incubation with wild anemones showed that the initial induced symbioses with the anemone taxon were stable. Anemones originally infected with tridacnid isolates either additionally acquired the anemone taxon or had the former outgrown by the latter. These results demonstrate the presence of a host-symbiont recognition mechanism, and possibly competition among potential algal symbionts in the Aiptasia-Symbiodinium association. Here we present a method that may be useful in monitoring the algal population dynamics in symbiotic corals in the field, along with an efficient method of rendering Aiptasia aposymbiotic for further laboratory investigation of Aiptasia-Symbiodinium symbioses.

Biological Conservation (4)

Dalen, L., A. Gotherstrom, et al. (2002). "Is the endangered Fennoscandian arctic fox (Alopex lagopus) population genetically isolated?" Biological Conservation **105**(2): 171.

http://www.sciencedirect.com/science/article/B6V5X-4561BX6-4/2/af3e3ebb8baae6e6e7f59bacf156b778

The arctic fox population in Fennoscandia is on the verge of going extinct after not being able to recover from a severe bottleneck at the end of the 19th century. The Siberian arctic fox population, on the other hand, is large and unthreatened. In order to resolve questions regarding gene flow between, and genetic variation within the populations, a 294 bp long part of the mitochondrial hypervariable region 1 was sequenced. This was done for 17 Swedish, 15 Siberian and two farmed foxes. Twelve variable nucleotide sites were observed, which resulted in 10 different haplotypes. Three haplotypes were found in Sweden and seven haplotypes were found in Siberia. An analysis of molecular variance showed a weak, but significant, differentiation between the populations. No difference in haplotype diversity was found between the populations. A phylogenetic analysis revealed that the three Swedish haplotypes were not monophyletic compared to the Siberian haplotypes. These results indicate a certain amount of gene flow between the two populations, both before and after the bottleneck. Restocking the Fennoscandian population with arctic foxes from Siberia might therefore be a viable option.

Gibbs, J. P. (1998). "Genetic structure of redback salamander Plethodon cinereus populations in

continuous and fragmented forests." Biological Conservation **86**(1): 77.

http://www.sciencedirect.com/science/article/B6V5X-3TP5RMP-8/2/f8be5e1e93abdd1cbd747b0e64438b8f

Fragmentation of natural habitats is increasing dramatically, yet its effects on the distribution of genetic variation in wild populations remain largely unknown. In this study, two woodland populations of the redback salamander Plethodon cinereus in Connecticut, USA, were contrasted using molecular and morphological markers. One population was from a landscape fragmented for 300 yr by human activities and another from a nearby, undisturbed landscape. Genetic differentiation, based on molecular markers, was marginally greater in the fragmented population than in the contiguous population, and, within the fragmented population, was greater among subpopulations lacking historical forest connections. Genetic divergence between subpopulations was also weakly related to geographic distance in the population occupying continuous forest, but not in the population occupying fragmented forest. Fragmentation enhanced morphotype diversity within populations of P. cinereus, whereas levels of molecular genetic diversity within subpopulations were apparently unaffected.

Jug, T., P. Berrebi, et al. (2005). "Distribution of non-native trout in Slovenia and their introgression with native trout populations as observed through microsatellite DNA analysis." Biological Conservation **123**(3): 381.

http://www.sciencedirect.com/science/article/B6V5X-4F7DJSC-4/2/a1857f2122b703e39680bdf3b997b738

In Slovenia, the Adriatic basin inhabited by native marble trout (S. marmoratus), and the Danubian basin inhabited by native Danubian lineage of brown trout (S. trutta) have been intensively affected by stocking with non-native trout strains. In order to assess spread of non-native strains and their introgression with native trout, a population study based on five microsatellite loci was applied across ten marble and ten brown trout populations, ranging from allegedly non-introgressed to heavily managed. On the basis of correspondence analysis, which revealed three clear groupings consisting of the Danubian and Atlantic lineages of brown trout and the marble trout, the alleles, characteristic of each grouping were identified and used for estimating genetic composition of each population according to the three possible origins. Among the wild populations, five marble and one brown trout populations were found to be pure; all the others were introgressed with exotic alleles (Atlantic and marmoratus alleles in the Danubian basin and Atlantic and Danubian in the Adriatic basin) that markedly dominate in intensively managed populations. As revealed by non-significant FIS values, panmixia between native and introduced fishes has for the most part already been reached. Our research showed that it is not only marble trout whose identity is endangered in Slovenia but also the existence of autochthonous Danubian brown trout is critically compromised, which is new information to be taken into account for local trout conservation.

Lee, C.-T., R. Wickneswari, et al. (2002). "Effect of selective logging on the genetic diversity of Scaphium macropodum." Biological Conservation **104**(1): 107.

http://www.sciencedirect.com/science/article/B6V5X-44R1B5P-B/2/e93c432185ada75f583513834ec69971

We examined the effect of selective logging on the genetic diversity of Scaphium macropodum using RAPD markers via two approaches: (1) to investigate the immediate effect by studying a

same population before and after logging, and (2) to determine the long term effect by comparing two regenerated stands with an adjacent unlogged stand, assuming that they were genetically identical before logging. Results showed no negative immediate impact for the first approach, probably due to the high abundance and heterogeneity of S. macropodum in the compartment investigated. However, for the latter approach, substantial genetic erosion (i.e. 31.5% reduction for Shannon diversity, H) was detected in one of the regenerated stands corresponding to its extremely low tree density for S. macropodum. This implies the possible occurrence of genetic drift and increased inbreeding due to population decline as a result of logging. However, the observed genetic differences among the three sub-populations having prevailed before logging cannot be totally discounted in the second approach. This study also demonstrates the use of tree density as a good surrogate measure of genetic diversity. The present harvesting system in Malaysia based on a general cutting limit need to be refined; the basis for determining cutting limit in a forest management unit should consider abundance of commercial species.

Biological Control (6)

Bobrowski, V. L., G. Pasquali, et al. (2002). "Characterization of two Bacillus thuringiensis isolates from South Brazil and their toxicity against Anticarsia gemmatalis (Lepidoptera: Noctuidae)." Biological Control **25**(2): 129.

http://www.sciencedirect.com/science/article/B6WBP-46MJSVB-5/2/af6e9d61a2e42289d325ee3f01562b22

Kong, H., C. Blackwood, et al. (2005). "The genetic characterization of Pseudomonas syringae pv. tagetis based on the 16S-23S rDNA intergenic spacer regions." Biological Control **32**(3): 356.

http://www.sciencedirect.com/science/article/B6WBP-4F5SB85-1/2/6e0aea81ceb85686c2dc4b7629fe6b34

Pseudomonas syringae pv. tagetis, a plant pathogen being considered as a biological control agent of Canada thistle (Cirsium arvense), produces tagetitoxin, an inhibitor of RNA polymerase which results in chlorosis of developing shoot tissues. Although the bacterium is known to affect several plant species in the Asteraceae and has been reported in several countries, little is known of its genetic diversity. The genetic relatedness of 24 strains of P. syringae pv. tagetis with respect to each other and to other P. syringae and Pseudomonas savastanoi pathovars was examined using 16S-23S rDNA intergenic spacer (ITS) sequence analysis. The size of the 16S-23S rDNA ITS regions ranged from 508 to 548 bp in length for all 17 P. syringae and P. savastanoi pathovars examined. The size of the 16S-23S rDNA ITS regions for all the P. syringae pv. helianthi and all the P. syringae pv. tagetis strains examined were 526 bp in length. Furthermore, the 16S-23S rDNA ITS regions of both P. syringae pv. tagetis and P. syringae pv. helianthi had DNA signatures at specific nucleotides that distinguished them from the 15 other P. syringae and P. savastanoi pathovars examined. These results provide strong evidence that P. syringae pv. helianthi is a nontoxigenic form of P. syringae pv. tagetis. The results also demonstrated that there is little genetic diversity among the known strains of P. syringae pv. tagetis. The genetic differences that do exist were not correlated with differences in host plant, geographical origin, or the ability to produce toxin.

Kong, H., C. D. Patterson, et al. (2004). "A PCR protocol for the identification of Pseudomonas syringae pv. tagetis based on genes required for tagetitoxin production." Biological Control **30**(1): 83.

http://www.sciencedirect.com/science/article/B6WBP-49V790D-3/2/4c78e3f5a581f17c2a5bbcb0096acce3

A polymerase chain reaction (PCR) protocol that can be used to distinguish Pseudomonas syringae pv. tagetis from other P. syringae pathovars, including those that induce apical chlorosis in several plants of the Asteracaea family and in pea, and closely related P. savastanoi pathovars was developed based on DNA sequences from P. syringae pv. tagetis that are required for tagetitoxin synthesis. PCR primer sets designated TAGTOX-9 and TAGTOX-10 in PCR amplifications with DNA from most strains of P. syringae pv. tagetis, produced amplicons of 507 and 733 bp, respectively. The same size amplicons were produced in PCR amplifications with bacterial cells isolated from chlorotic leaf tissue from Canada thistle (Cirsium arvense) plants infected with P. syringae pv. tagetis. Among 16 other P. syringae pathovars, only PCR amplifications with DNA from P. syringae pv. helianthi produced the same size amplicons with the respective primer sets. Low levels of the 507-bp amplicon were produced in PCR amplifications with the TAGTOX-9 primers and DNA from P. syringae pv. helianthi or the nontoxigenic strains of P. syringae pv. tagetis. These results suggest that P. syringae pv. helianthi, the most closely related pathovar to P. syringae pv. tagetis, may be a nontoxigenic form of P. syringae pv. tagetis. Results from PCR amplifications with the TAGTOX-9 and TAGTOX-10 primers provide strong evidence that the newly described Pseudomonas syringae pathovars, CT99B016C isolated from Canada thistle and PP105 and Pisum97-1 isolated from pea, which cause apical chlorosis in these respective hosts, are different from P. syringae pv. tagetis.

Sobhian, R., F. J. Ryan, et al. (2003). "DNA phenotyping to find a natural enemy in Uzbekistan for California biotypes of Salsola tragus L." Biological Control **28**(2): 222.

http://www.sciencedirect.com/science/article/B6WBP-48KVC5M-2/2/5f01759a2fb1d8bd0e2480b1b745fd2c

Salsola tragus L. (Russian thistle, Chenopodiaceae), a weed of Central Asian origin, has two biotypes in California, type A and type B. The gall midge Desertovellum stackelbergi Mamaev (Diptera: Cecidomyiidae), which attacks S. tragus in Uzbekistan, is a candidate biological control agent for this weed in the United States. In a field test conducted in Uzbekistan with plants of the two biotypes of S. tragus from California, both biotypes were attacked by the insect, although type A was the preferred host. Accessions of S. tragus from Uzbekistan, Greece, and Ukraine were similar to the California type A when compared using RAPD and ISSR analyses, while California type B was distinct. Since both California biotypes were hosts to the gall midge, further studies on the biology and host specificity of the insect are justified. Genetic characterization of target weeds can provide information useful for the selection of natural enemies.

van Beek, N., A. Lu, et al. (2003). "Effect of signal sequence and promoter on the speed of action of a genetically modified Autographa californica nucleopolyhedrovirus expressing the scorpion toxin LqhIT2." Biological Control **27**(1): 53.

http://www.sciencedirect.com/science/article/B6WBP-47MK74W-C/2/03fe1c34a6296d6567f0fe1743eea878

We report on the construction and optimization of recombinant Autographa californica nucleopolyhedrovirus engineered to express the insect-selective toxin IT2 from the scorpion Leiurus quinquestriatus hebraeus. We constructed a series of viruses expressing the synthetic Lqhit2 gene with different signal sequences or controlled by different promoters. The effect of the various viruses on speed of response was assayed in Heliothis virescens larvae. In addition, the performance of the optimum recombinant viral construct was compared with similar constructs carrying the Androctonus australis hector insect toxin controlled by two different promoters. There were no significant differences in speed of response of viruses with the early hr5/ie1 the early hr5/lef3, or the early/late hr5/39Kpromoter driving toxin expression. However, the choice of signal sequence resulted in significant effects. The signal sequence from the bombyxin gene of the silkworm Bombyx mori, proved nominally the best. When the signal sequences were used from the following genes, the viruses acted significantly slower: AcMNPV gp67, a lepidopteran adipokinetic hormone, a dipteran chymotrypsin, and the homologous Lqhit2. Finally, the signal sequences of the genes for cuticle protein II of Drosophila melanogaster and of the insect toxins of the scorpions A. australis hector and Hottentota judaicus performed very poorly. The speed of action of AcMNPV, carrying the synthetic Lqhit2 gene with the bombyxin secretion signal and driven by the hr5/ie1 promoter [Ac.LqhIT2(hr5/ie1)], was compared to that of the same virus carrying the AaIT gene under the control of the p10 promoter, or the hr5/ie1 promoter in H. virescens, Trichoplusia ni, and Spodoptera exigua larvae. All recombinant viruses elicited the response significantly faster than the common progenitor wild-type virus in all tests. The response elicited by Ac.LqhIT2(hr5/ie1) was nominally faster than that of both viruses expressing AaIT in all insects tested.

Zhou, L., K. L. Bailey, et al. (2004). "Plant colonization and environmental fate of the biocontrol fungus Phoma macrostoma." Biological Control **30**(3): 634.

http://www.sciencedirect.com/science/article/B6WBP-4C1FBWK-5/2/56e0ee5bc6acd9a56036d85de5b15e63

Several isolates of the fungus Phoma macrostoma demonstrated bioherbicidal activity against dandelion seedlings when applied to soil. Weed control ranged from 36 to 100% depending on the isolates and the doses applied. Using microbiological and molecular genetic techniques, the ability of these isolates to colonize target, and nontarget plants and to disperse and persist in soil were determined. PCR primers highly specific to the biocontrol isolates of P. macrostoma, were used to detect the isolates at rates of application between 4 and 1000 g/m2. Based on the results from representative isolates tested, it was concluded that P. macrostoma colonized root tissues of both resistant and susceptible crop species and a susceptible weed species grown in treated soil, and the frequency of fungal isolation declined with time. It was occasionally detected on untreated plant tissues, which may have resulted from either natural occurrences on seed, or contamination of soil. The biocontrol fungus appeared to have limited mobility in the soil since it was not often detected away from the area where it was placed. It persisted in the soil at detectable levels for up to 4 months, but then its presence declined with time. One year post application, P. macrostoma was either not present or significantly reduced in both soil and plant samples depending on the year of sampling. The results suggested that the isolates of P. macrostoma used for biological weed control would have minimal environmental impact due to its ubiquitous nature, limited mobility, and weak persistence over seasons.

Biologicals (1)

Baylis, S. A., N. Shah, et al. (2003). "Simian cytomegalovirus and contamination of oral poliovirus vaccines." Biologicals **31**(1): 63.

http://www.sciencedirect.com/science/article/B6WBS-481MHPP-8/2/bdbd1aa723661f3eb77ea4b2809f86bc

In the 1950s the use of primary rhesus macaque kidney cultures to propagate poliovirus for vaccine production led to the contamination of vaccines with simian virus 40 (SV40). African green monkey kidney (AGMK) cultures free of SV40 were used as an alternative cell substrate for vaccine manufacture. In this study we evaluate oral poliovirus seeds, vaccine bulks and vaccines themselves for the presence of a common contaminant of AGMK cultures, simian cytomegalovirus (SCMV). Using sensitive polymerase chain reaction (PCR) techniques, nearly half of the samples analysed were found to be contaminated with SCMV sequences. However, vaccine bulks, positive by PCR for SCMV failed to show any evidence of infectious virus in these studies. One poliovirus vaccine and one seed, propagated on rhesus macaque kidney cultures were found to be positive for the rhesus monkey CMV by PCR.

Biomaterials (10)

Brodkin, K. R., A. J. Garcia, et al. (2004). "Chondrocyte phenotypes on different extracellular matrix monolayers." Biomaterials **25**(28): 5929.

http://www.sciencedirect.com/science/article/B6TWB-4C59SWJ-1/2/0236cff5eb56ff513692bd4b4d7fc431

Chondrocytes undergo a process of dedifferentiation in monolayer culture that is characterized by a transition to a fibroblast-like phenotype. This behavioral change poses a challenge for tissue-engineered cartilage constructs, as approaches using autologous cells require expansion in vitro. Because chondrocytes express a variety of integrin receptors specific to different adhesive proteins, we hypothesized that chondrocytes expanded on various underlying protein monolayers would have different phenotypic responses. Bovine articular chondrocytes were cultured for up to 2 weeks on tissue culture plastic, fibronectin, collagen type I or collagen type II substrate in the presence or absence of ascorbate. Contrary to our hypothesis, the extracellular matrix protein substrates used in this study did not significantly alter the changes in chondrocyte morphology, gene expression, matrix formation, or cytoskeletal organization. Cells on all substrates assembled equivalent matrices, which may have subsequently regulated cell behavior. In cultures with ascorbate, populations of round and spread cells emerged after 1 week, with round cells expressing collagen type II and the differentiated phenotype and spread cells dedifferentiating. In cultures without ascorbate, chondrocytes rapidly adhered and spread onto organized fibronectin matrices via the [alpha]5[beta]1 integrin, which has been associated with survival and proliferation of chondrocytes in vitro. These findings indicate that expanding chondrocytes on protein monolayers may not be an effective solution to preventing dedifferentiation and improving autologous chondrocyte transplantation.

Girotto, D., S. Urbani, et al. (2003). "Tissue-specific gene expression in chondrocytes grown on three-dimensional hyaluronic acid scaffolds." Biomaterials **24**(19): 3265.

http://www.sciencedirect.com/science/article/B6TWB-48GVTJK-C/2/9a5baf2961f7d9088262ae08a8a295e5

The re-differentiation capacities of human articular and chick embryo sternal chondrocytes were evaluated by culture on HYAFF-11 and its sulphate derivative, HYAFF-11-S, polymers derived from the benzyl esterification of hyaluronate. Initial results showed that the HYAFF-11-S material promoted the highest rate of chondrocyte proliferation. RNA isolated from human and chick embryo chondrocytes cultured in Petri dishes, HYAFF-11 or HYAFF-11-S were subjected to semi-quantitative RT-PCR analyses. Human collagen types I, II, X, human Sox9 and aggrecan, chick collagen types I, II, IX and X were analysed. Results showed that human collagen type II mRNA expression was upregulated on HYAFF-11 biomaterials. In particular, a high level of collagen type IIB expression was associated with three-dimensional culture conditions, and the HYAFF-11 material was the most supportive for human collagen type X mRNA expression. Human Sox9 mRNA levels were constantly maintained in monolayer cell culture conditions over a period of 21 days, while these were upregulated when chondrocytes were cultured on HYAFF-11 and HYAFF-11S. Furthermore, chick collagen type IIA and IIB mRNA expression was detected after only 7 days of HYAFF-11 culture. Chick collagen type IX mRNA expression decreased in scaffold cultures over time. Histochemical staining performed in engineered cartilage revealed the presence of a de novo synthesized glycosaminoglycan-rich extracellular matrix; immunohistochemistry confirmed the deposition of collagen type II. This study showed that the three-dimensional HYAFF-11 culture system is both an effective chondrocyte delivery system for the treatment of articular cartilage defects, and an excellent in vitro model for studying cartilage differentiation.

Li, W.-J., R. Tuli, et al. (2005). "Multilineage differentiation of human mesenchymal stem cells in a three-dimensional nanofibrous scaffold." Biomaterials **26**(25): 5158.

http://www.sciencedirect.com/science/article/B6TWB-4FF8WM6-1/2/940ecce02898e3754459b641d024265e

Functional engineering of musculoskeletal tissues generally involves the use of differentiated or progenitor cells seeded with specific growth factors in biomaterial scaffolds. Ideally, the scaffold should be a functional and structural biomimetic of the native extracellular matrix and support multiple tissue morphogenesis. We have previously shown that electrospun, three-dimensional nanofibrous scaffolds that morphologically resemble collagen fibrils are capable of promoting favorable biological responses from seeded cells, indicative of their potential application for tissue engineering. In this study, we tested a three-dimensional nanofibrous scaffold fabricated from poly([epsilon]-caprolactone) (PCL) for its ability to support and maintain multilineage differentiation of bone marrow-derived human mesenchymal stem cells (hMSCs) in vitro. hMSCs were seeded onto pre-fabricated nanofibrous scaffolds, and were induced to differentiate along adipogenic, chondrogenic, or osteogenic lineages by culturing in specific differentiation media. Histological and scanning electron microscopy observations, gene expression analysis, and immunohistochemical detection of lineage-specific marker molecules confirmed the formation of three-dimensional constructs containing cells differentiated into the specified cell types. These results suggest that the PCL-based nanofibrous scaffold is a promising candidate scaffold for cell-based, multiphasic tissue engineering.

Mauney, J. R., C. Jaquiery, et al. (2005). "In vitro and in vivo evaluation of differentially demineralized cancellous bone scaffolds combined with human bone marrow stromal cells for tissue engineering." Biomaterials **26**(16): 3173.

http://www.sciencedirect.com/science/article/B6TWB-4DDXRBT-

4/2/8b7451687d7c8f614d2379c296dfd522

Mineralized and partially or fully demineralized biomaterials derived from bovine bone matrix were evaluated for their ability to support human bone marrow stromal cell (BMSC) osteogenic differentiation in vitro and bone-forming capacity in vivo in order to assess their potential use in clinical tissue-engineering strategies. BMSCs were either seeded on bone-derived scaffolds and cocultured in direct cell-to-scaffold contact, allowing for the exposure of soluble and insoluble matrix-incorporated factors, or cocultured with the scaffold preparations in a transwell system, exposing them to soluble matrix-incorporated factors alone. Osteoblast-related markers, alkaline phosphatase (ALP) activity and bone sialoprotein (BSP) and osteopontin (OP) mRNA expression were evaluated in BMSCs following 14 days of cocultivation in both systems. The data demonstrate that BMSCs from some donors express significantly higher levels of all osteoblast-related markers following cocultivation in direct cell-to-scaffold contact with mineralized scaffolds in comparison to fully demineralized preparations, while BMSCs from other donors display no significant differences in response to various scaffold preparations. In contrast, BMSCs cocultured independently with soluble matrix-incorporated factors derived from each scaffold preparation displayed significantly lower levels of ALP activity and BSP mRNA expression in comparison to untreated controls, while no significant differences were observed in marker levels between cells cocultured similarly with different biomaterial preparations. In addition, BMSCs were seeded directly on mineralized and partially or fully demineralized biomaterials and implanted in subcutaneous sites of athymic mice for 8 weeks to evaluate their in vivo bone-forming capacity. The ex vivo incorporation of BMSCs into all bone-derived scaffold preparations substantially increased the mean extent and frequency of samples containing de novo bone formation over similar nonseeded controls, as determined by histological and histomorphometrical analysis. No statistically significant differences were observed in the extent or frequency of bone formation between various scaffold preparations seeded with BMSCs from different donors. These results demonstrate that the in vivo osteoinductivity of bone-derived scaffolds can be modulated by ex vivo incorporated BMSCs and the extent of scaffold demineralization plays a significant role in influencing in vitro osteogenic differentiation of BMSCs depending on the coculture system and BMSC donor.

Mauney, J. R., D. L. Kaplan, et al. (2004). "Matrix-mediated retention of osteogenic differentiation potential by human adult bone marrow stromal cells during ex vivo expansion." Biomaterials **25**(16): 3233.

http://www.sciencedirect.com/science/article/B6TWB-4B9D7R3-1/2/d3aa4dd7c98696d154b54b4adfdc3cca

During prolonged cultivation ex vivo, adult bone marrow stromal stem cells (BMSCs) undergo two probably interdependent processes, replicative aging and a decline in differentiation potential. Recently, our results with primary human fibroblasts indicated that growth on denatured collagen (DC) matrix results in the reduction of the rate of cellular aging. The present study has been undertaken to test whether the growth of human BMSCs under the same conditions would translate into preservation of cellular aging-attenuated functions, such as the ability to express HSP70 in response to stress as well as of osteogenic differentiation potential. We report here that growth of BMSCs on a DC matrix versus tissue culture polystyrene significantly reduced one of the main manifestations of cellular aging, the attenuation of the ability to express a major protective stress response component, HSP70, increased the proliferation capacity of ex vivo expanded BMSCs, reduced the rate of morphological changes, and resulted in a dramatic increase in the retention of the potential to express osteogenic-specific functions and markers upon treatment with osteogenic stimulants. BMSCs are a promising and increasingly important cell source for tissue engineering as well as cell and gene therapeutic strategies. For use of BMSCs in these applications, ex vivo expansion is necessary to obtain a sufficient, therapeutically useful, number of cells; however, this results in the loss of differentiation potential.

This problem is especially acute in older patients where more extensive in vitro expansion of smaller number of stem/progenitor cells is needed. The finding that growth on certain biomaterials preserves aging-attenuated functions, enhances proliferation capacity, and maintains differentiation potential of BMSCs indicates a promising approach to address this problem.

Miot, S., T. Woodfield, et al. (2005). "Effects of scaffold composition and architecture on human nasal chondrocyte redifferentiation and cartilaginous matrix deposition." Biomaterials **26**(15): 2479.

http://www.sciencedirect.com/science/article/B6TWB-4D2FKCN-4/2/45910fa9c40074db9e4371be7cbb8d41

We investigated whether the post-expansion redifferentiation and cartilage tissue formation capacity of adult human nasal chondrocytes can be regulated by controlled modifications of scaffold composition and architecture. As a model system, we used poly(ethylene glycol)-terephthalate-poly(butylene)-terephthalate block copolymer scaffolds from two compositions (low or high PEG content, resulting in different wettability) and two architectures (generated by compression molding or three-dimensional (3D) fiber deposition) with similar porosity and mechanical properties, but different interconnecting pore architectures. Scaffolds were seeded with expanded human chondrocytes and the resulting constructs assessed immunohistochemically, biochemically and at the mRNA expression level following up to 4 weeks of static culture.For a given 3D architecture, the more hydrophilic scaffold enhanced cell redifferentiation and cartilaginous tissue formation after 4 weeks culture, as assessed by higher mRNA expression of collagen type II, increased deposition of glycosaminoglycan (GAG) and predominance of type II over type I collagen immunostain. The fiber-deposited scaffolds, with a more accessible pore volume and larger interconnecting pores, supported increased GAG deposition, but only if a more hydrophilic composition was used.By applying controlled and selective modifications of chemico-physical scaffold parameters, we demonstrate that both scaffold composition and architecture are instructive for expanded human chondrocytes in the generation of 3D cartilaginous tissues. The observed effects of composition and architecture were likely to have been mediated, respectively, by differential serum protein adsorption and efficiency of nutrient/waste exchange.

Ronziere, M.-C., S. Roche, et al. (2003). "Ascorbate modulation of bovine chondrocyte growth, matrix protein gene expression and synthesis in three-dimensional collagen sponges." Biomaterials **24**(5): 851.

http://www.sciencedirect.com/science/article/B6TWB-47DH8T7-J/2/b03e0c58ef6fedf36d241cfc22e36fde

This report completes a previous study on the growth and metabolism of fetal bovine epiphyseal chondrocytes cultured, within native or cross-linked collagen sponges carried out without the addition of fresh ascorbate. At low initial cell density (2.3 x 106 cells/cm3) cell proliferation and a low matrix deposition were observed, whereas at high initial cell density (2.3 x 107 cells/cm3) there was an absence of cell proliferation, but the deposition of a cartilage-like matrix was measured. In both cases, only traces of type I collagen (marker of chondrocyte dedifferentiation) were detected. In this report, we observed, after 1 month in culture with ascorbate, in both type of scaffolds and initial cell densities, an increase in cell proliferation (2-fold) and in expression of genes encoding for collagen types I, II, X and MMP-2 and -13, but no change in the level of matrix deposition (collagen and GAG). With regard to the proteins present, the main differences with or without ascorbate concerned the increase of neosynthesised type I collagen (up to 35% of the total collagen deposited in the sponge) and of the MMP-2 active form. In conclusion, these results show that ascorbate is an important factor to consider when preparing cartilage constructs

for its action on chondrocyte phenotype modulation and proliferation.

Saldanha, V. and D. A. Grande (2000). "Extracellular matrix protein gene expression of bovine chondrocytes cultured on resorbable scaffolds." Biomaterials **21**(23): 2427.

http://www.sciencedirect.com/science/article/B6TWB-418PMNJ-C/2/96f7d54051888f0832690164b62d083f

It has been demonstrated that using cultured chondrocytes that have been seeded onto various biomatrices can enhance the quality of the articular cartilage repair tissue. As tissue-engineering becomes increasingly more complex there is a need to understand how a specific biomaterial may influence gene expression. In this study several commonly used scaffold materials for cartilage tissue engineering were evaluated with respect to their influence on matrix gene expression. Primary cultures of bovine chondrocytes were established in monolayer then seeded onto polylactic acid (PLLA), polyglycolic acid (PGA), collagen matrices. The induction of collagen type I, collagen type II, and aggrecan was observed at various time points on these biomaterials using RT-PCR. The collagen type I gene was upregulated on collagen scaffolds throughout the culture period. PLLA and PGA showed initial induction followed by downregulation. Monolayer culture did not induce collagen I message. Collagen II genes were selectively upregulated after 72 and 96 h post seeding depending the scaffold material. Monolayer culture had strong induction of collagen II. The aggrecan protein was consistently expressed in all scaffold materials cultures and monolayer.

Wooley, P. H., R. Morren, et al. (2002). "Inflammatory responses to orthopaedic biomaterials in the murine air pouch." Biomaterials **23**(2): 517.

http://www.sciencedirect.com/science/article/B6TWB-44FCYG3-T/2/eb79b04e76fc8278ac8f7abdad573c2d

An in vivo model of the inflammatory response to orthopaedic biomaterials was used to examine cellular and cytokine responses to polymer particles of ultra high molecular weight polyethylene (UHMWPE) and polymethylmethacrylate (PMMA), and metal particles of cobalt-chrome (Co-Cr) and titanium alloy (Ti-6Al-4V). Responses were determined separately and in combinations, to examine interactions between different forms of biomaterials. Murine air pouches were injected with particle suspensions, and reactions evaluated using histological, immunological, and molecular techniques. All particulate biomaterials caused significant increases in membrane thickness compared with control (saline) air pouches, with the highest reaction seen in response to Ti-6Al-4V particles. A synergistic increase in membrane thickness was observed when PMMA was combined with UHMWPE, suggesting that multiple biomaterial stimuli markedly increase the inflammatory reaction. Cellular analysis indicated that all particles increased the absolute number and the percentage of macrophages in the membrane over the control level, with the most pronounced increase due to individual biomaterial occurring with UHMWPE particles. Cytokine analysis revealed that biomaterials provoked a strong IL-1 response. Ti-6Al-4V stimulated the highest IL-6 gene transcription and the lowest IL-1 gene transcription. The data suggest that synergism in the inflammatory response to biomaterials may be important in adverse responses to orthopaedic wear debris.

Yang, S.-Y., W. Ren, et al. (2002). "Diverse cellular and apoptotic responses to variant shapes of UHMWPE particles in a murine model of inflammation." Biomaterials **23**(17): 3535.

http://www.sciencedirect.com/science/article/B6TWB-45CW107-2/2/3afe396c3697f793d283bef2b3ebb82a

The wear of orthopaedic prostheses results in the release of a markedly heterogeneous assortment of particulate debris, with respect to both size and shape. Although particle size has been extensively examined, the role of particle shape in adverse inflammatory reactions to debris remains unclear. Using an in vivo murine model of inflammation, we assessed tissue responses to globular and to elongated ultra-high molecular weight polyethylene (UHMWPE) particles with a similar surface area, and investigated whether inflammation and cellular apoptosis varied with particle shape in the debris-tissue interaction. Histological changes of UHMWPE-stimulated pouch membrane were assessed using a computerized image analysis system. Quantitative real time PCR and ELISA were performed to assess mRNA expression and protein level of the cytokines, and TUNEL assays were conducted to quantify apoptotic cells. The data revealed that elongated particles generated more active inflammatory air pouches, stimulated more severe membrane proliferation and the inflammatory cellular infiltration compared to globular particles. Increased levels of IL-1[beta] and TNF[alpha] were detected in the lavage and homogenate of pouches stimulated with elongated particles in comparison to pouches with globular particles, and the apoptotic assay indicated more severe apoptotic changes within the inflammatory membrane provoked with elongated particles. Our results suggest that cellular responses to UHMWPE wear debris are dependent on the shape of the particles.

Biomolecular Engineering (4)

Bergquist, P. L., R. A. Reeves, et al. "Degenerate oligonucleotide gene shuffling (DOGS) and random drift mutagenesis (RNDM): Two complementary techniques for enzyme evolution." Biomolecular Engineering **In Press, Corrected Proof** http://www.sciencedirect.com/science/article/B6VRM-4FRTDG3-2/2/3f4a0d6f668672c1bf5355ab06d7fff6

Improvement of the biochemical characteristics of enzymes has been aided by misincorporation mutagenesis and DNA shuffling. Many gene shuffling techniques result predominantly in the regeneration of unshuffled (parental) molecules. We describe a procedure for gene shuffling using degenerate primers that allows control of the relative levels of recombination between the genes that are shuffled, and reduces the regeneration of unshuffled parental genes. This shuffling procedure avoids the use of endonucleases for gene fragmentation prior to shuffling and allows the inclusion of random mutagenesis of selected portions of the chimeric genes as part of the procedure. We illustrate the use of the shuffling technique with a family of [beta]-xylanase genes that possess widely different G + C contents. In addition, we introduce a new method (RNDM) for rapid screening of mutants from libraries where no adaptive selection has been imposed on the cells. They are identified only by their retention of enzymatic activity. The combination of RNDM followed by DOGS allows a comprehensive exploration of a protein's functional sequence space.

Hong, K., J. Sherley, et al. (2001). "Methylation of episomal plasmids as a barrier to transient gene expression via a synthetic delivery vector." Biomolecular Engineering **18**(4): 185.

http://www.sciencedirect.com/science/article/B6VRM-441N1K4-3/2/6d2f8de110ba2ac775896c2aa66bc335

Efficient and sustained transgene expression are desirable features for many envisioned gene therapy applications, yet synthetic vectors tested to date are rarely successful in achieving these properties. Substantial research efforts have focused on protection of plasmid DNA from nuclease attack as well as increasing nuclear transport of plasmids, resulting in significant but still limited gains. We show here that a further barrier to efficient and sustained expression exists for synthetic vectors: plasmid DNA methylation. We have investigated this barrier for transient expression of a green fluorescent protein (GFP) transgene delivered via Lipofectamine, by testing the effects of culturing C3A human hepatoblastoma cells with 5-Azacytidine (AzaC), an irreversible inhibitor of DNA methyltransferase. To control for loss of plasmids by dilution during mitosis, transfected cells were growth-arrested for 1 week and their subsequent GFP expression quantified by FACS. In the presence of AzaC, a significantly greater fraction of transfected cells remained GFP-positive and possessed higher levels of GFP production relative to AzaC-untreated cells. Additionally, we have applied a Methyl-Assisted PCR (MAP) assay to quantify a subset of methylated CpG sites in the GFP gene. When MAP was performed on plasmids isolated from transfected cells, the extent of methylation was found to be inversely related to the level of GFP expression.

Nguyen, G., N. Bukanov, et al. (2005). "Cloneless genomic DNA analysis: an efficient and simple methods for de novo genomic sequencing projects and gap filling." Biomolecular Engineering **21**(6): 135.

http://www.sciencedirect.com/science/article/B6VRM-4FCRFT8-1/2/da4c511e7d0bec976e941ed2e4cefbc6

The utility of using genomic DNA directly in agarose, i.e. cloneless libraries, in place of large clone libraries, radiation hybrid panels, or chromosome dissection was demonstrated. The advantage of the cloneless library approach is that, in principle, a targeted genomic resource can be developed rapidly for any genomic region using any genomic DNA sample. Here, a human chromosome 20 Not I fragment library was generated by slicing a pulsed field gel lane containing fractionating Not I cleaved DNA from a monosomic hybrid cell line into 2 mm pieces. A reliable PCR method using agarose embedded DNA was developed. InterAlu PCR generated unique patterns of products from adjacent slices (e.g. fractions). Further, the specificity of the interAlu products was demonstrated by FISH analysis and in other hybridization experiments to arrayed interAlu products. STS content mapping was used to order the fractions and also demonstrate the unique content of the library fractions.

Odeberg, J., T. Wood, et al. (2000). "A cDNA RDA protocol using solid-phase technology suited for analysis in small tissue samples." Biomolecular Engineering **17**(1): 1.

http://www.sciencedirect.com/science/article/B6VRM-41FKWH0-1/2/22d66c90aacf73276110ea6ff08e381f

cDNA representational difference analysis (cDNA RDA) is a PCR-based subtractive enrichment procedure for the cloning of differentially expressed genes. In this study, we have further developed the procedure to take advantage of solid-phase technology, and to facilitate the use of RDA when starting material is limited. Several parameters of the PCR-based generation of cDNA representations were investigated, and a solid-phase based purification step was introduced to simplify removal of digested adapter-ends and uncleaved fragments. The use of magnetic particles increased the speed of the method, and also eliminated the risk of carry-over contamination between iterative steps of subtraction and PCR amplification. The modified protocol was evaluated in monitoring differences in gene expression in (i) a rat system consisting of livers with and without growth hormone treatment, and in (ii) a human system consisting of

normal colon and colon cancer.

Bioorganic & Medicinal Chemistry (2)

Foister, S., M. A. Marques, et al. (2003). "Shape selective recognition of T.A base pairs by hairpin polyamides containing N-Terminal 3-Methoxy (and 3-Chloro) thiophene residues." Bioorganic & Medicinal Chemistry **11**(20): 4333.

http://www.sciencedirect.com/science/article/B6TF8-49FGJTF-B/2/315f6ba7ac71d8539962c90aabd121cd

Hairpin polyamides selectively recognize predetermined DNA sequences with affinities comparable to naturally occurring proteins. Internal side-by-side pairs of unsymmetrical aromatic rings within the minor groove of DNA distinguish each of the four Watson-Crick base pairs. In contrast, N-terminal ring pairs exhibit less specificity, with the exception of Im/Py targeting G.C base pairs. In an effort to explore the sequence specificity of new ring pairs, a series of hairpin polyamides containing 3-substituted-thiophene-2-carboxamide residues at the N-terminus was synthesized. An N-terminal 3-methoxy (or 3-chloro) thiophene residue paired opposite Py displayed 6- (and 3-) fold selectivity for T.A relative to A.T base pair, while disfavoring G,C base pairs by >200-fold. Our data suggests shape selective recognition with projection of the 3-thiophene substituent (methoxy or chloro) to the floor of the minor groove.

Izumikawa, M., M. Murata, et al. (2003). "Cloning of modular type I polyketide synthase genes from salinomycin producing strain of streptomyces albus." Bioorganic & Medicinal Chemistry **11**(16): 3401.

http://www.sciencedirect.com/science/article/B6TF8-48W2M9W-4/2/7086939f869a52fbd9c8a1804d2db3a6

Cloning of polyether polyketide synthase (PKS) genes for salinomycin biosynthesis was attempted from Streptomyces albus. Seven [beta]-ketoacyl synthase (KS) core regions were obtained by PCR amplification using primers designed based on the conserved KS domains of type I PKSs. Using the KS fragment as a probe, screening of an S. albus genomic DNA library was carried out by colony hybridization. From the positive cosmid clone isolated, a 4.5-kbBamHI fragment was subcloned and sequenced. It showed high homology with bacterial type I PKSs and was deduced to code for KS, malonyl transferase, and ketoreductase motifs. By gene disruption with this 4.5-kb BamHI fragment, the cloned gene was shown to be a part of the salinomycin biosynthetic gene cluster of S. albus.

Bioresource Technology (2)

Chachkhiani, M., P. Dabert, et al. (2004). "16S rDNA characterisation of bacterial and archaeal communities during start-up of anaerobic thermophilic digestion of cattle manure." Bioresource Technology **93**(3): 227.

http://www.sciencedirect.com/science/article/B6V24-4BH67T9-3/2/0fb79c6b00649cb560460f783c96c574

A laboratory-scale continuously stirred anaerobic thermophilic batch digester was inoculated with cattle manure. Bacterial and archaeal communities, as well as digester performances, were analysed during reactor start-up for about 20 days. Polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) was used for overall detection and for study of the dynamics of microbial populations. Dominant bacteria and archaea 16S rDNAs were sequenced from the sample on day 12. Ten bacteria and 3 archaea OTUs (operational taxonomic units) were identified from the 52 clones sequenced.Sequences corresponding to the dominant bacterial SSCP peak were phylogenetically close to the 16S rDNA sequence of Bacillus thermoterrestris, whereas sequences corresponding to the two dominant archaeal SSCP peaks were phylogenetically close to the 16S rDNA sequence of Methanoculleus thermophilicus and Methanosarcina thermophila.

Miyatake, F. and K. Iwabuchi "Effect of high compost temperature on enzymatic activity and species diversity of culturable bacteria in cattle manure compost." Bioresource Technology **In Press, Corrected Proof** http://www.sciencedirect.com/science/article/B6V24-4FJXN56-5/2/c2684e3ec49c6b9d6eb6415e29af1c39

To clarify the characteristics of thermophilic bacteria in cattle manure compost, enzymatic activity and species diversity of cultivated bacteria were investigated at 54, 60, 63, 66 and 70 [deg]C, which were dependent on composting temperature. The highest level of thermophilic bacterial activity was observed at 54 [deg]C. Following an increase in temperature to 63 [deg]C, a reduction in bacterial diversity was observed. At 66 [deg]C, bacterial diversity increased again, and diverse bacteria including Thermus spp. and thermophilic Bacillus spp. appeared to adapt to the higher temperature. At 70 [deg]C, bacterial activity measured as superoxide dismutase and catalase activity was significantly higher than at 66 [deg]C. However, the decomposition rate of protein in the compost was lower than the rate at 66 [deg]C due to the higher compost temperature.

Biosystems (3)

Klein, J. P., T. H. Leete, et al. (1999). "A biomolecular implementation of logically reversible computation with minimal energy dissipation." Biosystems **52**(1-3): 15.

http://www.sciencedirect.com/science/article/B6T2K-3XT0BGP-3/2/0e46aea817aebbccf43ec800ba366d20

Energy dissipation associated with logic operations imposes a fundamental physical limit on computation and is generated by the entropic cost of information erasure, which is a consequence of irreversible logic elements. We show how to encode information in DNA and use DNA amplification to implement a logically reversible gate that comprises a complete set of

operators capable of universal computation. We also propose a method using this design to connect, or 'wire', these gates together in a biochemical fashion to create a logic network, allowing complex parallel computations to be executed. The architecture of the system permits highly parallel operations and has properties that resemble well known genetic regulatory systems.

Sakamoto, K., D. Kiga, et al. (1999). "State transitions by molecules." Biosystems **52**(1-3): 81.

http://www.sciencedirect.com/science/article/B6T2K-3XT0BGP-B/2/c664531aadb6b7d80d1c2e861592081f

In our previous paper, we described a method by which a state machine is implemented by a single-stranded DNA molecule whose 3'-end sequence encodes the current state of the machine. Successive state transitions are performed in such a way that the current state is annealed onto an appropriate portion of DNA encoding the transition table of the state machine and the next state is copied to the 3'-end by extension with polymerase. In this paper, we first show that combined with parallel overlap assembly, a single series of successive transitions can solve NP-complete problems. This means that the number of necessary laboratory steps is independent from the problem size. We then report the results of two experiments concerning the implementation of our method. One is on isothermal reactions which greatly increase the efficiency of state transitions compared with reactions controlled by thermal cycles. The other is on the use of unnatural bases for avoiding out-of-frame annealing. The latter result can also be applied to many DNA-based computing paradigms.

Wang, L., Q. Liu, et al. (1999). "Surface-based DNA computing operations: DESTROY and READOUT." Biosystems **52**(1-3): 189.

http://www.sciencedirect.com/science/article/B6T2K-3XT0BGP-R/2/de37dfb0b58f0cd31874b19844cbebf6

DNA computing on surfaces is where complex combinatorial mixtures of DNA molecules are immobilized on a substrate and subsets are tagged and enzymatically modified (DESTROY) in repeated cycles of the DNA computation. A restriction enzyme has been chosen for the surface DESTROY operation. For the READOUT operation, both cycle sequencing and PCR amplification followed by addressed array hybridization were studied to determine the DNA sequences after the computations.

Carbohydrate Polymers (1)

Zhang, C. and K. Huang (2005). "Characteristic immunostimulation by MAP, a polysaccharide isolated from the mucus of the loach, Misgurnus anguillicaudatus." Carbohydrate Polymers **59**(1): 75.

http://www.sciencedirect.com/science/article/B6TFD-4DFK86B-3/2/91d8797f7a5e708ca00e5de45eec0d28

To evaluate the mechanism for the biological activity of a natural polysaccharide isolated from the mucus of the loach, Misgurnus anguillicudatus (MAP), the immunomodulatory of MAP was investigated by the methods of molecular biology and cellular biology. The results showed that MAP enhanced proliferation of T lymphocyte, IL-2 expression of Th1 cells, and IL-4 expression of Th2 cells. Time dependence of the secretion of cytokines showed that Th1 cell was the primary cellular target affected by MAP on T lymphocyte. However, MAP did not increase directly the proliferation of B cells and enhanced less IgM antibody production. Moreover, MAP improved the viability of peritoneal macrophages, stimulated TNF-[alpha] and IL-6 production and induced the inducible nitric oxide synthase (iNOS) transcription in macrophages. In addition, MAP exerted its immunomodulating activity at an optimal dose of 30 [mu]g/ml. At this concentration, MAP promoted farthest proliferation of spleen lymphocyte and macrophages. Consequently, MAP enhanced the immune system functions. In conclusion, the biological activity of the loach, which was as traditional Chinese medicine in folk remedies for the treatments of hepatitis, osteomyelitis, carbuncles, inflammations and cancers, as well as for the restoration to health in debilities by various pathogens and aging, may mainly result from MAP selectively activating T cells and macrophages and stimulating secretion of some cytokines.

Carbohydrate Research (1)

Burg, M. and J. Muthing (2001). "Characterization of cytosolic sialidase from Chinese hamster ovary cells: Part I: Cloning and expression of soluble sialidase in Escherichia coli." Carbohydrate Research **330**(3): 335.

http://www.sciencedirect.com/science/article/B6TFF-42HFP1G-6/2/24e34f21062d14aadb2c9bdeab327808

The cDNA of Chinese hamster ovary (CHO) cell cytosolic sialidase was amplified by RT-PCR and cloned into the pGEX-2T plasmid vector encoding for glutathione S-transferase (GST). Screening revealed transformed Escherichia coli clones with the constructed plasmid encoding the CHO cell sialidase sequence. After isopropyl-[beta]--thiogalacto-pyranoside (IPTG) induction, SDS-PAGE of the total protein extracts revealed a new protein of about 70 kDa, correlating with the molecular weight of a fusion protein composed of the GST (26 kDa) and the cloned cytosolic CHO cell sialidase (43 kDa). A soluble fusion protein was purified from sonified E. coli homogenates by one-step affinity chromatography on Glutathione Sepharose 4B, which showed sialidase activity towards 4-methyl-umbelliferyl-[alpha]--N-acetylneuraminic acid (MUF-Neu5Ac) substrate. Induction of cells with 0.1, 0.5, and 1.0 mM IPTG revealed highest total protein amounts after induction with 1.0 mM IPTG, but highest specific activity for affinity chromatography purified eluates from cultures induced with 0.1 mM IPTG. Therefore, large scale production was performed by inducing cells during exponential growth in a 25 L bioreactor for 3 h with 0.1 mM IPTG after chilling the cell suspension to 25[deg]C. The amount of 26.46 mg of 40-fold purified GST-sialidase with a specific activity of 0.999 U/mg protein was obtained from crude protein extracts by one-step affinity chromatography. 2-Deoxy-2,3-dehydro-N-acetylneuraminic acid (Neu5Ac2en) and Neu5Ac were competitive inhibitors for the sialidase, the former being the more effective one using MUF-Neu5Ac as the substrate. The cytosolic sialidase is capable of desialylating a wide spectrum of different types of gangliosides using a thin-layer chromatography overlay kinetic assay without detergents. This is the subject of the accompanying paper (Muthing, J.; Burg, M. Carbohydr. Res. 2001, 330, 347-356).

Chem Senses (2)

Koganezawa, M. and I. Shimada (2002). "Novel Odorant-binding Proteins Expressed in the Taste Tissue of the Fly." Chem Senses **27**(4): 319-332.

http://chemse.oupjournals.org/cgi/content/abstract/27/4/319

A taste tissue cDNA library of the fleshfly Boettcherisca peregrina was screened with a subtracted cDNA probe enriched with taste-receptor-tissue-specific cDNA. Seven genes were identified with sequence similarity to insect odorant-binding protein (OBP) genes. The predicted amino acid sequences of the genes contain the putative signal peptide sequence at the N-terminal and most of them conserve the six cysteines common to known insect OBPs. These genes show a high degree of sequence divergence with [~]20% amino acid identity. The most striking feature was that all seven of these genes are expressed mainly in the taste tissues, such as the labellum and tarsus, unlike the known insect OBP genes expressed in olfactory tissue. The predicted amino acid sequences had the highest degree of sequence similarity to the Drosophila melanogaster OBPs named pheromone binding protein-related proteins (PBPRPs). These gene products are here referred to as gustatory PBP-related proteins (GPBPRPs) 1-7. Homologous GPBPRP genes were found also in D. melanogaster by database search and are shown to be expressed in Drosophila taste tissues.

Nishimura, T., S. Teranishi, et al. (2002). "Glucocorticoid Enhances Na+/K+ ATPase mRNA Expression in Rat Olfactory Mucosa during Regeneration: A Possible Mechanism for Recovery from Olfactory Disturbance." Chem Senses **27**(1): 13-21.

http://chemse.oupjournals.org/cgi/content/abstract/27/1/13

Systemic or topical application of glucocorticoid is the treatment of choice for olfactory disturbance. Recently, Na+/K+ ATPase and glucocorticoid receptor immunoreactivity in the olfactory mucosa was reported. To elucidate a glucocorticoid action on Na+/K+ ATPase production, an animal model was produced by an intra-nasal application of 5% ZnSO4 solution to Wistar rats. Dexamethasone was injected i.p. (0.01 mg/100 g) for 14 days after the insult. Histologically, the regeneration process was completed on day 14 in both dexamethasone- and saline-injected control rats. We used a quantitative polymerase chain reaction (PCR) method to evaluate mRNA production of Na+/K+ ATPase and glucocorticoid receptor. In dexamethasone-injected rats, up-regulation of glucocorticoid receptor mRNA (95% more than control rats, P = 0.00068, unpaired t-test) and of Na+/K+ ATPase mRNA expression (76% more than control rats, P = 0.0042) was observed on day 14. The increased Na+/K+ ATPase expression in the regenerated olfactory mucosa is thought to be beneficial for an active uptake of K+, which is released during excitation, around olfactory neurons and for the transepithelial absorption of Na+ from olfactory mucus. Dexamethasone may thus contribute to the recovery of function after the morphological regeneration in part, at least, through its receptor by regulation of the ionic concentration in the olfactory mucosal microenvironment.

Chemico-Biological Interactions (9)

Blake, B. L., R. M. Philpot, et al. (1996). "Xenobiotic biotransforming enzymes in the central nervous system: an isoform of flavin-containing monooxygenase (FMO4) is expressed in rabbit brain." Chemico-Biological Interactions **99**(1-3): 253.

http://www.sciencedirect.com/science/article/B6T56-3XK16HH-N/2/2dc376d52b7d824216bf6a90d4502804

The flavin-containing monooxygenase (FMO, EC 1.14.13.8) is involved in the metabolism of a number of important xenobiotics including many which affect the central nervous system (CNS). Recently, reports in the literature concerning the amount, activity, location, and isozyme characteristics of this enzyme in the brain have presented conflicting evidence. In order to resolve some of the controversy surrounding FMO in the brain, a highly sensitive method for the detection of flavin-containing monooxygenase (FMO) mRNA in whole brain was employed. A poorly conserved region of FMO transcripts was used to design five sets of oligonucleotide primers. Each primer set was specific for one of the five currently known isoforms of FMO. Four and five isoforms, respectively, are expressed in rabbit liver and kidney, as determined by reverse transcription-polymerase chain reaction. However, only one set of primers amplified a specific rabbit brain cDNA fragment. The sequence of the amplification product affirmed its identity as a segment of FMO4 cDNA. Thus, the FMO of rabbit brain may consist of a single, as yet uncharacterized isozyme and, contrary to several recent reports, is likely to be expressed at low levels.

Gaedigk, A., P. Lekas, et al. (1998). "Novel sulfotransferases cloned by RT-PCR: real proteins or PCR artifacts?" Chemico-Biological Interactions **109**(1-3): 43.

http://www.sciencedirect.com/science/article/B6T56-3VWP2MP-4/2/0985441f9ee5173c4c5d2afa64897727

During studies designed to subclone human phenol sulfotransferase (STP and STM) sequences for use in heterologous E. coli-based expression systems, we designed two oligonucleotide primers that would allow for the simultaneous PCR amplification of expression cassettes containing the coding regions of the STP1, STP2 and STM cDNAs. Following total RNA isolation from human liver, reverse transcription of cDNA, PCR amplification under standard conditions, plasmid subcloning and restriction analysis to select for suitable ST recombinants, we recovered plasmids containing inserts corresponding to STP1, STP2 and STM. However, ten additional, closely related but apparently novel ST sequences were also isolated. Alignments of the three known ST sequences (and one published allelic variant) with these new clones revealed that each one appears to be a PCR-generated modular chimera possessing a combination of DNA segments derived from STP1, STP2 and STM. This observation should serve as an alert to the potential pitfalls of using PCR techniques for the cloning of highly related genes and their cDNA products, especially when PCR primer design allows for the amplification of multiple products in a single reaction.

Lo, H.-W. and F. Ali-Osman (1998). "Structure of the human allelic glutathione S-transferase-[pi] gene variant, hGSTP1*C, cloned from a glioblastoma multiforme cell line." Chemico-Biological Interactions **111-112**: 91.

http://www.sciencedirect.com/science/article/B6T56-3T149TG-9/2/ec735a60edaee6f3f929ae662e3ccea4

We recently reported the cloning of full-length cDNAs corresponding to mRNAs of three GST-[pi] genes, hGSTP1*A, hGSTP1*B and hGSTP1*C, as well as, the isolation of the full-length hGSTP1*C, of the human glutathione S-transferase-[pi] (GST-[pi]) gene that is characterized by a A->G transition at +1404 in exon 5 and a C->T transition at +2294 in exon 6. Although the promoter of the isolated gene was identical to that of the previously described GST-[pi] gene isolated from the MCF 7 and the HPB-ALL cell lines, both of which were hGSTP1*A, a number of structural differences were observed, including, nucleotide transitions, transversions, deletions and insertions, some of which created new restriction enzyme cleavage sites. A guanine insertion in the insulin response element, IRE, in intron 1 created an additional site for 5'-cytosine methylation. Seven repeat retinoic acid response element (RARE) consensus half sites, A(G)GG(T)TC(G)A at +1521 to +1644 were identified in the cloned hGSTP1*C. Five of the RARE half-sites had the minimal spacer nucleotide requirement for functionality and DNA mobility shift analysis with different pairs of the RARE half-sites and supershift studies using antibodies against RAR-[beta] showed significant binding of nuclear protein complexes from RA-treated cells to these RAREs. GST-[pi] gene expression was increased significantly in cells transfected with the GST-[pi] gene and treated with all-trans RA. These results contrast with those in a previous report in which RA was shown to suppress the GST-[pi] promoter, and indicate a complex mechanism of RA-mediated GST-[pi] gene regulation in tumor cells.

Martignoni, M., R. de Kanter, et al. (2004). "An in vivo and in vitro comparison of CYP induction in rat liver and intestine using slices and quantitative RT-PCR." Chemico-Biological Interactions **151**(1): 1.

http://www.sciencedirect.com/science/article/B6T56-4DX276P-1/2/975093134a2588904aa294cdcfc2e02b

Xenobiotics, including drugs, can influence cytochrome P450 (CYP) activity by upregulating the transcription of CYP genes. To minimize potential drug interactions, it is important to ascertain whether a compound will be an inducer of CYP enzymes early in the development of new therapeutic agents. In vivo and in vitro studies are reported that demonstrate the use of liver and intestinal slices as an in vitro model to predict potential CYP induction in vivo.Rat liver slices and intestinal slices were incubated, for 24 h and 6 h, respectively, with [beta]-naphthoflavone ([beta]NF), phenobarbital (PB) or dexamethasone (DEX). In an in vivo study, rats were treated with the same compounds for 3 days. In vivo and in vitro CYP mRNA levels were measured by using real-time quantitative reverse transcription-polymerase chain reaction (RT-PCR). In addition, CYP enzyme activities were determined in rat liver slices after 48 h incubation.In both rat liver and intestinal slices, [beta]NF significantly induced CYP1A1, CYP1A2 and CYP2B1 mRNA levels. PB significantly induced CYP2B1. In liver slices a minor induction of CYP1A1 and CYP3A1 by PB was observed, whereas DEX significantly induced CYP3A1, CYP2B1 and CYP1A2 mRNA levels.The induction profiles (qualitative and quantitative) observed in vivo and in vitro are quite similar. All together, these data demonstrate that liver and intestinal slices are a useful and predictive tool to study CYP induction.

Maugard, C. M., J. Charrier, et al. (1998). "Allelic deletion at glutathione S-transferase M1 locus and its association with breast cancer susceptibility." Chemico-Biological Interactions **111-112**: 365.

http://www.sciencedirect.com/science/article/B6T56-3T149TG-12/2/b45935a01db2c20aa59993dbdce1b205

Glutathione S-transferases (GSTs) are a family of isoenzymes involved in cellular detoxification. Previous studies have correlated the absence of the GSTM1 protein with an increased risk of developing some cancers, especially lung or bladder cancer, in heavy smokers. In this study, we determined GSTM1 gene polymorphisms in a French western population of 437 female controls

and 361 community breast cancer patients. Three distinct alleles of this gene may be identified: GST M1* A allele, GST M1* B allele, and GST M1* 0 allele (which is deleted). Null patients (GSTM1 0) are homozygous for the deletion. We determined in our two populations, patients with no, one or two GSTM1 alleles. The comparative analysis of our two populations did not demonstrate any statistically significant difference in GSTM1 allelotype distribution between the two groups (P=0.43), although the null genotype was the more frequent in patients. The predominance of the null genotype was significant in the oldest group of patients (>=55) (P=0.006), suggesting that GSTM1 null genotype may play an important role in breast cancer susceptibility in the elderly. This was not observed in the youngest age group, i.e. P=0.25), or in the patients aged from 40 to 55 years old (P=0.37). Our results also point out a putative protective role of the A allele in the older female control group (P=0.02), especially in subjects hemizygous for these alleles (P=0.03). A prospective study will be of interest to investigate the effect of dosage of the gene.

Murata, M., N. Ohta, et al. (2001). "The role of aldose reductase in sugar cataract formation: aldose reductase plays a key role in lens epithelial cell death (apoptosis)." Chemico-Biological Interactions **130-132**: 617.

http://www.sciencedirect.com/science/article/B6T56-42SGGWV-20/2/9217ed08d3abd86b7a72000f8471f3ff

Since aldose reductase is localized primarily in lens epithelial cells, osmotic insults induced by the accumulation of sugar alcohols occur first in these cells. To determine whether the accumulation of sugar alcohols can induce lens epithelial cell death, galactose-induced apoptosis has been investigated in dog lens epithelial cells. Dog lens epithelial cells were cultured in Dulbecco's modified Eagle's mimimum essential medium (DMEM) supplemented with 20% fetal calf serum (FCS). After reaching confluence at fifth passage, the medium was replaced with the same DMEM medium containing 50 mM -galactose and the cells were cultured for an additional 2 weeks. Almost all of the cells cultured in galactose medium were stained positively for apoptosis with the terminal deoxynucleotidyl transferance-mediated biotin-dUTP nick end labeling (TUNEL) technique. Agarose gel electrophoresis of these cells displayed obvious DNA fragmentation, known as a ladder formation. All of these apoptotic changes were absent in similar cells cultured in galactose medium containing 1 [mu]M of the aldose reductase inhibitor AL 1576. Addition of AL 1576 also reduced the cellular galactitol levels from 123+/-10 [mu]g/106 cells (n=5) to 3.9+/-1.9 [mu]g/106 cells (n=5). These observations confirm that galactose induced apoptosis occurs in dog lens epithelial cells. Furthermore, the prevention of apoptosis by an aldose reductase inhibitor suggests that this apoptosis is linked to the accumulation of sugar alcohols.

Ozawa, S., Y.-M. Tang, et al. (1998). "Genetic polymorphisms in human liver phenol sulfotransferases involved in the bioactivation of N-hydroxy derivatives of carcinogenic arylamines and heterocyclic amines." Chemico-Biological Interactions **109**(1-3): 237.

http://www.sciencedirect.com/science/article/B6T56-3VWP2MP-P/2/16d9cf75aa5f4cf511be0be13b7e9525

Three related forms of phenol sulfotransferase (PSULT), thermostable ST1A2 (SULT1A2hum) and ST1A3 (SULT1A1hum) and a thermolabile TL-PST (SULT1A3hum), are known to exist in human livers. Thermostable forms, whose activities are polymorphically distributed, have been shown to mediate the bioactivation of carcinogenic N-hydroxy arylamines and heterocyclic amines. To clarify the nature of the sulfation polymorphism, the study compared the expressed levels of ST1A2, ST1A3 and TL-PST mRNAs in human livers by the method of reverse-transcriptase polymerase chain reaction (RT-PCR), utilizing HindIII, BamHI and SnaBI sites which

were unique to the above PSULT cDNAs, respectively. Of the PCR products derived from human liver (n=26), 43-89, p-nitrophenol and dopamine sulfation rates ranged from 440-2670 and p-nitrophenol and dopamine sulfations. Relative levels of hepatic ST1A3 mRNA were non-normally distributed and correlated significantly with p-nitrophenol sulfation. In addition, variant forms of ST1A3 mRNA encoding Arg213His and Met223Val were detected in human livers. With regard to Arg213His, 28 individuals who had homozygous 213Arg alleles, 15 individuals who were heterozygotes and nine homozygous 213His individuals were found by a newly established genotyping method among 52 human liver samples. Frequency of 223Val allele was apparently lower than that of 213His allele, as no homozygous 223Val individual and only three individuals who were heterozygotes (223Met/Val) were observed among 52 individuals. These results suggest that regulation of p-nitrophenol sulfation occurs at the level of gene transcription of ST1A3 which is the major transcript of the three PSULT mRNAs and that a polygenic basis for the apparent genetic polymorphism of sulfation was likely because of the existence of ST1A3 variants.

Terada, T., Y. Sugihara, et al. (2003). "Further characterization of Chinese hamster carbonyl reductases (CHCRs)." Chemico-Biological Interactions **143-144**: 373.

http://www.sciencedirect.com/science/article/B6T56-47PPD21-9/2/c0e3ca60f33282e0b786954ac41cb66d

Three cDNAs encoding carbonyl reductases (CHC1-3) have been isolated and expressed in bacterial cells. The recombinant enzymes were further characterized with respect to substrate specificity, inhibitor sensitivity and response to pyruvate. In addition, the expression of the mRNAs of CHCRs encoding in brain, liver and kidney was analyzed by RT-PCR. Searches of EST files revealed that orthologues of both CHCR3 and human CBR3 are expressed in variety of human cells and tissues.

Terada, T., Y. Sugihara, et al. (2001). "Characterization of multiple Chinese hamster carbonyl reductases." Chemico-Biological Interactions **130-132**: 847.

http://www.sciencedirect.com/science/article/B6T56-42SGGWV-2P/2/ef67e5d4b4a55869a687e3f3cd439572

Carbonyl reductase (CR) is an enzyme which can catalyze the oxidoreduction of various carbonyl compounds in the presence of NAD(P)H. With the PCR method, using primers carrying the conserved nucleotide sequence among mammalian CRs, we isolated three different cDNAs (CHCR1, CHCR2 and CHCR3) which encode a unique carbonyl reductase from the Chinese hamster. The PCR products of CHCR1 and CHCR2 were clearly isolated with Bpu1102I, BspEI and XmaI restriction enzymes. The nucleotide-sequence of CHCR3 was completely different from those of CHCR1 and CHCR2. The predicted double-wound [beta][alpha][beta][alpha]-structures of the CHCRs suggests the presence of a typical NADP+-binding motif and is similar to the corresponding region of 3[alpha],20[beta]-hydroxysteroid dehydrogenase and mouse lung tetrameric carbonyl reductase. The deduced amino acid sequence of CHCR1 showed a high homology to CHCR2 (>96%) and the other mammalian CRs (>81%). However, CHCR3 showed a high homology to human CBR3 (>86%) and a relatively lower homology to the other CHCRs (2 and PGF2[alpha] from the analyses of enzymatic reaction products. The results of Western blotting and RT-PCR suggest these CHCRs have a tissue-dependent-distribution in the Chinese hamster.

Chemistry & Biology (5)

Aparicio, J. F., R. Fouces, et al. (2000). "A complex multienzyme system encoded by five polyketide synthase genes is involved in the biosynthesis of the 26-membered polyene macrolide pimaricin in Streptomyces natalensis." Chemistry & Biology **7**(11): 895.

http://www.sciencedirect.com/science/article/B6VRP-41PVY58-9/2/902f074a49b89dce5f348667d95a9f52

Background: Polyene macrolides are a class of large macrocyclic polyketides that interact with membrane sterols, having antibiotic activity against fungi but not bacteria. Their rings include a chromophore of 3-7 conjugated double bonds which constitute the distinct polyene structure. Pimaricin is an archetype polyene, important in the food industry as a preservative to prevent mould contamination of foods, produced by Streptomyces natalensis. We set out to clone, sequence and analyse the gene cluster responsible for the biosynthesis of this tetraene.Results: A large cluster of 16 open reading frames spanning 84985 bp of the S. natalensis genome has been sequenced and found to encode 13 homologous sets of enzyme activities (modules) of a polyketide synthase (PKS) distributed within five giant multienzyme proteins (PIMS0-PIMS4). The total of 60 constituent active sites, 25 of them on a single enzyme (PIMS2), make this an exceptional multienzyme system. Eleven additional genes appear to govern modification of the polyketide-derived framework and export. Disruption of the genes encoding the PKS abolished pimaricin production.Conclusions: The overall architecture of the PKS gene cluster responsible for the biosynthesis of the 26-membered polyene macrolide pimaricin has been determined. Eleven additional tailoring genes have been cloned and analysed. The availability of the PKS cluster will facilitate the generation of designer pimaricins by combinatorial biosynthesis approaches. This work represents the extensive description of a second polyene macrolide biosynthetic gene cluster after the one for the antifungal nystatin.

August, P. R., L. Tang, et al. (1998). "Biosynthesis of the ansamycin antibiotic rifamycin: deductions from the molecular analysis of the rif biosynthetic gene cluster of Amycolatopsis mediterranei S699." Chemistry & Biology **5**(2): 69.

http://www.sciencedirect.com/science/article/B6VRP-4B6165D-7R/2/5b787f66373e753dd145a16079ec57db

Background: The ansamycin class of antibiotics are produced by various Actinomycetes. Their carbon framework arises from the polyketide pathway via a polyketide synthase (PKS) that uses an unusual starter unit. Rifamycin (rif), produced by Amycolatopsis mediterranei, is the archetype ansamycin and it is medically important. Although its basic precursors (3-amino-5-hydroxy benzoic acid AHBA, and acetic and propionic acids) had been established, and several biosynthetic intermediates had been identified, very little was known about the origin of AHBA nor had the PKS and the various genes and enzymes that modify the initial intermediate been characterized.Results: A set of 34 genes clustered around the rifK gene encoding AHBA synthase were defined by sequencing all but 5 kilobases (kb) of a 95 kb contiguous region of DNA from A. mediterranei. The involvement of some of the genes in the biosynthesis of rifamycin B was examined. At least five genes were shown to be essential for the synthesis of AHBA, five genes were determined to encode the modular type I PKS that uses AHBA as the starter unit, and 20 or more genes appear to govern modification of the polyketide-derived framework, and rifamycin resistance and export. Putative regulatory genes were also identified. Disruption of the

PKS genes at the end of rifA abolished rifamycin B production and resulted in the formation of P8/1-OG, a known shunt product of rifamycin biosynthesis, whereas disruption of the orf6 and orf9 genes, which may encode deoxysugar biosynthesis enzymes, had no apparent effect. Conclusions: Rifamycin production in A. mediterranei is governed by a single gene cluster consisting of structural, resistance and export, and regulatory genes. The genes characterized here could be modified to produce novel forms of the rifamycins that may be effective against rifamycin-resistant microorganisms.

Kim, D., R. Kocz, et al. (1998). "On becoming a parasite: evaluating the role of wall oxidases in parasitic plant development." Chemistry & Biology **5**(2): 103.

http://www.sciencedirect.com/science/article/B6VRP-4B6165D-7V/2/e39a0295cc3aa883a860716d9bd57885

Background: The temporal and spatial control of the transition from vegetative to parasitic growth is critical to any parasite, but is essential to the sessile parasitic plants. It has been proposed that this transition in Striga spp. is controlled simply by an exuded oxidase that converts host cell-surface phenols into benzoquinones which act as developmental signals that mediate the transition. An understanding of this mechanism may identify the critical molecular events that made possible the evolution of parasitism in plants.Results: PoxA and PoxB are identified as the only apoplastic phenol oxidases in Striga asiatica seedlings, and the genes encoding them have been cloned and sequenced. These peroxidase enzymes are capable of oxidizing the 60 known inducing phenols into a small set of benzoquinones, and it is these quinones that induce parasitic development. Analysis of the reaction requirements and comparisons to host enzymes, however, lead us to argue that PoxA and PoxB are not necessary for host recognition.Conclusions: A new model is proposed where constitutive production of an activated oxygen species (in the case of Striga, H2O2) mediates host recognition. This strategy would allow a parasite to exploit abundant host enzymes to produce the diffusible recognition signals by converting a standard host defense into a parasitic offense.

Konz, D., A. Klens, et al. (1997). "The bacitracin biosynthesis operon of Bacillus licheniformis ATCC 10716: molecular characterization of three multi-modular peptide synthetases." Chemistry & Biology **4**(12): 927.

http://www.sciencedirect.com/science/article/B6VRP-4BWMKP8-12/2/f90f27a28b1c501808bd11ee5889b977

Background: The branched cyclic dodecylpeptide antibiotic bacitracin, produced by special strains of Bacillus, is synthesized nonribosomally by a large multienzyme complex composed of the three bacitracin synthetases BA1, BA2 and BA3. These enzymes activate and incorporate the constituent amino acids of bacitracin by a thiotemplate mechanism in a pathway driven by a protein template. The biochemical features of these enzymes have been studied intensively but little is known about the molecular organization of their genes.Results: The entire bacitracin synthetase operon containing the genes bacA-bacC was cloned and sequenced, identifying a modular structure typical of peptide synthetases. The bacA gene product (BA1, 598 kDa) contains five modules, with an internal epimerization domain attached to the fourth; bacB encodes BA2 (297 kDa), and has two modules and a carboxy-terminal epimerization domain; bacC encodes BA3, five modules (723 kDa) with additional internal epimerization domains attached to the second and fourth. A carboxy-terminal putative thioesterase domain was also detected in BA3. A putative cyclization domain was found in BA1 that may be involved in thiazoline ring formation. The adenylation/thioester-binding domains of the first two BA1 modules were overproduced and the detected amino-acid specificity coincides with the first two amino

acids in bacitracin. Disruption of chromosomal bacB resulted in a bacitracin-deficient mutant.Conclusions: The genes encoding the bacitracin synthetases BA1, BA2 and BA3 are organized in an operon, the structure of which reflects the modular architecture expected of peptide synthetases. In addition, a putative thiazoline ring formation domain was identified in the BA1 gene.

Waldron, C., P. Matsushima, et al. (2001). "Cloning and analysis of the spinosad biosynthetic gene cluster of Saccharopolyspora spinosa." Chemistry & Biology **8**(5): 487.

http://www.sciencedirect.com/science/article/B6VRP-4319MWC-8/2/9ea24535bf980bcce717d017b6b879af

Background: Spinosad is a mixture of novel macrolide secondary metabolites produced by Saccharopolyspora spinosa. It is used in agriculture as a potent insect control agent with exceptional safety to non-target organisms. The cloning of the spinosyn biosynthetic gene cluster provides the starting materials for the molecular genetic manipulation of spinosad yields, and for the production of novel derivatives containing alterations in the polyketide core or in the attached sugars.Results: We cloned the spinosad biosynthetic genes by molecular probing, complementation of blocked mutants, and cosmid walking, and sequenced an 80 kb region. We carried out gene disruptions of some of the genes and analyzed the mutants for product formation and for the bioconversion of intermediates in the spinosyn pathway. The spinosyn gene cluster contains five large open reading frames that encode a multifunctional, multi-subunit type I polyketide synthase (PKS). The PKS cluster is flanked on one side by genes involved in the biosynthesis of the amino sugar forosamine, in O-methylations of rhamnose, in sugar attachment to the polyketide, and in polyketide cross-bridging. Genes involved in the early common steps in the biosynthesis of forosamine and rhamnose, and genes dedicated to rhamnose biosynthesis, were not located in the 80 kb cluster.Conclusions: Most of the S. spinosa genes involved in spinosyn biosynthesis are found in one 74 kb cluster, though it does not contain all of the genes required for the essential deoxysugars. Characterization of the clustered genes suggests that the spinosyns are synthesized largely by mechanisms similar to those used to assemble complex macrolides in other actinomycetes. However, there are several unusual genes in the spinosyn cluster that could encode enzymes that generate the most striking structural feature of these compounds, a tetracyclic polyketide aglycone nucleus.

Chemosphere (3)

Conte, C., I. Mutti, et al. (1998). "DNA fingerprinting analysis by a PCR based method for monitoring the genotoxic effects of heavy metals pollution." Chemosphere **37**(14-15): 2739.

http://www.sciencedirect.com/science/article/B6V74-3VNHNVS-4/2/ecd803857bdc82b4492e56bf5af7c636

Environmental pollutants can have deleterious effects on living organisms. At high concentrations, or at high activities, they can cause acute toxicity damaging cells, tissues and organs. Chronic toxification, on the other hand, can also cause serious damage from bio-accumulation. Plants, as biological indicators, can measure both the actual and the potential effects of pollutants, when they are used to measure effects of heavy metals. We have applied a

system of "molecular fingerprinting" based on PCR (RAPD: Random Amplified Polymorphic DNA) to the evaluation of the genotoxic effects of heavy metals in order to estimate the environmental risk connected with their potential mutagenic effects in the model plant Arabidopsis thaliana, ecotype Columbia. Genomic DNA was utilised for RAPD analysis using random primers (10-mers). DNA from plants exposed to heavy metals solution displayed polymorphic bands which were not detectable in DNA of unexposed plants. The enhanced formation of RAPD polymorphisms was also observed in DNA of plant exposed in situ to an industrial pollution source. The comparison between "unexposed" and "exposed" genomes show that RAPD analysis can be used to evaluate how the environmental pollutants modify the structure of DNA in living organisms.

Londono, D. K., B. D. Siegfried, et al. (2004). "Atrazine induction of a family 4 cytochrome P450 gene in Chironomus tentans (Diptera: Chironomidae)." Chemosphere **56**(7): 701.

http://www.sciencedirect.com/science/article/B6V74-4BHCD8F-9/2/605a5ede2017d5b95e39d350347b8fd9

Cytochrome P450-dependent microsomal monooxygenase (P450) activity was measured in control and atrazine-exposed third instar midge larvae, Chironomus tentans. Significantly elevated O-demethylase activity was observed in gut homogenates taken from midges exposed to atrazine concentrations from 1 to 10 ppm for 90 h. No significant induction was observed at atrazine concentrations below 1 ppm. A region of a cytochrome P450 family 4 gene was amplified and sequenced from C. tentans larvae. Alignments of inferred amino acid sequences with other insect CYP4 gene homologues indicate a high degree of similarity. Northern blot analysis employing the CYP4 gene fragment as a probe showed an over-expression in C. tentans exposed to atrazine. The results support the previously identified inducibility of cytochrome P450-dependent activity and provide insight into the potential consequences of atrazine exposure to aquatic organisms.

Ma, Q. and K. T. Baldwin (2002). "A cycloheximide-sensitive factor regulates TCDD-induced degradation of the aryl hydrocarbon receptor." Chemosphere **46**(9-10): 1491.

http://www.sciencedirect.com/science/article/B6V74-44J69N3-1/2/ada0f8d5150b2f8aeeed9b9b49110220

Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology (1)

Minerds, K. L. and J. A. Donald (2001). "Natriuretic peptide receptors in the central vasculature of the toad, Bufo marinus." Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology **128**(2): 259.

http://www.sciencedirect.com/science/article/B6VNH-42D2CR1-8/2/00ac8deda34cc6939ca02776d319241d

Natriuretic peptide receptors in the central vasculature of the toad, Bufo marinus, were

characterized using autoradiographical, molecular, and physiological techniques. Specific 125I-rat ANP binding sites were present in the carotid and pulmonary arteries, the lateral aorta, the pre- and post-cava, and the jugular vein, and generally occurred in each layer of the blood vessel. The 125I-rat ANP binding was partially displaced by the specific natriuretic peptide receptor C ligand, C-ANF, which indicates the presence of two types of natriuretic peptide receptors in the blood vessels. This was confirmed by a RT-PCR study, which demonstrated that guanylyl cyclase receptor (NPR-GC) and NPR-C mRNAs are expressed in arteries and veins. An in vitro guanylyl cyclase assay showed that frog ANP stimulated the production of cGMP in arterial membrane fractions. Physiological recordings from isolated segments of the carotid and pulmonary arteries and the lateral aorta, which had been pre-constricted with arginine vasotocin, showed that rat ANP, frog ANP and porcine CNP relaxed the vascular smooth muscle with relatively similar potency. Together, the data show that the central vasculature contains two types of natriuretic peptide receptors (NPR-C and NPR-GC) and that the vasculature is a target for ANP and CNP.

Comparative Biochemistry and Physiology Part A: Physiology (1)

Ritchie, P. A., S. Lavoue, et al. (1997). "Molecular phylogenetics and the evolution of Antarctic notothenioid fishes." Comparative Biochemistry and Physiology Part A: Physiology **118**(4): 1009.

http://www.sciencedirect.com/science/article/B6T2P-3TGJRCW-4T/2/c94c96bea426368d19260277719e355c

The monophyly of the antarctic fish suborder Notothenioidei and the monophyly of its earliest family the Bovichtidae have been investigated with 12S and 16S mitochondrial DNA sequences. New data from Cottoperca, Pseudaphritis, Harpagifer and several outgroups, in addition to available sequences, show that the bovichtids are paraphyletic. Pseudaphritis is the sister group of all the non-bovichtid notothenioids. The same results are found from two independent genetic markers, the nuclear 28S rDNA and the 12S and 16S mitochondrial rDNA. This reliably refutes a previous hypothesis that placed Pseudaphritis as the sister group of all the remaining notothenioids (including Cottoperca and Bovichtus). Bootstrap analyses show that the Notothenioidei are monophyletic (although members of the suborder Trachinoidei have not been surveyed). Subsequent data from hemoglobin composition confirm the present relationships. After discussions between members of the European Science Foundation (ESF) network during its last two meetings, we point out here some fundamental aspects of comparative biology to improve understanding between the physiologist community and phylogeneticists. The most important points are differences in how the concept of homology is used and differences in the consideration of adaptation. When adaptation is evoked or questioned, endless speculations and untestable scenarios are often developed. We strongly advocate the use of phylogenetic trees for testing hypotheses of adaptation (through multiple character mapping). Such a "research program" in comparative biology has the power to improve knowledge because it can potentially lead to new experiments for testing adaptive hypotheses.

Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology (18)

Abe, Y., R. Nagata, et al. (2001). "Isolation, characterization and cDNA cloning of a one-lobed transferrin from the ascidianHalocynthia roretzi." Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology **128**(1): 73.

http://www.sciencedirect.com/science/article/B6T2R-43CCB4Y-8/2/f4b467e5f59b93266223439c30d00580

Transferrin was isolated from plasma of the ascidian Halocynthia roretzi by ion-exchange chromatography. The molecular weight of the plasma transferrin was determined to be 52K by SDS-polyacrylamide gel electrophoresis and gel filtration. Ascidian plasma transferrin was found to bind one mole of iron ion per mole of protein. The reductive S-pyridylethylated transferrin was subjected to Edman degradation analysis for determination of the N-terminal amino acid sequence, and it was also subjected to proteolytic fragmentation to yield peptide fragments, whose amino acid sequences were determined by Edman degradation analysis. Using the above amino acid sequences, a cDNA clone (1880 base pairs) encoding a protein of 372 amino acids containing a signal peptide of 21 amino acids was isolated from an H. roretzi hepatopancreas cDNA library. The reduced amino acid sequence contains the same sequences of the peptide fragments. A comparison of the amino acid sequence of ascidian transferrin with those of other members of the transferrin family revealed that the ascidian transferrin is composed of only the N-terminal lobe of two-lobed vertebrate transferrins. Thus, a one-lobed transferrin is present in the ascidian H. roretzi.

Ando, S., X.-H. Xue, et al. (1998). "Cloning and Sequencing of Complementary DNA for Fatty Acid Binding Protein from Rainbow Trout Heart." Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology **119**(1): 213.

http://www.sciencedirect.com/science/article/B6T2R-3WK3DWX-V/2/e9f40deab41fa0cf1d32538907f4dd39

A cDNA encoding a rainbow trout homologue of mammalian heart fatty acid binding protein (H-FABP) was isolated. The deduced protein sequence is 75% identical to that of rat H-FABP. The structural conservation of H-FABPs and their evolutionary relationship are discussed.

Bobe, J. and F. W. Goetz (2001). "Molecular cloning and expression of a TNF receptor and two TNF ligands in the fish ovary." Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology **129**(2-3): 475.

http://www.sciencedirect.com/science/article/B6T2R-43CCCV5-14/2/be8e449516bac67031dc4b60d0732a95

Using degenerative primers, partial cDNAs of a TNF (tumor necrosis factor) receptor and two TNF ligands were obtained by PCR of zebrafish and trout cDNAs, or cDNA libraries. These fragments were then used to screen cDNA libraries of appropriate tissues to obtain clones containing full coding sequences. A zebrafish cDNA was obtained that presumably codes for a 438 amino acid ovarian TNF receptor (OTR) that was identified as a death-domain-containing member of the TNF receptor family. On Northern blots, the OTR cDNA hybridized with a 3.4-kb transcript that is abundant in the zebrafish ovary but lightly detected in all other tissues tested. A zebrafish cDNA presumably coding for a 214 amino acid protein with sequence similarity to mammalian TRAIL (TNF-related apoptosis inducing ligand), was also isolated. In addition, a fragment of the brook trout TRAIL homologue was obtained. Finally, a full-length brook trout cDNA, that presumably codes for a 255 amino acid protein with sequence similarity to

mammalian TNF-alpha and lymphotoxin-alpha, was isolated. This study is the first report of a death-domain-containing TNF receptor and the first published report of a TNF ligand in fish.

Calduch-Giner, J. A., M. Mingarro, et al. (2003). "Molecular cloning and characterization of gilthead sea bream (Sparus aurata) growth hormone receptor (GHR). Assessment of alternative splicing." Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology **136**(1): 1.

http://www.sciencedirect.com/science/article/B6T2R-492702J-1/2/f1239be88db22df50e6a8b3da3305516

The full-length growth hormone receptor (GHR) of gilthead sea bream (Sparus aurata) was cloned and sequenced by RT-PCR and rapid amplification of 5'and 3'ends. The open reading frame codes for a mature 609 amino acid protein with a hydrophobic transmembrane region and all the characteristic motifs of GHRs. Sequence analysis revealed a 96 and 76% of amino acid identity with black sea bream (Acanthopagrus schlegeli) and turbot (Scophthalmus maximus) GHRs, respectively, but this amino acid identity decreases up to 52% for goldfish (Carassius auratus) GHR. By means of real-time PCR assays, concurrent changes in the hepatic expression of GHRs and insulin-like growth factor-I (IGF-I) was evidenced. Moreover, their regulation occurred in conjunction with the summer spurt of growth rates and circulating levels of GH and IGF-I. Search of alternative splicing was carried out exhaustively for gilthead sea bream GHR, but Northern blot and 3' RACE failed to demonstrate the occurrence of short alternative messengers. Besides, RT-PCR screening did not reveal deletions or insertions that could lead to alternative reading frames. In agreement with this, cross-linking assays only evidenced two protein bands that match well with the size of glycosylated and non-glycosylated forms of the full-length GHR. If so, it appears that alternative splicing at the 3'end does not occur in gilthead sea bream, although different messengers for truncated or longer GHR variants already exist in turbot and black sea bream, respectively. The physiological relevance of this finding remains unclear, but perhaps it points out large inter-species differences in the heterogeneity of the GHR population.

Donald, K. M., A. J. Day, et al. (2003). "Quantification of gene transcription and enzyme activity for functionally important proteolytic enzymes during early development in the Pacific oyster Crassostrea gigas." Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology **136**(3): 383.

http://www.sciencedirect.com/science/article/B6T2R-49F9WVK-2/2/69a2bd20bd9edf34e1ebeee08166d01a

Gene transcripts and enzyme activities were quantified for a selection of functionally important aminopeptidases at 2-day intervals throughout the first 72 days of development in the Pacific oyster Crassostrea gigas. Leucine aminopeptidase (LAP) and cathepsin B (CathB) gene transcripts were quantified using fluorogenic ('real time') PCR. LAP and CathB gene transcripts were detected at all time points. The proportion of CathB transcripts remained essentially constant and low throughout development (CtCtCt~23). CathB and cathepsin D (CathD) enzyme activities were measured biochemically. Whilst CathD activity peaked at day 19, LAP and CathB activities both peaked at day 24. The closely coupled increase in transcript and enzyme activity for LAP indicates regulation at the transcriptional level. Alternatively, the peak in enzyme activity for CathB without enhanced transcriptional activity suggests post-transcriptional regulation. Similar mechanisms of regulation for LAP and CathB have been observed in both plants and mammals, indicating widespread conservation.

Funkenstein, B. (2001). "Developmental expression, tissue distribution and hormonal regulation of fish (Sparus aurata) serum retinol-binding protein." Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology **129**(2-3): 613.

http://www.sciencedirect.com/science/article/B6T2R-43CCCV5-1N/2/e54d30dade81f9454ccf3c89fc94a1e1

Retinol-binding protein (RBP) is the specific carrier of retinol in vertebrates and forms a 1:1 complex with transthyretin (TTR). A cDNA encoding serum RBP was cloned from liver and 7-day larvae of the marine fish Sparus aurata. The mature protein is 176 amino acids long and shows sequence identity of 77-78%, 56%, 63% and 62% with rainbow trout, Xenopus, chicken and human RBP, respectively. Northern blot analysis of hepatic RBP revealed two transcripts: a major one of approximately 1.4-1.5 kb and a minor of approximately 0.7 kb. Distribution of RBP mRNA in various tissues was studied by RT-PCR and showed high expression in liver and skin, and low expression in brain, kidney and gill filament (20-35% of the level in liver). RBP expression in intestine, pyloric caeca, muscle and pituitary was estimated to be approximately 7-14% of the level in liver. The ontogeny of RBP expression in S. aurata was examined in unfertilized eggs, embryos and larvae by using RT-PCR followed by hybridization with a specific probe. RBP transcript was found in all larval stages studied. Very low levels of RBP mRNA were detected in unfertilized eggs and in embryos 8 h after fertilization with a gradual increase at 12 h and 15-16 h post-fertilization. A single injection of estradiol-17[beta] to S. aurata immature, bisexual fish or to adult males reduced steady-state levels of hepatic RBP by 37 and 25%, respectively. The same treatment induced vitellogenin expression. The present data suggest that in fish, liver is the main site of RBP synthesis, but that RBP may have an important function in fish skin. RBP is expressed early in embryonic development and in fish its expression can be down regulated by estrogen.

Goetz, F. W., B. Norberg, et al. (2004). "Characterization of the cod (Gadus morhua) steroidogenic acute regulatory protein (StAR) sheds light on StAR gene structure in fish." Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology **137**(3): 351.

http://www.sciencedirect.com/science/article/B6T2R-4C1C7TW-5/2/748edb742a95c13624b924707b738014

The full-length cDNA for the cod (Gadus morhua) StAR was cloned by RT-PCR and library screening using ovarian RNA. From the library screening, 2 size classes of cDNA were obtained; a 1577 bp cDNA (cStAR1) and a 2851 bp cDNA (cStAR2). The cStAR1 cDNA presumably encodes a protein of 286 amino acids. The cStAR2 cDNA was composed of 6 separated sequences that contained all of the coding regions of cStAR1 when added together, but also contained 5 noncoding regions not observed in cStAR1. Polymerase chain reactions of cod genomic DNA produced products slightly larger than cStAR2. The sequence of these products were the same as cStAR2 but revealed one additional noncoding region (intron). Thus, the fish StAR gene contains the same number of exons (7) and introns (6) as observed in mammals, but is approximately half the size of the mammalian gene. Using Northern analysis and RT-PCR, cStAR1 expression was observed only in testes, ovaries and head kidneys. Polymerase chain reaction products were also observed using cDNA from steroidogenic tissues and primers designed to regions specific for cStAR2, indicating that cStAR2 is expressed in tissues and may account for the presence of larger transcripts observed on Northern blots.

Hakansson, K., C. Huh, et al. (1996). "Mouse and rat cystatin C: Escherichia coli production, characterization and tissue distribution." Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology **114**(3): 303.

http://www.sciencedirect.com/science/article/B6T2R-3W3NDKC-W/2/99a55ea93e847ee6dacf2dd6044886b9

Recombinant mouse (Mus musculus) and rat (Rattus norvegicus) cystatin C were produced by expression in Escherichia coli, isolated and functionally characterized. The mouse and rat inhibitors were both fully active in titrations of papain. Determination of equilibrium constants for dissociation (Ki) for their complexes with the target proteinase, cathepsin B, produced values not largely different from that for human cystatin C (Ki 0.07-0.13 nM). Rabbit antisera against mouse and rat cystatin C were produced and used for improved affinity purification of the recombinant inhibitors. Affinity purified immunoglobulins isolated from the antiserum against mouse cystatin C were used for construction of a sensitive enzyme-linked immunosorbent assay. The assay was used to demonstrate a high degree of immunological cross-reactivity between mouse and rat cystatin C and could be used for cystatin C quantification in mouse and rat tissue homogenates. All tissues analyzed contained cystatin C, with a relative content very similar to that of human tissues. For all species, brain tissue contained the highest cystatin C amounts and liver the lowest, whereas kidney, spleen and muscle tissues were intermediate in content. In the mouse, a notable high cystatin C content in parotid gland tissue was observed. The high degree of similarity in distribution pattern and functional properties for mouse, rat and human cystatin C indicates that: a murine model should be relevant for studies of the human disease, hereditary cystatin C amyloid angiopathy.

Hunt, J. L. and P. Licht (1998). "Identification and structural characterization of a novel member of the vitamin D binding protein family." Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology **121**(4): 397.

http://www.sciencedirect.com/science/article/B6T2R-3VB3FC1-P/2/56a977ba39055dc2a05bd8fe885e8472

The apparent high degree of homology of a blood protein with a unique dual binding affinity for two distinct hormones, thyroxin (T4) and vitamin D, isolated from a turtle, Trachemys scripta (Family Emydidae) and mammalian vitamin D binding protein (DBP) prompted further interspecific comparison to better understand the structure of functional binding sites. Using polymerase-chain reaction (PCR) with primers derived from the putative nucleotide sequences encoding peptides from the degradation of the T. scripta protein, we cloned the cDNA. The mature turtle protein contains 466 amino acids, about eight residues more than in mammalian DBP. The nucleotide sequence of the coding region showed 63% nucleotide and 73% amino acid homology ([ap]53% identity) to mammalian DBP (human, rat, mouse, and rabbit). However, there was no significant homology to mammalian T4-binding globulin (TBG) or transthyretin (TTR). Comparisons with mammals help define further the requirements for the vitamin D and actin binding sites. Northern blots of RNA isolated from turtle tissue probed with the 5' portion of cDNA established expression of the transcript in liver, kidney, and brain (in order of abundance), in contrast to mammal sequences in which expression of DBP is largely confined to the liver.

Jimbo, M., T. Yanohara, et al. (2000). "The -galactose-binding lectin of the octocoral Sinularia lochmodes: characterization and possible relationship to the symbiotic dinoflagellates." Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology **125**(2): 227.

http://www.sciencedirect.com/science/article/B6T2R-408C90M-B/2/0fb8ffdda4a4edc36f0590808aec1644

A -galactose binding lectin (SLL-2) was isolated from Sinularia lochmodes, an octocoral, by a

combination of affinity chromatography on acid-treated agarose and FPLC on Superdex 200. SLL-2 agglutinated rabbit and horse erythrocytes while SLL-1, a minor component, reacted only with rabbit erythrocytes. SLL-2 is a glycoprotein with a molecular mass of 122 kDa and is composed of eight identical subunits (15 kDa). The sequence of the amino terminal region of SLL-2 did not show any apparent homology to the sequences of other animal and plant lectins. -Galactose, N-acetyl--galactosamine, lactose, and melibiose were moderate inhibitors to the agglutination of rabbit erythrocytes. In contrast, horse erythrocytes were much more susceptible to agglutination by SLL-2, which was inhibited by sugars and glycoproteins such as -galactose, N-acetyl--galactosamine, lactose, melibiose, and porcine stomach mucin. SLL-2 showed considerable tolerance to heating and kept its activity after heating at 80[deg]C for 60 min. In immuno-histochemical studies using an anti-SLL-2 antiserum and protein A gold conjugate, SLL-2 was found to be present in high amounts in the nematocysts. SLL-2 was also detected on the surface of symbiotic dinoflagellate, Symbiodinium sp. cells irrespective whether they were surrounded with or without host cells. These observations suggest the presence of lectin-mediated interaction between symbiotic dinoflagellates and S. lochmodes.

Johansen, K. A. and K. Overturf (2005). "Sequence, conservation, and quantitative expression of rainbow trout Myf5." Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology **140**(4): 533.

http://www.sciencedirect.com/science/article/B6T2R-4F3NXS3-3/2/0ac4607b746af4f4dc958a1622ada9eb

The success of rainbow trout as an aquaculture species is dependent on the ability to produce fish with large amounts of high-quality lean muscle. It is therefore important to understand not only the best conditions under which to raise the fish but also the molecular control of muscle growth. Vertebrate muscle growth is initiated by the specification of myogenic precursor cells into myoblasts. The myoblasts proliferate and fuse to form multinucleated myotubes, which mature into myofibers. A family of basic helix-loop-helix (bHLH) transcription factors, the Myogenic Regulatory Factors (MRFs), controls these events. In trout, two MRF-encoding genes, TMyoD (of which there are two) and Tmyogenin, have been identified. However, the primary MRF-encoding Myf5 is not yet sequenced. Here, using degenerate PCR and 5' and 3' RACE, the cDNA sequence of trout Myf5 (TMyf5) is identified. Translation of the cDNA reveals that TMyf5 is a bHLH protein with homology to Myf5 and MRFs in other organisms. It is expressed mainly in red and white muscle, suggesting that it shares functional homology to Myf5 in other species. The molecular control of muscle growth has been well-characterized in mammals, but there are differences in the growth of fish muscle, highlighting the need for characterization of MRFs in fish species, particularly those in which understanding muscle growth will have a positive impact on the economic potential of the species.

Kim, I., G. Y. Koh, et al. (1998). "Identification of Alternatively Spliced Na+-Ca2+ Exchanger Isoforms Expressed in the Heart." Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology **119**(1): 157.

http://www.sciencedirect.com/science/article/B6T2R-3WK3DWX-K/2/55412fd61ddae0a1e58e2b6edf5e2d96

Alternatively spliced isoforms of Na+-Ca2+ exchanger were found from various tissues and species. RT-PCR amplification was performed on the basis of our cloned mouse cardiac Na+-Ca2+ exchanger and four alternatively spliced isoforms of Na+-Ca2+ exchanger were identified. Three (NCX1.3, NCX1.4, and NCX1.12) of them were first identified in the heart, and one isoform (NCX1.12) was a novel spliced variant. These four spliced variants were present in the embryonic

and adult atria and ventricles. Different cell types of the heart expressed different spliced isoforms of Na+-Ca2+ exchanger. Southern blot analysis indicated that the Na+-Ca2+ exchanger gene existed as a single copy in the mouse genome. Thus, the Na+-Ca2+ exchanger isoforms expressed in mouse heart are consistent with being produced by alternative splicing and they may have different functions in various cell types in the mouse heart.

MacKenzie, S., C. P. Cutler, et al. (2002). "The effects of dietary sodium loading on the activity and expression of Na, K-ATPase in the rectal gland of the European Dogfish (Scyliorhinus canicula)." Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology **131**(2): 185.

http://www.sciencedirect.com/science/article/B6T2R-450012B-7/2/03a58f520c19a6a41d76515b54b6f62a

cDNA fragments of both the [alpha]- and [beta]-subunits of the Na, K-ATPase and a cDNA fragment of the secretory form of Na-K-Cl cotransporter from the European dogfish (Scyliorhinus canicula) were amplified and cloned using degenerate primers in RT-PCR. These clones were used along with a sCFTR cDNA from the related dogfish shark, Squalus acanthias to characterise the expression of mRNAs for these ion transporters in the dogfish rectal gland subsequent to an acute feeding episode. Following a single feeding event where starved dogfish were fed squid portions (20 g squid/kg fish), there was a delayed and transient 40-fold increase in the activity of Na, K-ATPase in crude rectal gland homogenates. Increases in enzyme activity were apparent 3 h after the feeding event and peaked at 9 h before returning to control values within 24 h. These increases in activity were accompanied by small and transient decreases in plasma sodium and chloride concentrations lasting up to 3 days. Significant increases in the expression of mRNAs for [alpha]- and [beta]-subunits of the Na, K-ATPase, the Na-K-Cl cotransporter and CFTR chloride channel were detected but not until 1-2 days after the feeding event. It is concluded that the transient increase in Na, K-ATPase activity is not attributable to increases in the abundance of [alpha]- and [beta]-subunit mRNAs but must be associated with some, as yet unknown, post-transcriptional activation mechanism.

Murayama, E., A. Okuno, et al. (2000). "Molecular cloning and expression of an otolith matrix protein cDNA from the rainbow trout, Oncorhynchus mykiss." Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology **126**(4): 511.

http://www.sciencedirect.com/science/article/B6T2R-40X8D55-W/2/e48e74efee413fde726ed820a895a84b

The fish otolith is a hard tissue consisting of calcium carbonate and organic matrices. The matrix proteins play important roles in otolith formation, but little is known about the nature of these proteins. In this study, matrix proteins were extracted from the otoliths of rainbow trout, Oncorhynchus mykiss, and chum salmon, Oncorhynchus keta. EDTA-soluble matrix proteins were separated by SDS-PAGE, revealing two major components in the otoliths of both species with apparent molecular masses of 55 and 43 kDa. N-terminal and some internal amino acid sequences of the 55-kDa otolith matrix protein were determined. A cDNA fragment encoding this protein of O. mykiss was amplified by reverse transcription PCR using two degenerate primers designed from the amino acid sequences. A cDNA encoding this protein was obtained by screening a saccular cDNA library using the amplified cDNA fragment as a probe. Nucleotide sequence analysis revealed that the cDNA clone has a sequence of 2.5 kb and the open reading frame encoding 344 amino acid residues. Northern blot analysis showed that mRNA of this protein is expressed specifically in the sacculus, and consistently during the day.

Orino, K., K. Eguchi, et al. (1997). "Sequencing of cDNA Clones that Encode Bovine Ferritin H and L Chains." Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology **118**(3): 667.

http://www.sciencedirect.com/science/article/B6T2R-3VBB5X0-W/2/80f949892e87f7788956e1f831490183

The molecular weight of the liver-type subunit (L) of bovine ferritin is much larger than that of the heart-type subunit (H) as determined by SDS-PAGE (L, 20.5 kDa; H, 18.4 kDa). The migration of these two subunits on SDS-PAGE gels, relative to each other, is opposite to that reported for ferritin L and H subunits in other mammalian species (L, 19 kDa; H, 21 kDa). To determine the cause of this anomaly, full-length cDNA clones of the bovine L and H chains were isolated from a bovine spleen [lambda] gt11 cDNA library and sequenced. The amino acid sequences of the L and H chains of bovine ferritin, deduced from their cDNA sequences, contained open reading frames coding for 174 and 180 amino acid residues with calculated molecular weights of 19,856 and 20,920 Da, respectively. The deduced amino acid sequence of the L chain shows 86%, 84%, 87%, 83% and 83% homology with the amino acid sequences of horse, human, rabbit, rat and mouse L chains, respectively. The H chain displays a higher homology with the human, rat and mouse H chains (91%, 92% and 93%, respectively). In addition, the bovine L chain did not contain the extra octapeptide present in rodent L chains, and bovine L and H chains did not react with concanavalin A. The bovine L and H chains expressed using a baculovirus expression system showed almost the same mobilities as those of bovine spleen ferritin, respectively, by SDS-PAGE. These results suggest that the much slower mobility of the bovine L chain compared with other mammalian L chains on SDS-PAGE cannot be attributed to insertion(s) of amino acid(s) or peptide(s) into the L chain, to the deletion(s) of them of it or to the addition of carbohydrate chain(s) but may result from significant differences in the binding affinity of SDS for bovine ferritin L chains.

Tsunemoto, K., K. Osatomi, et al. (2004). "Molecular characterization of cathepsin L from hepatopancreas of the carp Cyprinus carpio." Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology **137**(1): 107.

http://www.sciencedirect.com/science/article/B6T2R-4B945XP-5/2/4f62be2bb1b204ec6710890f3553012c

Purified cathepsin L from carp, Cyprinus carpio, consists of a 28 kDa single-chain form that is different from the 24 and 5 kDa mammalian two-chain form. We cloned cathepsin L from carp hepatopancreas. The sequence consisted of a 1490 bp cDNA and a 1014 bp open reading frame, encoding a deduced protein of 337 amino acids that is likely processed to an active enzyme (single-chain form) with 222 amino acids. Its similarity to other types of vertebrate cathepsin L is less than 69%. Mammalian cathepsin L is further processed to a two-chain form, but possibly this is not the case with carp cathepsin L: the P1 site where cleavage occurred in the two-chain form of mammalian cathepsin L contains a serine, while carp cathepsin L processes a valine. Therefore, carp cathepsin L may have a different mechanism of action from mammalian cathepsin L.

Tsuzuki, S., T. Masuta, et al. (1997). "Structure and Expression of Bombyxin E1 Gene: A Novel Family Gene that Encodes Bombyxin-IV, an Insect Insulin-Related Neurosecretory Peptide." Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology **117**(3):

409.

http://www.sciencedirect.com/science/article/B6T2R-3SG3M4Y-C/2/2b8887aac95a7610c5421163d6d81d21

A bombyxin gene encoding precursor molecule for bombyxin-IV, one of the insulin-related neurosecretory peptide of the silkmoth Bombyx mori, has been cloned and characterized. The nucleotide sequence of this gene and its deduced amino acid sequence deviate moderately from those characterized previously for the family A, B, C and D bombyxin genes. The gene encoding the bombyxin-IV precursor was therefore defined into a novel family E and designated as gene E1. The bombyxin E1 transcript in Bombyx brain was shown to locate in four pairs of medial neurosecretory cells, which also produce other bombyxin family mRNAs, and the amount of the E1 transcript did not change markedly during the fifth larval instar. Genomic Southern hybridization indicated that the Bombyx haploid genome contained a single copy of the bombyxin family E gene.

Young Choi, C. and A. Takemura (2001). "Molecular cloning and expression of connexin 32.3 cDNA in the ovary from the red seabream (Pagrus major)." Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology **129**(4): 767.

http://www.sciencedirect.com/science/article/B6T2R-43C5N6W-7/2/119b6f4d4206747868e776d22def314a

This study examined connexin (Cx) gene activity in relation to oocyte maturation in the red seabream (Pagrus major) ovary. Mixed primers for the polymerase chain reaction (PCR) were designed based on the high sequence homology of selected regions of known Cx genes. PCR-amplified cDNA fragments generated by 3' and 5' rapid amplication of cDNA ends (RACE) were combined to generate full-length cDNA sequences. The 1212-bp cDNA has an open reading frame encoding 282 amino acids, with a molecular mass of 32.3 kDa (red seabream Cx32.3). Hydropathy plots of red seabream Cx32.3 show the four typical major hydrophobic and four major hydrophilic regions of Cx proteins. Typical Cx consensus sequences are observed in the first and second extracellular loops. The ovarian follicles of matured female seabream were incubated in the presence of 17[alpha],20[beta]-dihydroxy-4-pregnen-3-one (DHP, 10 ng/ml), gonadotropin (GtH)-I (300 ng/ml) and GtH-II (300 ng/ml). Northern blot analysis of poly(A)+ RNA extracted from the ovarian follicles were hybridized with red seabream Cx32.3 and [beta]-actin probes. The transcription level of PmCx32.3 in the presence of DHP, PmGtH-I and PmGtH-II was significantly higher than in the control.

Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology (1)

Minerds, K. L. and J. A. Donald (1997). "Lack of evidence for functional natriuretic peptide receptors in the heart of the cane toad, Bufo marinus." Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology **118**(2): 233.

http://www.sciencedirect.com/science/article/B6T2S-41TNDY4-M/2/de2b1b2c98a1101b2ea4d72f106948ae

Several studies have shown that the heart of species from each vertebrate class contains natriuretic peptide binding sites which suggests that ANP released from the heart may act in a paracrine/autocrine fashion. The present study used a set of techniques to study cell surface receptors in order to investigate the presence and nature of NPRs in the heart of the cane toad, Bufo marinus. Autoradiographical studies of both atria and ventricle showed no variation between total and non-specific binding, indicating a lack of NP binding sites in these tissues. This was confirmed with in vitro binding studies in which increasing concentrations of ANP did not compete for any specific binding. Increasing concentrations of ANP did not increase cGMP generation and physiological experiments showed that both ANP and CNP had no effect on the force or rate of contraction of a sino-atrial preparation. Molecular expression studies, however, showed that mRNA for NPRs was expressed in the heart, in spite of the lack of evidence for NPR on the cell surface. Overall, this study showed that no functional NPRs are present in the heart, and provided evidence that the heart is not a target organ for NP action in B. marinus.

Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology (5)

Heater, S. J., J. L. P. Oehlers, et al. (2004). "DNA polymerase [beta] mRNA and protein expression in Xiphophorus fish." Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology **138**(3): 325.

http://www.sciencedirect.com/science/article/B6W89-4DF44HD-2/2/6f135f2e4df4ae8fd87d138a0e0fef67

Herein we report Xiphophorus DNA polymerase [beta] (XiphPol[beta]) mRNA and protein expression levels in brain, liver, gill, and testes tissues from Xiphophorus maculatus, Xiphophorus helleri, and Xiphophorus couchianus parental line fish and two different tumor-bearing Xiphophorus interspecies hybrids. Polymerase [beta] protein levels in the Xiphophorus tissues were measured by Western blot, and mRNA was measured with a quantitative real time RT-PCR method which employed cRNA construction to produce accurate calibration curves. We found significant differences in both mRNA and protein levels between the tumor-bearing hybrid animals and the three parental species. However, there were no significant differences in either mRNA levels or protein expression observed between the parental species. Thus, interspecies hybridization results in dysregulation of Pol[beta] expression and this may manifest a modulation in DNA repair capability and susceptibility to latent tumorigenesis.

Luo, Q., M. Ban, et al. (2005). "Distinct effects of 4-nonylphenol and estrogen-17[beta] on expression of estrogen receptor [alpha] gene in smolting sockeye salmon." Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology **140**(1): 123.

http://www.sciencedirect.com/science/article/B6W89-4FHJYDV-3/2/81949daa546c8b1c2f675d423f072e88

Xenoestrogens such as 4-nonylphenol (4-NP) have been shown to affect the parr-smolt transformation, but their mechanisms of action are not known. We therefore examined effects of 4-NP and estradiol-17[beta] (E2) on expression of estrogen receptor (ER) [alpha] gene in the liver, gill, pituitary and brain of sockeye salmon to elucidate molecular mechanisms of 4-NP and E2 and developmental differences in response during smolting. Fish were treated twice within a

week with 4-NP (15 and 150 mg/kg BW), E2 (2 mg/kg BW) or only vehicle at three stages of smolting, pre-smolting in March, early smolting in April and late smolting in May. The absolute amounts of ER[alpha] mRNA were determined by real-time PCR. The basal amounts of ER[alpha] mRNA peaked in April in the liver, gill and pituitary. In March, E2 extensively increased the amounts in the liver, while 4-NP had no effects at this stage. In contrast, 4-NP (but not E2) decreased liver ER[alpha] mRNA in April. 4-NP also decreased the amount of ER[alpha] mRNA in the gill in April. In the pituitary, 4-NP increased ER[alpha] mRNA in March but decreased it in May. There were no significant effects in the brain. Changes in basal ER[alpha] mRNA observed in this study indicate that estrogen responsiveness of tissues may change during salmon smolting. Furthermore, 4-NP and E2 have different effects on expression of ER[alpha] gene in the liver and gill during smolting, and the response is dependent on smolt stage.

Spencer, F., L. Chi, et al. (2001). "Temporal relationships among uterine pituitary adenylate cyclase-activating polypeptide, decidual prolactin-related protein and progesterone receptor mRNAs expressions during decidualization and gestation in rats." Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology **129**(1): 25.

http://www.sciencedirect.com/science/article/B6W89-437B3GV-3/2/0fff593c4ee66675e08951a6a2a49174

Pituitary adenylate cyclase-activating peptide (PACAP), a novel compound with vasoactive intestinal polypeptide-like activity, was recently shown to be localized in the neuronal endings of the human uterus. The purpose of the present study was to assess the functional presence of PACAP mRNA in the decidual endometrium and its relationship to the expression levels of decidual prolactin-related protein (dPRP) and the progesterone receptor mRNAs during decidualization and pregnancy in Sprague-Dawley rats. PACAP was constitutively and temporally expressed in the decidual endometrium and gravid uterus. The time-dependent correlated expression levels of PACAP, dPRP and the progesterone receptor were induced by the neurogenic reproductive signals, i.e. the vagino-cervical/deciduogenic stimuli of decidualization and by the normal equivalent stimuli of mating/blastocyst implantation of gestation. Correlation among the mRNA expression levels of PACAP, dPRP and the progesterone receptor and the coordinated inhibitory actions of the anti-progesterone (RU-486) suggest that there is also correlated time-dependent steroid regulation of the mRNA levels of PACAP, dPRP and the progesterone receptor in the decidual and pregnant uteri. One possible functional meaning for the time-related localization of endometrial/uterine PACAP could be to facilitate endometrial blood flow and increase the availability of metabolic substrates to the developing deciduoma or embryo. The study demonstrates the potential importance of PACAP expression in the regulation of the maternal feto-placental component and suggests a prominent reproductive role for the neuropeptide in mammalian pregnancy.

Yada, T. and T. Azuma (2002). "Hypophysectomy depresses immune functions in rainbow trout." Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology **131**(1): 93.

http://www.sciencedirect.com/science/article/B6W89-44W8K9Y-9/2/3d153c0a173d0a50b441ba4e10b19e0a

As the immune system is known to be influenced by the endocrine system, the effects of hypophysectomy on immune functions were examined in the rainbow trout (Oncorhynchus mykiss). Superoxide anion (O2-) production, accompanied by phagocytosis, was significantly decreased in leucocytes isolated from the head kidney 7 days after hypophysectomy. Significant reduction was also observed in plasma immunoglobulin (Ig) M levels, whereas no change was observed in plasma lysozyme activity. The number of Ig-secreting leucocytes in peripheral blood

had decreased after hypophysectomy, although total leucocyte number was not affected. The percentage of Ig-producing leucocytes as assessed by flow cytometry using a monoclonal antibody to trout IgM showed significant reduction in the head kidney. However, hypophysectomy did not affect the number of Ig-producing leucocytes in spleen, thymus or peripheral blood. By RT-PCR, expression of two growth hormones (GH I and II) and prolactin (PRL) mRNA was detected in lymphoid tissues, such as head kidney, spleen, thymus and intestine, as well as in leucocytes from blood and head kidney, indicating the local production of these hormones. These results indicate important roles of hypophyseal hormones produced not only in the pituitary, but also in the lymphoid tissues, in the maintenance of the immune functions in trout.

Yada, T., K. Muto, et al. (2004). "Effects of prolactin and growth hormone on plasma levels of lysozyme and ceruloplasmin in rainbow trout." Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology **139**(1-3): 57.

http://www.sciencedirect.com/science/article/B6W89-4DK64SB-2/2/d528a4ffad09aaf8d23eec43339d3c1d

In vivo and in vitro effects of prolactin (PRL) and growth hormone (GH) on plasma levels of lysozyme and ceruloplasmin were examined in the rainbow trout (Oncorhynchus mykiss). Hypophysectomy had no effect on the plasma lysozyme level. Implantation of PRL- or GH-containing cholesterol pellets increased the lysozyme level in a dose-related manner. After hypophysectomy and sham operation, plasma ceruloplasmin was elevated above the level in intact fish, suggesting inflammation caused by the surgery. PRL or GH treatment significantly attenuated the increased level of ceruloplasmin in the operated fish. Expression of lysozyme mRNA was detected in the leucocytes isolated from the peripheral blood by RT-PCR. In vitro administration of PRL or GH showed no effect on the proliferation of isolated leucocytes or on the total protein content; however, lysozyme activity in the medium increased in a dose-related manner. These results suggest that PRL and GH directly stimulate lysozyme production without affecting the proliferation of leucocytes, and the attenuated ceruloplasmin level increased in response to inflammation.

Comparative Immunology, Microbiology and Infectious Diseases (7)

Baloda, S. B., K. Krovacek, et al. (1995). "Detection of aerolysin gene in Aeromonas strains isolated from drinking water, fish and foods by the polymerase chain reaction." Comparative Immunology, Microbiology and Infectious Diseases **18**(1): 17.

http://www.sciencedirect.com/science/article/B6T5H-3Y6PFXV-2S/2/e5089bb3dd74d67f490fb1955627b0db

A polymerase chain reaction (PCR) technique was used to assay the presence of the aerolysin gene in a total of 89 Aeromonas hydrophila and A. sobria strains isolated from drinking water, fish and foods. These strains were also characterized for the production of virulence factors such as haemolysin, protease and cytotoxin. The primers used in the PCR targeted a 209-bp fragment of the aer gene coding for the [beta]-haemolysin and detected template DNA only in haemolytic A. hydrophila strains. The cell-free culture supernatants of these aerolysin-positive A. hydrophila strains were also cytotoxic to the HeLa and McCoy cells. The haemolytic A. sobria and non-

haemolytic A. hydrophila were consistently negative in the PCR assay. Primer specificity was determined in the PCR by using a control haemolytic Escherichia coli, Streptococcus pyogenes and a restriction endonuclease assay. The PCR clearly identified the aerolysin-producing strains of A. hydrophila and may have application as a rapid species-specific virulence test.

Foley, J. E., C. M. Leutenegger, et al. (2003). "Evidence for modulated immune response to Anaplasma phagocytophila sensu lato in cats with FIV-induced immunosuppression." Comparative Immunology, Microbiology and Infectious Diseases **26**(2): 103.

http://www.sciencedirect.com/science/article/B6T5H-469W2K8-4/2/3e0dec198f147d0e00f2156417e3187c

Gonzalez-Rey, C., A. M. Belin, et al. (2003). "RAPD-PCR and PFGE as tools in the investigation of an outbreak of beta-haemolytic Streptococcus group A in a Swedish hospital." Comparative Immunology, Microbiology and Infectious Diseases **26**(1): 25.

http://www.sciencedirect.com/science/article/B6T5H-45XR89W-1/2/b836789fd99cbcd03f053aba3ffce5b8

Osek, J., P. Gallien, et al. (1999). "The use of polymerase chain reaction for determination of virulence factors of Escherichia coli strains isolated from pigs in Poland." Comparative Immunology, Microbiology and Infectious Diseases **22**(3): 163.

http://www.sciencedirect.com/science/article/B6T5H-3WK3RT6-1/2/086df03f6f7877d35002768d34d91081

E. coli strains isolated from pigs with postweaning diarrhea or edema disease were tested by phenotypic and genotypic methods for the presence of virulence antigens and genes, respectively. The slide agglutination and ELISA analyses were used for determination of F4, F5, F6, F17, and F41 fimbriae whereas the prevalence of fimbrial fedA and toxin eltI, estI, estII, stx1, stx2 and stx2e genes were recorded by the means of PCR. Only F4 antigen (ac variant) was found in strains of the serogroup O149:K91 isolated from pigs with diarrhea. PCR analyses showed that the fedA gene encoding F18 fimbriae was present in 61,9% of strains isolated from pigs with diarrhea and in 84.2% of strains isolated from pigs with edema disease. The eltI genes encoding heat-labile toxin I (LTI) were present only in 9 out of 21 strains recovered from pigs with diarrhea. Shiga toxin 2 variant (stx2e) genes were found in six isolates from edema disease and also in one strain from diarrhea. The PCR test used in the study was a sensitive and valuable method for determination of virulence factors of E. coli strains.

Tempesta, M., M. Camero, et al. (2004). "Experimental infection of goats at different stages of pregnancy with caprine herpesvirus 1." Comparative Immunology, Microbiology and Infectious Diseases **27**(1): 25.

http://www.sciencedirect.com/science/article/B6T5H-487MTS9-2/2/dfdeccb3524b2dd63e79c83ff5c24d27

Three goats from a group of five caprine herpesvirus 1 (CpHV.1) seronegative pregnant goats

were inoculated intranasally with a virulent BA.1 strain of CpHV.1. Goat n.1 was infected on day 45 of pregnancy, goat n.2 on day 92 and goat n.3 on day 127. Each of the three goats produced a single foetus 10-60 days after infection. Foetus n.1 was never found and so it could not be examined for virological findings. Goat n.2 delivered at term of gestation and CpHV.1 was detected by PCR and isolated from most of the foetal organs. Foetus n.3 was partially autolysed and the virus was only detected by PCR but not isolated from foetal organs. The results confirm the damaging effect of CpHV.1 infection on pregnancy, the difficulty in diagnosing the CpHV.1 induced abortion, and the importance developing appropriate prophylactic programmes.

Tempesta, M., A. Pratelli, et al. (1999). "A preliminary study on the pathogenicity of a strain of caprine herpesvirus-1." Comparative Immunology, Microbiology and Infectious Diseases **22**(2): 137.

http://www.sciencedirect.com/science/article/B6T5H-3VM10K4-5/2/80a63d5b374e1b8020fd7df3bbc242de

Yokoyama, E., Y. Shibusawa, et al. "Influence of bacteriocin-like substance, generation times, and genetic profiles of Listeria innocua on the isolation of Listeria monocytogenes." Comparative Immunology, Microbiology and Infectious Diseases **In Press, Corrected Proof**
http://www.sciencedirect.com/science/article/B6T5H-4FNP2J7-1/2/f06a8f7885998b75b7742391a6a765f7

Inhibition of isolation of Listeria monocytogenes by bacteriocin-like substance (BLS)-producing Listeria innocua after enrichment culture was investigated. When 26 L. monocytogenes strains were examined in combination with eight L. innocua strains using the spot on lawn method, 52/208 (25.0%) combinations showed the growth inhibition of L. monocytogenes. When two Listeria species were cultured simultaneously in selective enrichment broth, inhibition of isolation of L. monocytogenes was observed in 12/52 of the combinations at 24 h (23.1%), in 24/52 at 48 h (46.2%) and in 30/52 (57.7%) after 7 days of incubation. The randomly amplified polymorphic DNA profiles showed no interstrain similarities between either strains of the BLS-producing L. innocua or the BLS-sensitive L. monocytogenes strains. Therefore inhibition by BLS-producing L. innocua of isolation of L. monocytogenes after enrichment culture is unlikely to be dependent upon a particular genetic profile.ResumeL'inhibition de l'isolation de Listeria monocytogenes par une substance telle que la bacteriocine (BLS) produisant de la Listeria innocua apres une culture enrichie fut etudiee. Lorsque 26 souches de L. monocytogenes furent examinees en combinaison avec 8 souches de L. innocua en utilisant la plaque d'une methode de tapis, 52 combinaisons sur 208 (25.0%) montrerent une inhibition croissante de L. monocytogenes. Lorsque 2 varietes de Listeria furent cultivees simultanement dans un bouillon enrichi selectif, une inhibition de l'isolement de L. monocytogenes fut observee dans 12 combinaisons sur 52 en 24 h (23.1%), 24 sur 52 en 48 h (46.2%) et 30 sur 52 (57.7%), apres 7 jours d'incubation. Les profils d'une amplification aleatoire polymorphique de l'ADN ne montrerent pas de similarites intersouches entre les souches de BLS produisant de la L. innocua ou les souches sensibles BLS de L. monocytogenes. Par consequent, une inhibition par BLS produisant de la L. innocua provenant de l'isolement de L. monocytogenes apres une culture enrichie est improbable, selon qu'il s'agit d'un profil genetique particulier.

Bregitzer, P. and D. Tonks (2003). "Inheritance and Expression of Transgenes in Barley." Crop Sci. **43**(1): 4-12.

http://crop.scijournals.org/cgi/content/abstract/43/1/4

Empirical assessments of transgene inheritance and phenotypic expression will assist in the development of efficient breeding strategies for transgenic germplasm, and guide research into the improvement of transformation techniques. The inheritance of a barley yellow dwarf virus (BYDV) coat protein gene and bar, and the expression of bar as measured by resistance to glufosinate-ammonium damage, was studied in the T1 and T3 generations of barley (Hordeum vulgare L.) populations derived from seven independent transformation events. Most populations deviated from Mendelian inheritance patterns, and several showed evidence of transgene silencing. To further study transgene behavior, several transgenic lines were crossed to a diverse set of nontransgenic cultivars and breeding lines to produce single cross- and backcross-derived populations. In these populations, the inheritance of glufosinate-ammonium resistance generally fit Mendelian expectations for single, dominant loci. Quantitative measurements of glufosinate-ammonium resistance showed heritable variability for glufosinate-ammonium resistance both among and within individual transformation events, but no variability could be attributed to the different genetic backgrounds of the nontransgenic parents. It is concluded that, although transgenic parents such as these can be used in a breeding program, transformation systems that result in greater stability of transgene behavior are desirable.

Butruille, D. V., H. D. Silva, et al. (2004). "Response to Selection and Genetic Drift in Three Populations Derived from the Golden Glow Maize Population." Crop Sci. **44**(5): 1527-1534.

http://crop.scijournals.org/cgi/content/abstract/44/5/1527

Reciprocal recurrent selection (RRS) in maize (Zea mays L.) is used to develop populations with superior combining ability, and it is usually assumed that these populations must be genetically distinct for RRS to be effective. Starting from two randomly derived subpopulations, GG(A) and GG(B), of the open-pollinated maize population Golden Glow' (GGC0), we conducted six cycles of full-sib RRS for grain yield and moisture. Our objectives were to (i) document selection response; (ii) evaluate inter and intrapopulation genetic diversity; and (iii) review selection response relative to gene action, heterosis, inbreeding, and genetic drift. We performed a generation means analysis (GMA) using GG(A), GG(B), and a third population of Golden Glow developed by 21 cycles of mass selection for prolificacy, GG(MP). Field performance was evaluated at four environments in Wisconsin. We analyzed allele frequency changes using simple sequence repeats (SSRs) and amplified fragment length polymorphisms (AFLPs). Selection response for grain yield of GG(A) x GG(B) population crosses was 5.3 g plant-1 cycle-1. Grain moisture and root lodging decreased by -0.5% cycle-1 and -5.2% cycle-1, while prolificacy increased by 0.03 ears cycle-1. After six cycles of RRS, GG(A) and GG(B) diverged genetically from each other, as well as from GG(MP)C21 and Golden Glow. Gene diversity within GG(A) and GG(B) decreased. However, total gene diversity over both GG(A) and GG(B) did not change over cycles of RRS. Genetic drift did not appear to seriously impede selection response. Most of the selection response, regardless of trait, selection method, or subpopulation, was attributed to additive genetic effects.

Cornelious, B. K. and C. H. Sneller (2002). "Yield and Molecular Diversity of Soybean Lines Derived from Crosses of Northern and Southern Elite Parents." Crop Sci. **42**(2): 642-647.

http://crop.scijournals.org/cgi/content/abstract/42/2/642

Genetic diversity is low in southern United States elite soybean [Glycine max (L.) Merr.] cultivars. Multiple sources of diversity will be required to effectively diversify this gene pool. The objective in this study was to evaluate the genetic diversity and yield of familiess derived from crosses between northern elite (NE) by southern elite (SE) parents. Lines were derived from 10 crosses of NE x SE parents. Molecular markers were used to estimate genetic distance between each line and its SE parent. Yield and agronomic traits were measured in field trials from 1997 to 1999 in six of the crosses. The association of diversity with line yield, expressed relative to yield of the SE parent was determined with regression. On average, the use of NE parents reduced yield, relative to using other SE parents. Some crosses and NE parents were better than others and produced families with yield that exceeded that of their SE parent, indicating that some genes from the NE parents were superior to the genes in the SE parent. At least one line with yield either superior or similar to their SE parent was found in each cross. The finding of positive transgressive segregants in some crosses and the results of the regression analyses indicate that most of the NE parents posses some yield genes that are likely to be superior to those of the SE parents. Our approach to selecting for diversity and yield may be applicable to large introgression programs where diversity from many sources is desired.

Helguera, M., I. A. Khan, et al. (2003). "PCR Assays for the Lr37-Yr17-Sr38 Cluster of Rust Resistance Genes and Their Use to Develop Isogenic Hard Red Spring Wheat Lines." Crop Sci. **43**(5): 1839-1847.

http://crop.scijournals.org/cgi/content/abstract/43/5/1839

Rust resistance genes Lr37, Sr38, and Yr17 are located within a segment of Triticum ventricosum (Tausch) Cess. chromosome 2NS translocated to the short arm of bread wheat chromosome 2AS. Characterization of this chromosome segment by 13 restriction fragment length polymorphism (RFLP) markers indicated that the 2NS translocation replaced approximately half of the short arm of chromosome 2A (distal 25-38 centimorgans, cM). The objective of this study was to develop polymerase chain reaction (PCR) assays based on RFLP marker cMWG682 to facilitate the transfer of this cluster of rust resistance genes into commercial wheat (Triticum aestivum L.) cultivars. DNA sequence was obtained from the A-, B-, D-, and N-alleles of cMWG682 and was used to design N-allele specific primers. The 2NS fragment amplified by PCR primers cosegregated with the presence of the RFLP-2NS band in all backcross populations. A cleaved amplified polymorphic sequence (CAPS) was used to develop a marker for the 2A-allele. This marker can be used to differentiate homozygous and heterozygous plants carrying the 2NS translocation in the final cycle of backcross introgression or in screenings for homozygous plants in segregating populations. Finally, a third PCR assay was developed by means of TaqMan technology as a high-throughput alternative for selection of the 2NS/2AS translocation in large segregating populations in breeding programs that have access to real time PCR equipment. These molecular markers were used to develop four hard red spring isogenic lines homozygous for the 2NS chromosome segment. One of the isogenic lines, derived from Anza,' did not show the expected resistance in spite of the presence of all the RFLP markers for the 2NS chromosome segment. Analysis of F1 hybrids suggested that a suppressor of the Lr37 gene is present in Anza. These isogenic lines will provide a valuable tool to test the effects of the large 2NS translocation on quality and agronomic performance.

Hoeck, J. A., W. R. Fehr, et al. (2003). "Molecular Marker Analysis of Seed Size in Soybean." Crop Sci. **43**(1): 68-74.

http://crop.scijournals.org/cgi/content/abstract/43/1/68

Seed size is an important attribute of soybean [Glycine max (L.) Merr.] for some food uses. The objectives of this study were to identify simple-sequence-repeat (SSR) markers associated with quantitative trait loci for seed size (SSQTL) and to compare the effectiveness of phenotypic selection and marker-assisted selection for seed size among individual F2 plants. Three small-seeded lines were crossed to parents with normal seed size to form three two-parent populations. The parents of the populations were screened with 178 SSR markers to identify polymorphism. Population 1 (Pop 1) had 75 polymorphic SSR markers covering 1306 centimorgans (cM), Pop 2 had 70 covering 1143 cM, and Pop 3 had 82 covering 1237 cM. Seed size of each population was determined with 100 F2 plants grown at Isabela, Puerto Rico, and their F2-derived lines grown in two replications at three environments. Single-factor analysis of variance and multiple regression were used to determine significant marker-SSQTL associations. Population 1 had 12 markers that individually accounted for 8.1 to 14.9% of the variation for seed size combined across environments, Pop 2 had 16 markers that individually accounted for 7.8 to 36.5% of the variation, and Pop 3 had 22 markers that individually accounted for 8.6 to 28.8% of the variation. Three marker loci that had significant SSQTL associations in this study also were significant in previous research, and 13 markers had unique SSQTL associations. The relative effectiveness of phenotypic and marker-assisted selection among F2 plants varied for the three populations. Averaged across the three populations, phenotypic selection for seed size was as effective and less expensive than marker-assisted selection.

Nelsen, N. S., Z. Li, et al. (2004). "Genomic Polymorphism Identifies a Subtilisin-Like Protease near the Rhg4 Locus in Soybean." Crop Sci. **44**(1): 265-273.

http://crop.scijournals.org/cgi/content/abstract/44/1/265

Soybean [Glycine max (L.) Merr.] germplasm PI 437654 exhibits broad resistance to soybean cyst nematode (Heterodera glycines Ichinohe, SCN). Probes derived from PI 437654 bacterial artificial chromosome (BAC) clone 15G19 at the Rhg4 locus detected a restriction fragment length polymorphism (RFLP) between resistant and susceptible germplasms. Detailed RFLP analysis using restriction fragments from BAC clone 15G19 associated the polymorphism with an 8-kb BamHI fragment containing the promoter region and partial coding sequence of a novel soybean subtilisin-like protease, GmSUB1. Complete sequence of GmSUB1 was determined (GenBank AY277949). Regulatory elements for root gene expression, pathogen response, coordinated multiple-gene expression, and a novel 90-bp direct repeat were identified. GmSUB1 shows 74% similarity to Arabidopsis thaliana AIR3. Hybridization analyses indicate that PI 437654 contains only full-length copies of GmSUB1, whereas susceptible germplasm Williams 82' contains both full-length and truncated copies of the gene. A 4-fold increase in GmSUB1 copy number, and a corresponding 2- to 3-fold increase in steady state GmSUB1 mRNA levels, was observed in PI 437654 compared with Williams 82. Localization and polymorphism of GmSUB1 within the Rhg4 resistance region, and increases in GmSUB1 gene copy number and expression in PI 437654 compared with Williams 82 infers a functional role in the pathogen response. GmSUB1 is believed to be secreted into the extracellular matrix, and may function in reorganization of cell wall components during plant development and in the defense response.

Pedersen, J. F., D. B. Marx, et al. (2003). "Use of A3 Cytoplasm to Reduce Risk of Gene Flow through Sorghum Pollen." Crop Sci. **43**(4): 1506-1509.

http://crop.scijournals.org/cgi/content/abstract/43/4/1506

A critical impediment to field testing and deployment of transgenic sorghum [Sorghum bicolor (L.) Moench] is the threat of gene flow to weedy relatives through pollen. A technique using sorghum with A3 cytoplasmic male sterility to control transgene flow through pollen while using

nontransgenic pollinators is described and an experiment was designed to evaluate the risk of viable pollen flow using A3 hybrids under field conditions. Seed set under pollinating bags (an indicator of fertile pollen) was evaluated at the University of Nebraska Field Laboratory at Ithaca, NE, in 2001 and 2002 on selfed F2 progeny grown from open pollinated seed of 12 F1 hybrids produced in A1 and A3 cytoplasm. The F2 seed was produced in hybrid yield trials in 1997 and 1998 at Ithaca, NE. In each evaluation year, the experimental design was a split-split plot with seed production year the main plot factor, hybrid as the subplot factor, and cytoplasm as the sub-subplot factor. Cytoplasm effects were highly significant, with percent seed set on A1 F2 individuals averaging 74%, and on A3 F2 individuals averaging 0.04%. Upper confidence limits (P = 0.05) for percent seed set were 1.32% or less for the progeny from all A3 hybrids. Polymerase chain reaction (PCR) analysis confirmed that four male fertile individuals (from a population of 1007) were detected with A3 cytoplasm. These results support the hypothesis that gene flow through pollen can be severely restricted but not eliminated in sorghum by the use of A3 cytoplasmic male sterility.

Raker, C. M. and D. M. Spooner (2002). "Chilean Tetraploid Cultivated Potato, Solanum tuberosum, is Distinct from the Andean Populations: Microsatellite Data." Crop Sci. **42**(5): 1451-1458.

http://crop.scijournals.org/cgi/content/abstract/42/5/1451

This study tests the genetic difference between landrace populations of Solanum tuberosum L. subsp. tuberosum and subsp. andigenum (Juz. & Bukasov) Hawkes using nuclear DNA microsatellites. Microsatellite loci were amplified in subsp. andigenum (35 accessions), subsp. tuberosum (35 accessions), and other cultivated and wild species (22 accessions). A total of 208 alleles were scored from 18 microsatellite loci spread throughout all 12 chromosomes of potato. Using an infinite allele model and a least squares method of analysis, microsatellite loci separated subsp. tuberosum from subsp. andigenum, and cultivated and wild species. These results support the genetic difference of these two populations and their recognition at some classification level.

Schmidt, M. A., G. S. Martin, et al. (2004). "Increased Transgene Expression by Breeding and Selection in White Clover." Crop Sci. **44**(3): 963-967.

http://crop.scijournals.org/cgi/content/abstract/44/3/963

To determine if standard breeding methodology is applicable to transgenes, phenotypic recurrent selection was used to select for increased transgene expression in white clover, Trifolium repens L. Plants were transformed with nptII and gusA, and selected on 100 mg L-1 of kanamycin. Independently transformed plants were intercrossed, and the progeny was germinated on 200, 300, or 400 mg L-1 of kanamycin. Those seedlings surviving on 400 mg-1 were in turn intercrossed, and the progeny was selected on 300, 400, or 500 mg L-1 of kanamycin. NPTII levels were measured in each selected population, and Southern blots were made from individuals in each population. The highest-expressing individual in the T2 had levels of NPTII that were more than four times higher than those in the highest parent. With selection on increasing levels of kanamycin, average expression across each generation went from 0.033 ng {micro}g-1 NPTII in the parents to 0.095 ng {micro}g-1 in the selected T1 plants to 0.539 ng {micro}g-1 in the selected T2 plants. Southern hybridization suggested that plants displaying a heightened level of nptII expression in the T1 and T2 fell into two categories. The first contained one particular transgenic event, implicating the importance of other genomic factors in modulating gene expression. Alternatively, the plants had an accumulation of various nptII loci, suggesting an association between multiple transgene copies and high expression levels. On the basis of these results, selection for transgene expression appears to be a viable option for plant breeding

programs.

Smalley, M. D., W. R. Fehr, et al. (2004). "Quantitative Trait Loci for Soybean Seed Yield in Elite and Plant Introduction Germplasm." Crop Sci. **44**(2): 436-442.

http://crop.scijournals.org/cgi/content/abstract/44/2/436

Genetic improvement for yield in soybean [Glycine max (L.) Merr.] has been accomplished by breeding within a narrow elite gene pool. Plant introductions (PIs) may be useful for obtaining additional increases in yield if unique and desirable alleles at quantitative trait loci (QTL) can be identified. The objectives of the study were to identify QTL for yield in elite and PI germplasm and to determine if the PIs possessed favorable alleles for yield. Allele frequencies were measured with simple sequence repeat (SSR) markers in three populations, designated AP10, AP12, and AP14, that differed in their percentage of PI parentage. AP10 had 40 PI parents, AP12 had 40 PI and 40 elite parents, and AP14 had 40 elite parents. Four cycles of recurrent selection for yield had been conducted in the three populations. Allele frequencies of the highest-yielding C4 lines in the three populations were compared with the parents used to form the populations of the initial cycles. Allele flow was simulated to account for genetic drift. Fifty-four SSRs were associated with 43 yield QTL. Seven of the QTL had been identified in previous research. Sixteen favorable marker alleles were unique to the PI parents. The genes associated with the unique PI alleles merit further investigation for their potential to increase yield of soybean cultivars.

Current Biology (9)

Corcos, D., O. Dunda, et al. (1995). "Pre-B-cell development in the absence of [lambda]5 in transgenic mice expressing a heavy-chain disease protein." Current Biology **5**(10): 1140.

http://www.sciencedirect.com/science/article/B6VRT-4D5X1XC-6B/2/185db7f2335073beb64aad0963c0a0f7

Background: Heavy-chain diseases (HCDs) are human lymphoproliferative neoplasias that are characterized by the secretion of truncated immunoglobulin heavy chains devoid of light chains. We have previously proposed -- by analogy to the process by which mutated growth factor receptors can be oncogenic -- that because the genetic defects in HCDs result in the production of abnormal membrane-associated heavy chains lacking an antigen-binding domain, these abnormal B-cell antigen receptors might engage in ligand-independent signalling. Normal pre-B-cell development requires the presence of the pre-B-cell receptor, formed by the association of [mu] heavy chains with two polypeptides -- so-called surrogate light chains, Vpre-B and [lambda]5 -- that are homologous to the variable and constant portions of immunoglobulin light chains, respectively. To assess whether amino-terminal truncation of membrane-associated heavy chains results in their constitutive activation, we have examined the ability of a HCD-associated [mu] protein to promote pre-B-cell development in transgenic mice.Results When the [mu] HCD transgene is introduced into SCID mice, CD43- pre-B cells develop normally. To determine whether this pre-B-cell development requires surrogate light chains, we backcrossed mice expressing full-length or truncated [mu] transgenes with [lambda]5-deficient mice. Our results show that the truncated heavy chain, but not the normal chain, is able to promote pre-B-cell development in the absence of [lambda]5. We also show that truncated [mu] chains

spontaneously aggregate at the surface of bone marrow cells.Conclusion Expression of the truncated [mu] heavy chain overrides a tightly controlled step of pre-B-cell development, which strongly suggests that a constitutive signal is delivered by the truncated [mu] chain disease protein. The self-aggregation of [mu] chain disease proteins might account for this constitutive activation. We conclude that amino-terminal truncation of heavy chains could play a role in the genesis of HCD neoplasia if it occurs at an appropriate stage of B-cell differentiation, namely in a mature B cell.

Heim, R. and R. Y. Tsien (1996). "Engineering green fluorescent protein for improved brightness, longer wavelengths and fluorescence resonance energy transfer." Current Biology **6**(2): 178.

http://www.sciencedirect.com/science/article/B6VRT-4CB6PVS-6/2/9aacee2345f4b6d676e924c8a3d8878d

Background: Variants of the green fluorescent protein (GFP) with different colors would be very useful for simultaneous comparisons of multiple protein fates, developmental lineages and gene expression levels. The simplest way to shift the emission color of GFP is to substitute histidine or tryptophan for the tyrosine in the chromophore, but such blue-shifted point mutants are only dimly fluorescent. The longest wavelengths previously reported for the excitation and emission peaks of GFP mutants are 488 and 511 nm, respectively.Results Additional substitutions, mainly in residues 145-163, have improved the brightness of the blue-shifted GFP mutants with histidine and tryptophan in place of tyrosine 66. Separate mutations have pushed the excitation and emission peaks of the most red-shifted mutant to 504 and 514 nm, respectively. At least three different colors of GFP mutants can now be cleanly distinguished from each other under the microscope, using appropriate filter sets. A fusion protein consisting of linked blue- and green-fluorescent proteins exhibits fluorescence resonance energy transfer, which is disrupted by proteolytic cleavage of the linker between the two domains.Conclusion Our results demonstrate that the production of more and better GFP variants is possible and worthwhile. The production of such variants facilitates multicolor imaging of differential gene expression, protein localization or cell fate. Fusions between mutants of different colors may be useful substrates for the continuous in situ assay of proteases. Demonstration of energy transfer between GFP variants is an important step towards a general method for monitoring the mutual association of fusion proteins.

Huang, F. Z., A. E. Bely, et al. (2001). "Stochastic WNT signaling between nonequivalent cells regulates adhesion but not fate in the two-cell leech embryo." Current Biology **11**(1): 1.

http://www.sciencedirect.com/science/article/B6VRT-428DKHP-1/2/763173edc9cc475e17a77f185bae97fa

Background: In the leech Helobdella robusta, an annelid worm, the early pattern of cell divisions is stereotyped. The unequal first cleavage yields cells AB and CD, which differ in size, cytoplasmic inheritance, normal fate, and developmental potential.Results: Here we report a dynamic and transcription-independent pattern of WNT signaling in the two-cell stage of H. robusta. Surprisingly, HRO-WNT-A is first expressed in a stochastic manner, such that either AB or CD secretes the protein in each embryo. This stochastic phase is followed by a deterministic phase during which first AB, then CD expresses HRO-WNT-A. When contact between the cells is reduced or eliminated, both AB and CD express HRO-WNT-A simultaneously. Finally, bathing embryos in anti-HRO-WNT-A antibody during first cleavage reduces the adhesion between cells AB and CD.Conclusions: Our findings show that the stochastic phase of HRO-WNT-A signaling in the two-cell stage of Helobdella is negatively regulated by cell-cell contact and that this early signaling affects cell adhesion without affecting cell fate. We speculate that the primordial function of wnt class genes may have been to regulate cell-cell adhesion and that the nuclear signaling

components of the wnt pathway arose later in association with the evolution of diverse cell types.

Kayser, M., S. Brauer, et al. (2000). "Melanesian origin of Polynesian Y chromosomes." Current Biology **10**(20): 1237.

http://www.sciencedirect.com/science/article/B6VRT-41N57JK-1D/2/6db4159551b6da3b025df3415c9f1920

Background: Two competing hypotheses for the origins of Polynesians are the 'express-train' model, which supposes a recent and rapid expansion of Polynesian ancestors from Asia/Taiwan via coastal and island Melanesia, and the 'entangled-bank' model, which supposes a long history of cultural and genetic interactions among Southeast Asians, Melanesians and Polynesians. Most genetic data, especially analyses of mitochondrial DNA (mtDNA) variation, support the express-train model, as does linguistic and archaeological evidence. Here, we used Y-chromosome polymorphisms to investigate the origins of Polynesians.Results: We analysed eight single nucleotide polymorphisms (SNPs) and seven short tandem repeat (STR) loci on the Y chromosome in 28 Cook Islanders from Polynesia and 583 males from 17 Melanesian, Asian and Australian populations. We found that all Polynesians belong to just three Y-chromosome haplotypes, as defined by unique event polymorphisms. The major Y haplotype in Polynesians (82% frequency) was restricted to Melanesia and eastern Indonesia and most probably arose in Melanesia. Coalescence analysis of associated Y-STR haplotypes showed evidence of a population expansion in Polynesians, beginning about 2,200 years ago. The other two Polynesian Y haplotypes were widespread in Asia but were also found in Melanesia.Conclusions: All Polynesian Y chromosomes can be traced back to Melanesia, although some of these Y-chromosome types originated in Asia. Together with other genetic and cultural evidence, we propose a new model of Polynesian origins that we call the 'slow-boat' model: Polynesian ancestors did originate from Asia/Taiwan but did not move rapidly through Melanesia; rather, they interacted with and mixed extensively with Melanesians, leaving behind their genes and incorporating many Melanesian genes before colonising the Pacific.

Kittler, R., M. Kayser, et al. (2003). "Molecular Evolution of Pediculus humanus and the Origin of Clothing." Current Biology **13**(16): 1414.

http://www.sciencedirect.com/science/article/B6VRT-49BYF2J-S/2/0c8e3bd4e7de24a2032696b240067a33

The human head louse (Pediculus humanus capitis) and body louse (P. humanus corporis or P. h. humanus) are strict, obligate human ectoparasites that differ mainly in their habitat on the host [1 and 2]: the head louse lives and feeds exclusively on the scalp, whereas the body louse feeds on the body but lives in clothing. This ecological differentiation probably arose when humans adopted frequent use of clothing, an important event in human evolution for which there is no direct archaeological evidence. We therefore used a molecular clock approach to date the origin of body lice, assuming that this should correspond with the frequent use of clothing. Sequences were obtained from two mtDNA and two nuclear DNA segments from a global sample of 40 head and body lice, and from a chimpanzee louse to use as an outgroup. The results indicate greater diversity in African than non-African lice, suggesting an African origin of human lice. A molecular clock analysis indicates that body lice originated not more than about 72,000 +/- 42,000 years ago; the mtDNA sequences also indicate a demographic expansion of body lice that correlates with the spread of modern humans out of Africa. These results suggest that clothing was a surprisingly recent innovation in human evolution.

Lowell, S., P. Jones, et al. (2000). "Stimulation of human epidermal differentiation by Delta-Notch signalling at the boundaries of stem-cell clusters." Current Biology **10**(9): 491.

http://www.sciencedirect.com/science/article/B6VRT-408JDCB-G/2/327e8acb9bb451588146370e20428cf3

Background: Human epidermis is renewed throughout life from stem cells in the basal layer of the epidermis. Signals from the surrounding keratinocytes influence the differentiation of the stem cells, but the nature of the signals is unknown. In many developing tissues, signalling mediated by the transmembrane protein Delta1 and its receptor Notch1 inhibits differentiation. Here, we investigated the role of Delta-Notch signalling in postnatal human epidermis.Results: Notch1 expression was found in all living epidermal layers, but Delta1 expression was confined to the basal layer of the epidermis, with highest expression in those regions where stem cells reside. By overexpressing Delta1 or DeltaT, a truncated form of Delta1, in primary human keratinocytes and reconstituting epidermal sheets containing mixtures of Delta-overexpressing cells and wild-type cells, we found that cells expressing high levels of Delta1 or DeltaT failed to respond to Delta signals from their neighbours. In contrast, wild-type keratinocytes that were in contact with neighbouring cells expressing Delta1 were stimulated to leave the stem-cell compartment and initiate terminal differentiation after a few rounds of division. Delta1 promoted keratinocyte cohesiveness, whereas DeltaT did not.Conclusions: We propose that high Delta1 expression by epidermal stem cells has three effects: a protective effect on stem cells by blocking Notch signalling; enhanced cohesiveness of stem-cell clusters, which may discourage intermingling with neighbouring cells; and signalling to cells at the edges of the clusters to differentiate. Notch signalling in epidermal stem cells thus differs from other progenitor cell populations in promoting, rather than suppressing, differentiation.

Nunes-Duby, S. E., M. A. Azaro, et al. (1995). "Swapping DNA strands and sensing homology without branch migration in [lambda] site-specific recombination." Current Biology **5**(2): 139.

http://www.sciencedirect.com/science/article/B6VRT-4DFJW0T-8/2/2e6814f225ab1e48f2db09d62e9310da

Background: Many site-specific recombinases act by forming and resolving branched Holliday junction intermediates. Previous findings have been consistent with models involving branch migration across the 'overlap region' of obligate homology, located between the staggered sites where the two single-strand exchanges occur. We have investigated the validity of such models in the case of bacteriophage [lambda] site-specific recombination.Results By using synthetic [lambda] att-site Holliday junctions, incorporating sequence heterologies that impose constraints on branch migration, we have found that the optimal position of the junction for either top-strand or bottom-strand resolution by [lambda] integrase (Int) is not at the ends, but close to the middle of the seven base-pair overlap region. A minor shift of the branch point around the central base pair caused a remarkable switch in resolution bias. Our findings suggest that branch migration is limited to the central one to three base pairs of the overlap region. They lead to a new model for [lambda] site-specific recombination, in which there are two symmetrical swaps of two to three nucleotides each, linked by a central isomerization step that causes a change of the stacking interactions between the four junction arms. On the basis of isolated strand-joining reactions carried out by Int in the presence or absence of base complementarity, we propose that sequence homology is sensed during the annealing step prior to strand joining. The new model eliminates mechanistic complications associated with large helical rotations required by branch-migration models.Conclusion The results reported here suggest that the recognition of sequence homology in Int-dependent site-specific recombination does not rely primarily on branch migration. The property of cleaving Holliday junctions a few base pairs away from the crossover

puts [lambda] Int into the same category as endonucleases that cleave Holliday junctions in homologous recombination.

Poinar, H., M. Kuch, et al. (2003). "Nuclear Gene Sequences from a Late Pleistocene Sloth Coprolite." Current Biology **13**(13): 1150.

http://www.sciencedirect.com/science/article/B6VRT-4909176-Y/2/0046673165b0f3b4820948641bac5830

The determination of nuclear DNA sequences from ancient remains would open many novel opportunities such as the resolution of phylogenies, the sexing of hominid and animal remains, and the characterization of genes involved in phenotypic traits. However, to date, single-copy nuclear DNA sequences from fossils have been determined only from bones and teeth of woolly mammoths preserved in the permafrost [1]. Since the best preserved ancient nucleic acids tend to stem from cold environments [2 and 3], this has led to the assumption that nuclear DNA would be retrievable only from frozen remains. We have previously shown that Pleistocene coprolites stemming from the extinct Shasta sloth (Nothrotheriops shastensis, Megatheriidae) contain mitochondrial (mt) DNA from the animal that produced them as well as chloroplast (cp) DNA from the ingested plants [4]. Recent attempts to resolve the phylogeny of two families of extinct sloths by using strictly mitochondrial DNA has been inconclusive [5]. We have prepared DNA extracts from a ground sloth coprolite from Gypsum Cave, Nevada, and quantitated the number of mtDNA copies for three different fragment lengths by using real-time PCR. We amplified one multicopy and three single-copy nuclear gene fragments and used the concatenated sequence to resolve the phylogeny. These results show that ancient single-copy nuclear DNA can be recovered from warm, arid climates. Thus, nuclear DNA preservation is not restricted to cold climates.

Shibata, S. and J. T. Lee (2004). "Tsix Transcription- versus RNA-Based Mechanisms in Xist Repression and Epigenetic Choice." Current Biology **14**(19): 1747.

http://www.sciencedirect.com/science/article/B6VRT-4DG5TDG-T/2/7a5488015b4f7ae56e164a705b6abd44

Recent inquiries have revealed a surprisingly large number (>2500) of naturally occurring antisense transcripts [1, 2, 3 and 4], but their function remains largely undiscovered. A better understanding of antisense mechanisms is clearly needed because of their potentially diverse roles in gene regulation and disease [5, 6, 7 and 8]. A well-documented case occurs in X inactivation, the mechanism by which X-linked gene expression is equalized between XX females and XY males [9]. The antisense gene Tsix [6] determines X chromosome choice and represses the noncoding silencer, Xist [10, 11 and 12]. In principle, Tsix action may involve RNA, the act of transcription, or local chromatin. Here, we create novel Tsix alleles to distinguish transcription- versus RNA-based mechanisms. When Tsix transcription is terminated before Xist (TsixTRAP), Tsix cannot block Xist upregulation, suggesting the importance of overlapping antisense transcription. To separate the act of transcription from RNA, we knocked in Tsix cDNA in the reverse orientation (TsixcDNA) to restore RNA levels in cis without concurrent transcription across Xist. However, TsixcDNA cannot complement TsixTRAP. Surprisingly, both mutations disrupt choice, indicating that this epigenetic step requires transcription. We conclude that the processed antisense RNA does not act alone and that Tsix function specifically requires antiparallel transcription through Xist. A mechanism of transcription-based feedback regulation is proposed.

Deep Sea Research Part II: Topical Studies in Oceanography(1)

Riemann, L., G. F. Steward, et al. (1999). "Bacterial community composition during two consecutive NE Monsoon periods in the Arabian Sea studied by denaturing gradient gel electrophoresis (DGGE) of rRNA genes." Deep Sea Research Part II: Topical Studies in Oceanography **46**(8-9): 1791.

http://www.sciencedirect.com/science/article/B6VGC-3Y0RMGY-C/2/3e7916c1e45f22b3a75ee8271b9027a6

Horizontal and vertical variations in bacterial community composition were examined in samples collected during two Joint Global Ocean Flux Study (JGOFS) Arabian Sea cruises in 1995. The cruises, 11 months apart, took place during two consecutive NE Monsoon periods (January and December). Bacteria were harvested by filtration from samples collected in the mixed layer, mid-water, and deep sea at stations across the study area. Total bacterial community genomic DNA was analyzed by PCR amplification of 16S rRNA gene fragments, followed by denaturing gradient gel electrophoresis (DGGE). In total, 20 DGGE bands reflecting unique or varying phylotypes were excised, cloned and sequenced. Amplicons were dominated by bacterial groups commonly found in oceanic waters (e.g., the SAR11 cluster of [alpha]-Proteobacteria and cyanobacteria), but surprisingly none of the sequenced amplicons were related to [gamma]-Proteobacteria or to members of the Cytophaga-Flavobacter-Bacteroides phylum. Amplicons related to magnetotactic bacteria were found for the first time in pelagic oceanic waters. The DGGE banding patterns revealed a dominance of [ap]15 distinguishable amplicons in all samples. In the mixed layer the bacterial community was dominated by the same [ap]15 phylotypes at all stations, but unique phylotypes were found with increasing depth. Except for cyanobacteria, comparison of the bacterial community composition in surface waters from January and December 1995 showed only minor differences, despite significant differences in environmental parameters. These data suggest a horizontal homogeneity and some degree of seasonal predictability of bacterial community composition in the Arabian Sea.

Desalination (1)

Gabelich, C. J., T. I. Yun, et al. (2004). "The effect of naturally occurring biopolymers on polyamide membrane fouling during surface water treatment." Desalination **161**(3): 263.

http://www.sciencedirect.com/science/article/B6TFX-4C237ST-6/2/19c6c89fcd29bc6fad7f74e3ab46d043

Parallel experiments using a blend of surface waters were conducted to evaluate differential fouling rates among reverse osmosis (RO) membranes when operated under pilot- vs. full-scale conditions. Testing was conducted using a 230 L/min conventional (rapid mix/flocculation/sedimentation/filtration) package plant (CPP) and a 2,000 ML/d fullscale treatment plant (FTP) as pretreatment to separate RO membrane test units. Coagulation consisted of 10 mg/L alum (as Al2(SO4)3-14H2O) and 2.0 mg/L cationic polymer. A2.5-3.0 mg/L free-chlorine residual was maintained at the filter effluent and converted to chloramines through ammonium sulfate addition (3:1 chlorine-to-ammonia w/w ratio). Membrane performance was

based on normalized flux and salt rejection data. Membrane surface analyses included scanning electron microscopy, energy-dispersive spectroscopy, and attenuated total reflectance Fourier transform infrared spectroscopy. Microbial activity and community analyses were conducted through (a) fluorescence staining with 4',6'-diamidino-2-phenylindole, (b) polymerase-chain reaction amplification of isolated bacterial DNA, and (c) microscopic taxonomic identification. Results indicated that the RO membrane fed by the CPP fouled at least three times faster than the RO membrane fed by the FTP. The differential fouling between the two process streams was determined to be from lack of maintenance in the CPP influent piping that led to the establishment of biological communities consisting of algae, microbes, and, potentially, freshwater clams. These communities produced low levels of natural polymers, which when presented to the polyamide RO membrane surface, resulted in rapid fouling.

Development (12)

Bort, R., J. P. Martinez-Barbera, et al. (2004). "Hex homeobox gene-dependent tissue positioning is required for organogenesis of the ventral pancreas." Development **131**(4): 797-806.

http://dev.biologists.org/cgi/content/abstract/131/4/797

In animal development, digestive tissues emerge from different positions of the endoderm as a result of patterning signals from overlying mesoderm. Although embryonic tissue movement during gastrulation generates an initial positional relationship between the endoderm and mesoderm, the role of subsequent endoderm movement against the mesoderm in patterning is unknown. At embryonic day 8.5 in the mouse, proliferation of cells at the leading edge of ventral-lateral endoderm, where the liver and ventral pancreas emerge, helps close off the foregut. During this time, the endoderm grows adjacent to and beyond the cardiogenic mesoderm, an inducer of the liver program and an inhibitor of the pancreas program. The homeobox gene Hex is expressed in this endoderm cell domain and in the liver and ventral pancreas buds, after organogenesis. We have found that in Hex-/- embryos, there is a complete failure in ventral pancreatic specification, while the liver program is still induced. However, when Hex-null ventral endoderm is isolated prior to its interaction with cardiogenic mesoderm and is cultured in vitro, it activates early pancreas genes. We found that Hex controls the proliferation rate, and thus the positioning, of the leading edge of endoderm cells that grow beyond the cardiogenic mesoderm, during gut tube closure. Thus, Hex-controlled positioning of endoderm cells beyond cardiogenic mesoderm dictates ventral pancreas specification. Other endodermal transcription factors may also function morphogenetically rather than by directly regulating tissue-specific programs.

Chen, H.-H., J. W. Yip, et al. (2002). "Differential expression of a transcription regulatory factor, the LIM domain only 4 protein Lmo4, in muscle sensory neurons." Development **129**(21): 4879-4889.

http://dev.biologists.org/cgi/content/abstract/129/21/4879

In the stretch-reflex system, proprioceptive sensory neurons make selective synaptic connections with different subsets of motoneurons, according to the peripheral muscles they supply. To examine the molecular mechanisms that may influence the selection of these synaptic targets, we constructed single-cell cDNA libraries from sensory neurons that innervate antagonist muscles. Differential screening of these libraries identified a transcription regulatory co-factor of the LIM

homeodomain proteins, the LIM domain only 4 protein Lmo4, expressed in most adductor but few sartorius sensory neurons. Differential patterns of Lmo4 expression were also seen in sensory neurons supplying three other muscles. A subset of motoneurons also expresses Lmo4 but the pattern of expression is not specific for motor pools. Differential expression of Lmo4 occurs early, as neurons develop their characteristic LIM homeodomain protein expression patterns. Moreover, ablation of limb buds does not block Lmo4 expression, suggesting that an intrinsic program controls the early differential expression of Lmo4. LIM homeodomain proteins are known to regulate several aspects of sensory and motor neuronal development. Our results suggest that Lmo4 may participate in this differentiation by regulating the transcriptional activity of LIM homeodomain proteins.

Coles, E., J. Christiansen, et al. (2004). "A vertebrate crossveinless 2 homologue modulates BMP activity and neural crest cell migration." Development **131**(21): 5309-5317.

http://dev.biologists.org/cgi/content/abstract/131/21/5309

Previous work has revealed that proteins that bind to bone morphogenetic proteins (BMPs) and inhibit their signalling have a crucial role in the spatial and temporal regulation of cell differentiation and cell migration by BMPs. We have identified a chick homologue of crossveinless 2, a Drosophila gene that was identified in genetic studies as a promoter of BMP-like signalling. Chick Cv-2 has a conserved structure of five cysteine-rich repeats similar to those found in several BMP antagonists, and a C-terminal Von Willebrand type D domain. Cv-2 is expressed in the chick embryo in a number of tissues at sites at which elevated BMP signalling is required. One such site of expression is premigratory neural crest, in which at trunk levels threshold levels of BMP activity are required to initiate cell migration. We show that, when overexpressed, Cv-2 can weakly antagonise BMP4 activity in Xenopus embryos, but that in other in vitro assays Cv-2 can increase the activity of co-expressed BMP4. Furthermore, we find that increased expression of Cv-2 causes premature onset of trunk neural crest cell migration in the chick embryo, indicative of Cv-2 acting to promote BMP activity at an endogenous site of expression. We therefore propose that BMP signalling is modulated both by antagonists and by Cv-2 that acts to elevate BMP activity.

Dalpe, G., L. W. Zhang, et al. (2004). "Conversion of cell movement responses to Semaphorin-1 and Plexin-1 from attraction to repulsion by lowered levels of specific RAC GTPases in C. elegans." Development **131**(9): 2073-2088.

http://dev.biologists.org/cgi/content/abstract/131/9/2073

Plexins are functional receptors for Semaphorin axon guidance cues. Previous studies have established that some Plexins directly bind RACGTP and RHO. Recent work in C. elegans showed that semaphorin 1 (smp-1 and smp-2) and plexin 1 (plx-1) are required to prevent anterior displacement of the ray 1 cells in the male tail (Fujii et al., 2002; Ginzburg et al., 2002). We show genetically that plx-1 is part of the same functional pathway as smp-1 and smp-2 for male ray positioning. RAC GTPase genes mig-2 and ced-10 probably function redundantly, whereas unc-73, which encodes a GEF for both of these GTPases, is required cell autonomously for preventing anterior displacement of ray 1 cells. RNAi analysis indicates that rho-1-encoded RHO GTPase, plus let-502 and K08B12.5-encoded RHO-kinases, are also required to prevent anterior displacement of ray 1 cells, suggesting that different kinds of RHO-family GTPases act similarly in ray 1 positioning. At low doses of wild-type mig-2 and ced-10, the Semaphorin 1 proteins no longer act through PLX-1 to prevent anterior displacements of ray 1, but have the opposite effect, acting through PLX-1 to mediate anterior displacements of ray 1. These results suggest that Plexin 1 senses levels of distinct RHO and RAC GTPases. At normal levels of RHO

and RAC, Semaphorin 1 proteins and PLX-1 prevent a forward displacement of ray 1 cells, whereas at low levels of cycling RAC, Semaphorin 1 proteins and PLX-1 actively mediate their anterior displacement. Endogenously and ectopically expressed SMP-1 and SMP-2 suggest that the hook, a major source of Semaphorin 1 proteins in the male tail, normally attracts PLX-1-expressing ray 1 cells.

Ginzburg, V. E., P. J. Roy, et al. (2002). "Semaphorin 1a and semaphorin 1b are required for correct epidermal cell positioning and adhesion during morphogenesis in C. elegans." Development **129**(9): 2065-2078.

http://dev.biologists.org/cgi/content/abstract/129/9/2065

The semaphorin family comprises secreted and transmembrane proteins involved in axon guidance and cell migration. We have isolated and characterized deletion mutants of C. elegans semaphorin 1a (Ce-sema-1a or smp-1) and semaphorin 1b (Ce-sema-1b or smp-2) genes. Both mutants exhibit defects in epidermal functions. For example, the R1.a-derived ray precursor cells frequently fail to change anterior/posterior positions completely relative to their sister tail lateral epidermal precursor cell R1.p, causing ray 1 to be formed anterior to its normal position next to ray 2. The ray cells, which normally separate from the lateral tail seam cell (SET) at the end of L4 stage, remains connected to the SET cell even in adult mutant males. The ray 1 defects are partially penetrant in each single Ce-sema-1 mutant at 20{degrees}C, but are greatly enhanced in Ce-sema-1 double mutants, suggesting that Ce-Sema-1a and Ce-Sema-1b function in parallel to regulate ray 1 position. Both mutants also have defects in other aspects of epidermal functions, including head and tail epidermal morphogenesis and touch cell axon migration, whereas, smp-1 mutants alone have defects in defecation and brood size. A feature of smp-1 mutants that is shared with mutants of mab-20 (which encodes Sema-2a) is the abnormal perdurance of contacts between epidermal cells.

Kang, D., F. Huang, et al. (2003). "A hedgehog homolog regulates gut formation in leech (Helobdella)." Development **130**(8): 1645-1657.

http://dev.biologists.org/cgi/content/abstract/130/8/1645

Signaling by the hedgehog (hh)-class gene pathway is essential for embryogenesis in organisms ranging from Drosophila to human. We have isolated a hh homolog (Hro-hh) from a lophotrochozoan species, the glossiphoniid leech, Helobdella robusta, and examined its expression by reverse transcription polymerase chain reaction (RT-PCR) and whole-mount in situ hybridization. The peak of Hro-hh expression occurs during organogenesis (stages 10-11). No patterned expression was detected within the segmented portion of the germinal plate during the early stages of segmentation. In stage 10-11 embryos, Hro-hh is expressed in body wall, foregut, anterior and posterior midgut, reproductive organs and in a subset of ganglionic neurons. Evidence that Hro-hh regulates gut formation was obtained using the steroidal alkaloid cyclopamine, which specifically blocks HH signaling. Cyclopamine induced malformation of both foregut and anterior midgut in Helobdella embryos, and no morphologically recognizable gonads were seen. In contrast, no gross abnormalities were observed in the posterior midgut. Segmental ectoderm developed normally, as did body wall musculature and some other mesodermal derivatives, but the mesenchymal cells that normally come to fill most of the coelomic cavities failed to develop. Taken with data from Drosophila and vertebrates, our data suggest that the role of hh-class genes in gut formation and/or neural differentiation is ancestral to the bilaterians, whereas their role in segmentation evolved secondarily within the Ecdysozoa.

Mansfield, J. H., J. E. Wilhelm, et al. (2002). "Ypsilon Schachtel, a Drosophila Y-box protein, acts antagonistically to Orb in the oskar mRNA localization and translation pathway." Development **129**(1): 197-209.

http://dev.biologists.org/cgi/content/abstract/129/1/197

Subcellular localization of mRNAs within the Drosophila oocyte is an essential step in body patterning. Yps, a Drosophila Y-box protein, is a component of an ovarian ribonucleoprotein complex that also contains Exu, a protein that plays an essential role in mRNA localization. Y-box proteins are known translational regulators, suggesting that this complex might regulate translation as well as mRNA localization. Here we examine the role of the yps gene in these events. We show that yps interacts genetically with orb, a positive regulator of oskar mRNA localization and translation. The nature of the genetic interaction indicates that yps acts antagonistically to orb. We demonstrate that Orb protein is physically associated with both the Yps and Exu proteins, and that this interaction is mediated by RNA. We propose a model wherein Yps and Orb bind competitively to oskar mRNA with opposite effects on translation and RNA localization.

Payne, T., S. D. Johnson, et al. (2004). "KNUCKLES (KNU) encodes a C2H2 zinc-finger protein that regulates development of basal pattern elements of the Arabidopsis gynoecium." Development **131**(15): 3737-3749.

http://dev.biologists.org/cgi/content/abstract/131/15/3737

Flowers of the parthenocarpic knuckles mutant are conditionally male sterile and contain ectopic stamens and carpels that originate from placental tissue within developing gynoecia. The mutation was mapped to a 123 Kb interval on chromosome 5 using molecular markers. All aspects of the knuckles phenotype could be complemented by a genomic fragment from the region which contained the annotated MAC12.2 gene. A guanine to adenine transition within a predicted C2H2 zinc finger-encoding region of MAC12.2 causes the second of two critical zinc-binding cysteine residues to be replaced by a tyrosine. Transgenic plants in which translational fusions of the GUS reporter to KNUCKLES were driven by the presumptive KNUCKLES promoter indicate that the gene is expressed first in developing carpel primordia, and later in stamens and ovules of flower buds. In situ hybridization experiments showed a broader pattern of transcript localization, suggesting that post-transcriptional regulatory mechanisms may limit KNUCKLES protein accumulation and localization. Based on genetic evidence and the presence of a carboxy-terminal motif demonstrated by others to function as an active repression domain, we propose that KNUCKLES might function as a transcriptional repressor of cellular proliferation that regulates floral determinacy and relative size of basal pattern elements along the proximo-distal axis of the developing Arabidopsis gynoecium.

Perron, M., S. Boy, et al. (2003). "A novel function for Hedgehog signalling in retinal pigment epithelium differentiation." Development **130**(8): 1565-1577.

http://dev.biologists.org/cgi/content/abstract/130/8/1565

Sonic hedgehog is involved in eye field separation along the proximodistal axis. We show that Hh signalling continues to be important in defining aspects of the proximodistal axis as the optic vesicle and optic cup mature. We show that two other Hedgehog proteins, Banded hedgehog and

Cephalic hedgehog, related to the mouse Indian hedgehog and Desert hedgehog, respectively, are strongly expressed in the central retinal pigment epithelium but excluded from the peripheral pigment epithelium surrounding the ciliary marginal zone. By contrast, downstream components of the Hedgehog signalling pathway, Gli2, Gli3 and X-Smoothened, are expressed in this narrow peripheral epithelium. We show that this zone contains cells that are in the proliferative state. This equivalent region in the adult mammalian eye, the pigmented ciliary epithelium, has been identified as a zone in which retinal stem cells reside. These data, combined with double labelling and the use of other retinal pigment epithelium markers, show that the retinal pigment epithelium of tadpole embryos has a molecularly distinct peripheral to central axis. In addition, Gli2, Gli3 and X-Smoothened are also expressed in the neural retina, in the most peripheral region of the ciliary marginal zone, where retinal stem cells are found in Xenopus, suggesting that they are good markers for retinal stem cells. To test the role of the Hedgehog pathway at different stages of retinogenesis, we activated the pathway by injecting a dominant-negative form of PKA or blocking it by treating embryos with cyclopamine. Embryos injected or treated at early stages display clear proximodistal defects in the retina. Interestingly, the main phenotype of embryos treated with cyclopamine at late stages is a severe defect in RPE differentiation. This study thus provides new insights into the role of Hedgehog signalling in the formation of the proximodistal axis of the eye and the differentiation of retinal pigment epithelium.

Tsarovina, K., A. Pattyn, et al. (2004). "Essential role of Gata transcription factors in sympathetic neuron development." Development **131**(19): 4775-4786.

http://dev.biologists.org/cgi/content/abstract/131/19/4775

Sympathetic neurons are specified during their development from neural crest precursors by a network of crossregulatory transcription factors, which includes Mash1, Phox2b, Hand2 and Phox2a. Here, we have studied the function of Gata2 and Gata3 zinc-finger transcription factors in autonomic neuron development. In the chick, Gata2 but not Gata3 is expressed in developing sympathetic precursor cells. Gata2 expression starts after Mash1, Phox2b, Hand2 and Phox2a expression, but before the onset of the noradrenergic marker genes Th and Dbh, and is maintained throughout development. Gata2 expression is affected in the chick embryo by Bmp gain- and loss-of-function experiments, and by overexpression of Phox2b, Phox2a, Hand2 and Mash1. Together with the lack of Gata2/3 expression in Phox2b knockout mice, these results characterize Gata2 as member of the Bmp-induced cluster of transcription factors. Loss-of-function experiments resulted in a strong reduction in the size of the sympathetic chain and in decreased Th expression. Ectopic expression of Gata2 in chick neural crest precursors elicited the generation of neurons with a non-autonomic, Th-negative phenotype. This implies a function for Gata factors in autonomic neuron differentiation, which, however, depends on co-regulators present in the sympathetic lineage. The present data establish Gata2 and Gata3 in the chick and mouse, respectively, as essential members of the transcription factor network controlling sympathetic neuron development.

Woods, R. G., K. E. Roper, et al. (2004). "Gene expression during early ascidian metamorphosis requires signalling by Hemps, an EGF-like protein." Development **131**(12): 2921-2933.

http://dev.biologists.org/cgi/content/abstract/131/12/2921

Hemps, a novel epidermal growth factor (EGF)-like protein, is expressed during larval development and early metamorphosis in the ascidian Herdmania curvata and plays a direct role in triggering metamorphosis. In order to identify downstream genes in the Hemps pathway we used a gene expression profiling approach, in which we compared post-larvae undergoing normal metamorphosis with larval metamorphosis blocked with an anti-Hemps antibody. Molecular

profiling revealed that there are dynamic changes in gene expression within the first 30 minutes of normal metamorphosis with a significant portion of the genome (approximately 49%) being activated or repressed. A more detailed analysis of the expression of 15 of these differentially expressed genes through embryogenesis, larval development and metamorphosis revealed that while there is a diversity of temporal expression patterns, a number of genes are transiently expressed during larval development and metamorphosis. These and other differentially expressed genes were localised to a range of specific cell and tissue types in Herdmania larvae and post-larvae. The expression of approximately 24% of the genes that were differentially expressed during early metamorphosis was affected in larvae treated with the anti-Hemps antibody. Knockdown of Hemps activity affected the expression of a range of genes within 30 minutes of induction, suggesting that the Hemps pathway directly regulates early response genes at metamorphosis. In most cases, it appears that the Hemps pathway contributes to the modulation of gene expression, rather than initial gene activation or repression. A total of 151 genes that displayed the greatest alterations in expression in response to anti-Hemps antibody were sequenced. These genes were implicated in a range of developmental and physiological roles, including innate immunity, signal transduction and in the regulation of gene transcription. These results suggest that there is significant gene activity during the very early stages of H. curvata metamorphosis and that the Hemps pathway plays a key role in regulating the expression of many of these genes.

Zhu, J., K. Motejlek, et al. (2002). "{beta}8 integrins are required for vascular morphogenesis in mouse embryos." Development **129**(12): 2891-2903.

http://dev.biologists.org/cgi/content/abstract/129/12/2891

In order to assess the in vivo function of integrins containing the {beta}8 subunit, we have generated integrin {beta}8-deficient mice. Ablation of {beta}8 results in embryonic or perinatal lethality with profound defects in vascular development. Sixty-five percent of integrin {beta}8-deficient embryos die at midgestation, with evidence of insufficient vascularization of the placenta and yolk sac. The remaining 35% die shortly after birth with extensive intracerebral hemorrhage. Examination of brain tissue from integrin {beta}8-deficient embryos reveals abnormal vascular morphogenesis resulting in distended and leaky capillary vessels, as well as aberrant brain capillary patterning. In addition, endothelial cell hyperplasia is found in these mutant brains. Expression studies show that integrin {beta}8 transcripts are localized in endodermal cells surrounding endothelium in the yolk sac and in periventricular cells of the neuroepithelium in the brain. We propose that integrin {beta}8 is required for vascular morphogenesis by providing proper cues for capillary growth in both yolk sac and embryonic brain. This study thus identifies a molecule crucial for vascular patterning in embryonic yolk sac and brain.

Developmental Biology (15)

Haines, B. P., R. Gupta, et al. "The NLRR gene family and mouse development: Modified differential display PCR identifies NLRR-1 as a gene expressed in early somitic myoblasts." Developmental Biology **In Press, Corrected Proof** http://www.sciencedirect.com/science/article/B6WDG-4FNCVTW-1/2/5c93b90434653798c619b28fb64cd0d5

During vertebrate embryogenesis, the somites form by segmentation of the trunk mesoderm,

lateral to the neural tube, in an anterior to posterior direction. Analysis of differential gene expression during somitogenesis has been problematic due to the limited amount of tissue available from early mouse embryos. To circumvent these problems, we developed a modified differential display PCR technique that is highly sensitive and yields products that can be used directly as in situ hybridisation probes. Using this technique, we isolated NLRR-1 as a gene expressed in the myotome of developing somites but not in the presomitic mesoderm. Detailed expression analysis showed that this gene was expressed in the skeletal muscle precursors of the myotome, branchial arches and limbs as well as in the developing nervous system. Somitic expression occurs in the earliest myoblasts that originate from the dorsal lip in a pattern reminiscent of the muscle determination gene Myf5, but not at the ventral lip, indicating that NLRR-1 is expressed in a subset of myotome cells. The NLRR genes comprise a three-gene family encoding glycosylated transmembrane proteins with external leucine-rich repeats, a fibronectin domain, an immunoglobulin domain and short intracellular tails capable of mediating protein-protein interaction. Analysis of NLRR-3 expression revealed regulated expression in the neural system in developing ganglia and motor neurons. NLRR-2 expression appears to be predominately confined to the adult. The regulated embryonic expression and cellular location of these proteins suggest important roles during mouse development in the control of cell adhesion, movement or signalling.

Hamdoun, A. M., G. N. Cherr, et al. (2004). "Activation of multidrug efflux transporter activity at fertilization in sea urchin embryos (Strongylocentrotus purpuratus)." Developmental Biology **276**(2): 452.

http://www.sciencedirect.com/science/article/B6WDG-4DJBP6T-1/2/47adc2c79e907615cfb8b87c90af4132

This study presents functional and molecular evidence for acquisition of multidrug transporter-mediated efflux activity as a consequence of fertilization in the sea urchin. Sea urchin eggs and embryos express low levels of efflux transporter genes with homology to the multidrug resistance associated protein (mrp) and permeability glycoprotein (p-gp) families of ABC transporters. The corresponding efflux activity is low in unfertilized eggs but is dramatically upregulated within 25 min of fertilization; the expression of this activity does not involve de novo gene expression and is insensitive to inhibitors of transcription and translation indicating activation of pre-existing transporter protein. Our study, using specific inhibitors of efflux transporters, indicates that the major activity is from one or more mrp-like transporters. The expression of activity at fertilization requires microfilaments, suggesting that the transporters are in vesicles and moved to the surface after fertilization. Pharmacological inhibition of mrp-mediated efflux activity with MK571 sensitizes embryos to the toxic compound vinblastine, confirming that one role for the efflux transport activity is embryo protection from xenobiotics. In addition, inhibition of mrp activity with MK571 alone retards mitosis indicating that mrp-like activity may also be required for early cell divisions.

Holland, N. D., T. V. Venkatesh, et al. (2003). "Amphink2-tin, an amphioxus homeobox gene expressed in myocardial progenitors: insights into evolution of the vertebrate heart." Developmental Biology **255**(1): 128.

http://www.sciencedirect.com/science/article/B6WDG-4816430-5/2/06a20caf404113b84b200ac348198baa

We isolated a full-length cDNA clone of amphioxus AmphiNk2-tin, an NK2 gene similar in sequence to vertebrate NK2 cardiac genes, suggesting a potentially similar function to Drosophila tinman and to vertebrate NK2 cardiac genes during heart development. During the neurula stage of amphioxus, AmphiNk2-tin is expressed first within the foregut endoderm, then transiently in

muscle precursor cells in the somites, and finally in some mesoderm cells of the visceral peritoneum arranged in an approximately midventral row running beneath the midgut and hindgut. The peritoneal cells that express AmphiNk2-tin are evidently precursors of the myocardium of the heart, which subsequently becomes morphologically detectable ventral to the gut. The amphioxus heart is a rostrocaudally extended tube consisting entirely of myocardial cells (at both the larval and adult stages); there are no chambers, valves, endocardium, epicardium, or other differentiated features of vertebrate hearts. Phylogenetic analysis of the AmphiNk2-tin sequence documents its close relationship to vertebrate NK2 class cardiac genes, and ancillary evidence suggests a relationship with the Drosophila NK2 gene tinman. Apparently, an amphioxus-like heart, and the developmental program directing its development, was the foundation upon which the vertebrate heart evolved by progressive modular innovations at the genetic and morphological levels of organization.

Kearney, J. B., S. R. Wheeler, et al. (2004). "Gene expression profiling of the developing Drosophila CNS midline cells." Developmental Biology **275**(2): 473.

http://www.sciencedirect.com/science/article/B6WDG-4DFBSN9-2/2/1db3535cfa35454f46f44738590183ff

The Drosophila CNS midline cells constitute a specialized set of interneurons, motorneurons, and glia. The utility of the CNS midline cells as a neurogenomic system to study CNS development derives from the ability to easily identify CNS midline-expressed genes. For this study, we used a variety of sources to identify 281 putative midline-expressed genes, including enhancer trap lines, microarray data, published accounts, and the Berkeley Drosophila Genome Project (BDGP) gene expression data. For each gene, we analyzed expression at all stages of embryonic CNS development and categorized expression patterns with regard to specific midline cell types. Of the 281 candidates, we identified 224 midline-expressed genes, which include transcription factors, signaling proteins, and transposable elements. We find that 58 genes are expressed in mesectodermal precursor cells, 138 in midline primordium cells, and 143 in mature midline cells--50 in midline glia, 106 in midline neurons. Additionally, we identified 27 genes expressed in glial and mesodermal cells associated with the midline cells. This work provides the basis for future research that will generate a complete cellular and molecular map of CNS midline development, thus allowing for detailed genetic and molecular studies of neuronal and glial development and function.

Krovel, A. V. and L. C. Olsen (2004). "Sexual dimorphic expression pattern of a splice variant of zebrafish vasa during gonadal development." Developmental Biology **271**(1): 190.

http://www.sciencedirect.com/science/article/B6WDG-4CB083R-4/2/e1989093de162a78044c1f68a1b4b030

In Drosophila, the RNA helicase VASA (VAS) is required for both germ line formation and oocyte differentiation. While the murine VAS homologue is required for spermatogenesis, it is dispensable for germ line formation. The molecular basis for this apparently dual role of VAS in germ line ontogeny is, however, unclear. Recent evidence indicates that fish, like flies, employs VAS both in early and late stages of the germ line development and that there is a sex-linked differential expression of splice variants. We show here that the longer of two splice variants of zebrafish vas is transiently downregulated in the germ line around the time when the germ cells reach the developing gonad. Using transgenic vas:EGFP fish lines, which allow us to distinguish between male and female individuals, we show that the long splice variant reappears in both sexes at around day 25 and is subsequently downregulated during male gonadal development. Our data further suggest that there is a switch from maternal to zygotic expression of the long

splice variant of vas as sexual dimorphic development commences.

Laird, D. J. and I. L. Weissman (2004). "Telomerase maintained in self-renewing tissues during serial regeneration of the urochordate Botryllus schlosseri." Developmental Biology **273**(2): 185.

http://www.sciencedirect.com/science/article/B6WDG-4CX031V-1/2/cc5eb924021be09d9e081576fa513588

Telomerase is critical for the protection of germ line and stem cell chromosomes from fatal shortening during replication. In most organisms, telomerase activity is suppressed in progressively committed cells and falls to basal rates in terminally differentiated lineages. The colonial ascidian Botryllus schlosseri propagates asexually and sexually, presumably from pools of stem cells that self-renew throughout the 2- to 5-year colony life span. Asexual budding takes place continuously from the parental body wall. When the colony reaches a critical size, sexual reproduction commences with the generation of gonads. Here, we establish the existence of 6-15 kb telomeres on the ends of Botryllus chromosomes. We develop a real-time quantitative PCR telomeric repeat amplification protocol (TRAP) assay that reliably detects 0.2-100 TPG units in cells and tissues. We find highest levels of enzymatic activity in the gonads, developing embryos, and tissues containing the earliest asexual buds. Telomerase activity appears to be suppressed in later buds during organogenesis and falls to basal rates in mature zooids. We postulate that this pattern reflects maximum telomere restoration in somatic stem cells of early buds and suppression of telomerase activity in progenitors and terminally differentiated cells, indicative of an alternate role for stem cells as repeated body regenerators in colonial life histories.

Millen, K. J., J. H. Millonig, et al. (2004). "Roof plate and dorsal spinal cord dl1 interneuron development in the dreher mutant mouse." Developmental Biology **270**(2): 382.

http://www.sciencedirect.com/science/article/B6WDG-4C76C1G-1/2/457ba64104db88cb6e178a3c56ed6276

The establishment of neural circuits in the spinal cord depends on the differentiation of functionally distinct types of neurons in the embryonic neural tube. A number of genes have recently been shown to control the generation of dorsal interneurons through inductive signals provided by the roof plate. The roof plate is a transient signaling center on the dorsal midline of the neural tube that coordinates dorsal CNS development through the action of local peptide signals, primarily the bone morphogenic proteins (BMPs) and the Wingless-related genes (Wnts). The role of the roof plate has become evident through studies of mutations of genes in these gene families, and through several spontaneously occurring mouse mutants, including dreherJ (drJ), all of which cause dorsal neural tube defects. We previously demonstrated that the roof plate is missing in the dreher mouse. Positional cloning of the dreher locus demonstrated that an inactivating point mutation in the LIM homeodomain (HD) transcription factor encoded by the Lmx1a gene, is responsible for the dreherJ phenotype [Nature, 403 (2000) 764]. Here we report that Lmx1a is first expressed at E8.5 in a small number of cells in the lateral neural plate. As the neural tube closes, Lmx1a expression is restricted to the roof plate. In drJ/drJ, although non-functional Lmx1a is correctly expressed at E8.5-E9.5, its expression is lost in the spinal cord roof plate by E10.5. Coincident with the loss of Lmx1a expression, Bmp expression fails, and the generation and differentiation of the dorsal-most spinal cord neurons, the dl1 interneurons, is abnormal. In drJ/drJ embryos, defects are evident in the number of dl1 progenitors, as well as in their migration to form the lateral and medial nuclei, and axon patterning, through mechanisms that apparently involve defects in early steps of neuronal polarity. Consistent with the general hypothesis that a failure of roof plate formation and function results in deficits in dorsal patterning of the neural tube, the dreher affects the generation and differentiation of the dl1 interneuron

population.

Morck, C., M. Rauthan, et al. (2004). "pha-2 encodes the C. elegans ortholog of the homeodomain protein HEX and is required for the formation of the pharyngeal isthmus." Developmental Biology **272**(2): 403.

http://www.sciencedirect.com/science/article/B6WDG-4CP68N2-3/2/202818a0aabb712f8e60d714ca77ba5f

The pha-2 mutant was isolated in 1993 by Leon Avery in a screen for worms with visible defects in pharyngeal feeding behavior. In pha-2 mutant worms, the pharyngeal isthmus is abnormally thick and short and, in contrast to wild-type worms, harbors several cell nuclei. We show here that pha-2 encodes a homeodomain protein and is homologous to the vertebrate homeobox gene, Hex (also known as Prh). Consistent with a function in pharyngeal development, the pha-2 gene is expressed in the pharyngeal primordium of Caenorhabditis elegans embryos, particularly in pm5 cells that form the bulk of the isthmus. We show that in the pha-2 mutant there is a failure of the pm5 cells to elongate anteriorly while keeping their nuclei within the nascent posterior bulb to form the isthmus during the 3-fold embryonic stage. We also present evidence that pha-2 regulates itself positively in pm5 cells, that it is a downstream target of the forkhead gene pha-4, and that it may also act in the isthmus as an inhibitor of the ceh-22 gene, an Nkx2.5 homolog. Finally, we have begun characterizing the regulation of the pha-2 gene and find that intronic sequences are essential for the complete pha-2 expression profile. The present report is the first to examine the expression and function of an invertebrate Hex homolog, that is, the C. elegans pha-2 gene.

Natale, D. R., A. J. M. Paliga, et al. (2004). "p38 MAPK signaling during murine preimplantation development." Developmental Biology **268**(1): 76.

http://www.sciencedirect.com/science/article/B6WDG-4BJ1YSP-3/2/962bdca2a4156edeac6ff12f4a66ecab

Mitogen-activated protein kinase (MAPK) pathways mediate some important cellular processes and are likely to also regulate preimplantation development. The role of p38 MAP kinase signaling during murine preimplantation development was investigated in the present study. p38 MAPK, p38-regulated or -activated kinase (PRAK; MK5), map kinase-activated protein kinase 2 (MK2), and heat shock protein 25 (hsp25) mRNAs and proteins were detected throughout preimplantation development. Two-cell stage embryos cultured in the presence of SB220025 and SB203580 (specific inhibitors of p38 MAPK [alpha]/[beta]), progressed to the eight-cell stage with the same frequency as controls; however, treated embryos halted their development at the 8- to 16-cell stage. In addition, embryos treated with p38 MAPK inhibitors displayed a complete loss of MK2 and hsp25 phosphorylation and also a complete loss of filamentous actin as indicated by the absence of rhodamine-phalloidin staining. In these inhibitor-treated groups, the embryos were composed of a mixture of compacting and noncompacting cells, and the embryos were one to two cell divisions behind controls. Treated embryos remained viable as the developmental blockade was rescued by removing embryos from the drug treatment and placing them in drug-free medium until they progressed to the blastocyst stage. This study demonstrates that p38 MAPK activity is required to support development through the murine preimplantation interval.

Stein, P., P. Svoboda, et al. (2003). "Transgenic RNAi in mouse oocytes: a simple and fast approach to

study gene function." Developmental Biology **256**(1): 188.

http://www.sciencedirect.com/science/article/B6WDG-47WD69K-H/2/f877dbf2d9c8ff8bbd329d25974d0283

Double-strand RNA (dsRNA)-mediated posttranscriptional gene silencing, also known as RNA interference (RNAi), is a powerful tool to inhibit gene expression in several experimental model systems, including Arabidopsis, Caenorhabditis, and Drosophila. We previously described that the microinjection of Mos dsRNA into fully grown mouse oocytes results in the specific degradation of Mos mRNA in a time- and concentration-dependent manner. We report here a transgenic RNAi approach that is suitable to study gene function during mouse oocyte development and differentiation. The oocyte-specific Zp3 promoter was used to drive the expression of a long hairpin dsRNA (~500 bp) targeting Mos mRNA. Transgenic founder animals appeared healthy, but while males were fertile, females were not, in accordance with the known Mos null phenotype. The amount of Mos mRNA in the transgenic F1 females was reduced by >90%, whereas there was no decrease in the nontargeted tissue plasminogen activator (Plat) mRNA. Moreover, the maturation-associated increase in mitogen-activated protein (MAP) kinase activity was not observed, and the metaphase II eggs underwent spontaneous parthenogenetic activation, thus recapitulating the Mos null phenotype. This approach provides a powerful method to study the functions of any oocyte-synthesized gene during oocyte development and early embryogenesis.

Talian, J. C. and P. S. Zelenka (1991). "Calpactin I in the differentiating embryonic chicken lens: mRNA levels and protein distribution." Developmental Biology **143**(1): 68.

http://www.sciencedirect.com/science/article/B6WDG-4DKTPB0-6/2/9dda2a5a1e9d832ac72202a187dd23f7

Calpactin I, one of the EDTA-extractable proteins of the lens membrane, binds phospholipid and actin in a calcium-dependent manner. It is also a known substrate of the pp60src kinase. Analysis of embryonic chicken lens RNA with a bovine calpactin I-specific cDNA probe revealed the presence of a ~1.8 Kb calpactin mRNA in the lens cells. Six-day embryonic chicken lenses were microdissected into central epithelium, equatorial epithelium, and fiber cells. Total cytoplasmic RNA was isolated from these samples and calpactin I mRNA levels were determined by the polymerase chain reaction (PCR) following reverse transcription (RT). Quantitative PCR indicates that the calpactin I mRNA levels in the equatorial epithelium are greater than in the central epithelium by a factor of 12.7 +/- 2.7. Calpactin I mRNA in fiber cells is an additional 3.5 +/- 1.5 times greater than in the equatorial epithelium. Whole mounts of embryonic chicken lens epithelia and histological sections of whole lenses were also examined with an antibody directed against chicken calpactin I. Calpactin I was predominantly localized in a punctate distribution in equatorial epithelial cells and near the plasma membrane of elongate fiber cells. The elevated levels of calpactin I mRNA observed in the equatorial epithelium and fiber cells and the immunological localization of the protein suggest a possible role of calpactin I in the elongation of fiber cells during lens differentiation.

Tanaka, K. J., K. Matsumoto, et al. (2004). "CiYB1 is a major component of storage mRNPs in ascidian oocytes: implications in translational regulation of localized mRNAs." Developmental Biology **272**(1): 217.

http://www.sciencedirect.com/science/article/B6WDG-4CJXT1T-1/2/c599acf524c87adeca7acc1f10f709b3

In ascidian eggs, the existence of several localized maternal cytoplasmic determinants has been proposed and the importance of localized mRNAs for tissue differentiation has been demonstrated. We previously identified the ascidian Y-box proteins (CiYB1, 2 and 3), homologues of which are known to be involved in the storage of maternal mRNA in oocytes of other organisms. In this study, we found that CiYB1 protein is abundant in the gonad, egg, and embryo. Purification of messenger ribonucleoprotein (mRNP) particles from the gonad revealed that CiYB1 was one of their major components. A significant change in the distribution of CiYB1 protein from stored mRNP particles in the gonad to the ribosome fraction in eggs and embryos was observed. This change correlates most likely with the shift of stored maternal mRNAs to polyribosomes. Moreover, we found that CiYB1 colocalized with Cipem and Ci-macho1 mRNAs, which are localized at the posterior end of the embryo at the cleavage stage. Cipem and Ci-macho1 mRNAs were co-immunoprecipitated with CiYB1 in the oocyte and embryo lysates. The formation of a complex between Cipem mRNA and CiYB1 protein resulted in translational repression in the in vitro translation system. Our results indicate that associating with CiYB1 protein contributes to the translational control of the localized mRNA in eggs and embryos.

Warburton, D., R. Seth, et al. (1992). "Epigenetic role of epidermal growth factor expression and signalling in embryonic mouse lung morphogenesis." Developmental Biology **149**(1): 123.

http://www.sciencedirect.com/science/article/B6WDG-4F030CR-7T/2/fd76d6bdc31e6be20bc5a3ae29764cf0

A major unsolved problem in developmental biology is to determine when and how time- and position-restricted instructions are signaled and received during secondary embryonic inductions such as branching morphogenesis. The mouse embryonic lung rudiment was used to test the hypothesis that endogenous peptide growth factors, specifically epidermal growth factor (EGF), serve as instructive epigenetic signals for morphogenesis. The presence of EGF precursor mRNA transcripts was detected using the reverse-transcriptase-coupled polymerase chain reaction both in E11-E17-day mouse embryo lung tissues in vivo and in E11-day lung cultured for up to 7 days in vitro under chemically defined, serum-free conditions. Immunolocalization identified a position-restricted distribution of EGF in and around the primitive airways both during in vivo lung morphogenesis and in culture. EGF receptors (EGFR) coimmunolocalized with EGF in the primitive airways. Addition of exogenous EGF to lungs in culture resulted in significant concentration-dependent stimulation of branching morphogenesis, DNA, RNA, and protein content, and in [3H]thymidine incorporation into DNA. Conversely, the addition of tyrphostin (specific EGF receptor kinase antagonist) to lungs in culture resulted in concentration-dependent inhibition of branching morphogenesis, DNA, RNA, and protein content, and in [3H]thymidine incorporation into DNA without apparent cytotoxicity. The inhibition of the EGF signal by tyrphostin was confirmed by immunoprecipitation of tyrosine phosphoproteins. We conclude that early mouse embryo lungs express EGF transcripts and corresponding EGF peptides in a specific position-restricted distribution which coimmunolocalizes with EGFR in the primitive airways, while stimulatory and inhibitory studies indicate a functional role for the transduced EGF signal in the epigenetic regulation of lung branching morphogenesis. We speculate that the peptide growth factor EGF serves a function in secondary embryonic morphogenetic inductions, which may be modulated by interaction with other growth factors.

Wright, P. W., L. C. Bolling, et al. (2003). "ePAD, an oocyte and early embryo-abundant peptidylarginine deiminase-like protein that localizes to egg cytoplasmic sheets." Developmental Biology **256**(1): 74.

http://www.sciencedirect.com/science/article/B6WDG-47X6RT9-4/2/b6849f3d3c1d9e483dd3318c46280c56

Selected for its high relative abundance, a protein spot of MW ~75 kDa, pI 5.5 was cored from a Coomassie-stained two-dimensional gel of proteins from 2850 zona-free metaphase II mouse eggs and analyzed by tandem mass spectrometry (TMS), and novel microsequences were identified that indicated a previously uncharacterized egg protein. A 2.4-kb cDNA was then amplified from a mouse ovarian adapter-ligated cDNA library by RACE-PCR, and a unique 2043-bp open reading frame was defined encoding a 681-amino-acid protein. Comparison of the deduced amino acid sequence with the nonredundant database demonstrated that the protein was ~40% identical to the calcium-dependent peptidylarginine deiminase (PAD) enzyme family. Northern blotting, RT-PCR, and in situ hybridization analyses indicated that the protein was abundantly expressed in the ovary, weakly expressed in the testis, and absent from other tissues. Based on the homology with PADs and its oocyte-abundant expression pattern, the protein was designated ePAD, for egg and embryo-abundant peptidylarginine deiminase-like protein. Anti-recombinant ePAD monospecific antibodies localized the molecule to the cytoplasm of oocytes in primordial, primary, secondary, and Graafian follicles in ovarian sections, while no other ovarian cell type was stained. ePAD was also expressed in the immature oocyte, mature egg, and through the blastocyst stage of embryonic development, where expression levels began to decrease. Immunoelectron microscopy localized ePAD to egg cytoplasmic sheets, a unique keratin-containing intermediate filament structure found only in mammalian eggs and in early embryos, and known to undergo reorganization at critical stages of development. Previous reports that PAD-mediated deimination of epithelial cell keratin results in cytoskeletal remodeling suggest a possible role for ePAD in cytoskeletal reorganization in the egg and early embryo.

Zerlin, M., A. Milosevic, et al. (2004). "Glial progenitors of the neonatal subventricular zone differentiate asynchronously, leading to spatial dispersion of glial clones and to the persistence of immature glia in the adult mammalian CNS." Developmental Biology **270**(1): 200.

http://www.sciencedirect.com/science/article/B6WDG-4C4WXCW-2/2/54f3d1692748ceebfd2a41ecd45b5136

The subventricular zone (SVZ) of the developing mammalian forebrain gives rise to astrocytes and oligodendrocytes in the neocortex and white matter, and neurons in the olfactory bulb in perinatal life. We have examined the developmental fates and spatial distributions of the descendants of single SVZ cells by infecting them in vivo at postnatal day 0-1 (P0-1) with a retroviral "library". In most cases, individual SVZ cells gave rise to either oligodendrocytes or astrocytes, but some generated both types of glia. Members of glial clones can disperse widely through the gray and white matter. Progenitors continued to divide after stopping migration, generating clusters of related cells. However, the progeny of a single SVZ cell does not differentiate synchronously: individual clones contained both mature and less mature glia after short or long intervals. For example, progenitors that settled in the white matter generated three types of clonal oligodendrocyte clusters: those composed of only myelinating oligodendrocytes, of both myelinating oligodendrocytes and non-myelinating oligodendrocytes, or of only non-myelinating cells of the oligodendrocyte lineage. Thus, some progenitors do not fully differentiate, but remain immature and may continue to cycle well into adult life.

Dig Dis Sci (1)

Shitoh, K., K. Koinuma, et al. (2004). "Mutation of beta-catenin does not coexist with K-ras mutation in

colorectal tumorigenesis." Dig Dis Sci **49**(10): 1631-3.

Alterations of the APC, K-ras, and beta-catenin genes are defined as early events in colorectal tumorigenesis. These alterations are well-known as constitutents of Vogelstein's pathway, however, the relationship among them is unclear. For understanding colorectal tumorigenesis it is important to evaluate their relationship. We analyzed the relationship between beta-catenin and K-ras gene mutations in clinical colorectal samples. Sixty-four cases of colorectal cancers (44 proximal, 20 distal) without a family history of colorectal cancer were used for this study. We purified genomic DNAs from fresh surgical samples and, thus, analyzed the mutations of beta-catenin (exon 3) and K-ras (codons 12 and 13) by PCR direct sequencing method using Big Dye terminator cycle sequencing with AmpliTaq polymerase FS. We found 27% (17/64) K-ras mutations (proximal 25%, 11/44; distal 30%, 6/20). The frequency of beta-catenin mutations was 11% (7/64; proximal 9%, 4/44; distal 15%, 3/20). All cases with beta-catenin mutation had no mutation of K-ras. All sites of beta-catenin mutation have been reported previously (codons 33, 34, 41, 45). In cell lines, it has been reported previously that beta-catenin and K-ras play the same roles in activation of cyclin D1 transcription. Our results may support this report and suggest that some colorectal cancers with beta-catenin mutation will progress without K-ras mutation. Further study may disclose a new pathway or new mechanism of colorectal tumorigenesis.

Domestic Animal Endocrinology (4)

Cordano, P., H. M. Hammon, et al. (2000). "mRNA of insulin-like growth factor (IGF) quantification and presence of IGF binding proteins, and receptors for growth hormone, IGF-I and insulin, determined by reverse transcribed polymerase chain reaction, in the liver of growing and mature male cattle." Domestic Animal Endocrinology **19**(3): 191.

http://www.sciencedirect.com/science/article/B6T62-41JM94F-5/2/04374e7b5c1cd6887435f1633a356a4f

Plasma insulin-like growth factor-I (IGF-I) concentrations were related to hepatic levels of IGF-I mRNA measured by competitive reverse transcription polymerase chain reaction (PCR) (RT-PCR) in neonatal (8 d old) calves, veal calves, fattened castrated bulls and mature intact bulls. Furthermore, the presence of mRNAs of IGF-II and of receptors for IGF-I (IGF-IR), growth hormone (GHR) and insulin (IR), as well as mRNAs of IGF binding proteins (IGFBP-1, -2 and -3) were assessed by RT-PCR. Hepatic IGF-I mRNA levels and plasma IGF-I concentrations in veal calves, fattened castrated bulls and in intact bulls were 4 to 8 times higher than in 8-d old calves and were 2 to 3 times higher in calves fed colostrum than in calves fed only milk replacer. Hepatic IGF-I mRNA concentrations were closely correlated ($r = 0.92$) with plasma IGF-I concentrations, suggesting that hepatic IGF-I production largely determines plasma IGF-I levels. The presence of IGF II, IGF-IR, GHR, IR and IGFBP-1, -2 and -3 mRNA was confirmed in the liver of 8-d old calves and older cattle as well, and among newborn calves their presence was independent of differences in nutrition. In conclusion, the major hepatic components of the GH-IGF axis were present in neonatal calves, but the IGF-I expression and therefore also plasma IGF-I levels were relatively low.

Lucy, M. C., S. D. Hauser, et al. (1993). "Variants of somatotropin in cattle: gene frequencies in major dairy breeds and associated milk production." Domestic Animal Endocrinology **10**(4): 325.

http://www.sciencedirect.com/science/article/B6T62-47RSDPG-6/2/9e7d6a024952e983571a9a3d6a243617

The amino acid sequence of bovine somatotropin (bST) varies at position 127 where either valine or leucine is found. The frequencies of leucine127 and valine127 bST gene alleles in cows (n = 302) and sires (n = 70) from major dairy breeds (Holstein, Brown Swiss, Guernsey, Jersey, and Ayrshire) were determined using DNA extracted from whole blood or spermatozoa. A 428 base pair fragment of the bST gene was amplified using polymerase chain reaction (PCR) and variants of the bST gene were detected as polymorphisms by Alu I restriction endonuclease digestion of PCR products. Restriction enzyme DNA fragments for the leucine127 variant were 265, 96, 51, and 16 base pair and for the valine127 variant were 265, 147, and 16 base pair as a polymorphism of bST was present in the 147 base pair DNA fragment. Frequencies of leucine127 and valine127 alleles for cows (n=302) were 1.0 and 0 for Brown Swiss.93 and.07 for Holstein.92 and.08 for Guernsey.79 and.21 for Ayrshire, and.56 and.44 for Jersey, respectively. In Holstein sires used for artificial insemination (n=70), the frequency of leucine127 and valine127 alleles was.96 and.04. Estimates of transmitting ability for milk production tended to be greater for Holstein cows that were homozygous for leucine127 bST and Jersey cows that were homozygous for valine127 bST whereas Holstein sires with different bST genotypes were similar. In summary, frequencies of alleles for the bST gene were not similar in different dairy breeds and estimates of milk production were correlated with bST gene variant in cows but not sires.

Malayer, J. R. and V. M. Woods (1998). "Expression of Estrogen Receptor and Maintenance of Hormone-Responsive Phenotype in Bovine Fetal Uterine Cells." Domestic Animal Endocrinology **15**(3): 141.

http://www.sciencedirect.com/science/article/B6T62-3V4KPGW-1/2/c6b23533c85b8c4c634289c2881c6053

Objectives were to establish conditions for preparation, growth, and maintenance of a primary culture cell model of fetal uterine cells, and to determine whether cells maintained under those conditions would maintain their capacity to respond to estrogen stimulation in vitro. Fetal uteri (n = 19) were enzymatically dispersed and grown on Type 1 collagen in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum. Fetal-uterine cells appeared fibroblast-like and exhibited positive immunostaining for both vimentin and cytokeratin. Effects of gestational age (GA), passage number (p), and hormonal treatment on appearance of specific mRNAs were determined by RT-PCR; relative concentrations of products determined by densitometry were analyzed as the ratio of target cDNA to the GAPDH loading control. Cells expressed mRNAs for estrogen receptor (ER), TGF-[beta], EGF-R, PRL-R, IL-1 [alpha], and IL-6. ER mRNA was greater at 185-200 than at 100-110 d GA ($P < 0.01$). All specific mRNAs examined were greater in p5 cells than p2 at both 100-110 ($P < 0.01$) and 185-200 d GA ($P < 0.02$). There was no effect of estradiol on these specific mRNAs in cells from 100-110 d GA; at 185-200 d GA, there was an estradiol (1.0 n) effect both at 6 hr ($P < 0.001$) and 24 hr ($P < 0.02$). Overall, there was an effect of 8-br-cAMP (1 m; 6 h) on specific mRNAs in cells at both 100-110 ($P < 0.001$) and 185-200 d GA ($P < 0.001$). In p5 cells from Day 185-200 GA, there was increased cell proliferation ($P < 0.001$) in response to estradiol (1 n; 24 hr). These data suggest that primary fetal uterine cells retain their age-specific and hormone-responsive phenotype under these in vitro conditions.

Voge, J. L., C. A. T. Santiago, et al. (2004). "Quantification of insulin-like growth factor binding protein mRNA using real-time PCR in bovine granulosa and theca cells: effect of estradiol, insulin, and

gonadotropins." Domestic Animal Endocrinology **26**(3): 241.

http://www.sciencedirect.com/science/article/B6T62-4B6TVHD-5/2/b8332e2e585b5579dd5e277481c615a6

The effects of estradiol, insulin, and gonadotropins on levels of insulin-like growth factor binding protein (IGFBP)-2, -3, -4, and -5 mRNA levels in bovine granulosa and theca cells were evaluated in vitro using serum-free medium containing various hormone treatments arranged in four different experiments. Amounts of IGFBP-2, -3, -4 and -5 mRNA were quantitated using fluorescent quantitative real-time RT-PCR. In small-follicle (1-5 mm) granulosa cells, follicle-stimulating hormone (FSH) in the presence or absence of insulin increased (PPP7.9 mm) granulosa cells, insulin alone increased (PP>0.10). Estradiol (3 and 300 ng/ml) decreased (PPP>0.10) on IGFBP-2, -3, or -5 mRNA levels. Estradiol decreased (P<0.05) IGFBP-2, -3, and -4 mRNA levels but had no effect on IGFBP-5 mRNA levels in theca cells. LH had no effect on levels of IGFBP-2, -3, -4, or -5 mRNA in theca cells. These results indicate that expression of IGFBP-2, -3, -4, and -5 mRNA by granulosa and theca cells are differentially regulated by estradiol, insulin and gonadotropins, therefore discretely modulating the amount of bioavailable IGFs to these cells depending upon the specific hormonal stimuli. In particular, these studies are the first in cattle to show that estradiol selectively inhibits IGFBP-2, -3, and -4 gene expression in theca cells, inhibits IGFBP-5 gene expression in large-follicle granulosa cells, and stimulates IGFBP-2 gene expression in small-follicle granulosa cells.

Electrochimica Acta (1)

Matsunaga, T., M. Okochi, et al. (1999). "Construction of an automated DNA detection system for manipulation of Microcystis spp. using specific DNA probe immobilized on magnetic particles." Electrochimica Acta **44**(21-22): 3779.

http://www.sciencedirect.com/science/article/B6TG0-3WV9N09-P/2/9fd72598711cd8434da30e3c54a3c28e

Fully-automated detection system for manipulation of bloom-forming genera of cyanobacteria, Microcystis spp., was developed using a specific DNA probe designed based on sequence polymorphism within the 16S ribosomal DNA (rDNA) of this strain. Specific DNA sequences in 16S rDNA for Microcystis spp were determined by sequence data analysis and two probes were designed for detection of Microcystis spp. Microcystis 16S rDNA was amplified by PCR and applied to the detection system where target DNA was selectively recognized by species-specific hybridization using two DNA probes, a DNA probe conjugated on magnetic particles and a digoxigenin-labeled DNA probe. Using alkaline phosphatase labeled anti-digoxigenin antibody probe, target DNA can be detected by luminescence. In this detection method, the difference in only a few base pairs of target DNA can be discriminated and specific detection of Microcystis spp. was achieved. The appropriate annealing temperature was 60[deg]C. A computer controlled fully-automated system was used for detection of Microcystis spp and detection efficiency equivalent to that of a manual approach was obtained.

Environment International (1)

Jensen, L. B., Y. Agerso, et al. (2002). "Presence of erm genes among macrolide-resistant Gram-positive bacteria isolated from Danish farm soil." Environment International **28**(6): 487.

http://www.sciencedirect.com/science/article/B6V7X-472BHGG-1/2/494f519500afea5c8aa921ac383b3c9e

The presence of erm genes was investigated among macrolide-resistant Gram-positive bacteria isolated from soil samples collected from four Danish farms that had been treated with animal waste. Soil samples were collected before, a few days after spread and 1 months and 5 months later. In 33% (9/27) of these isolates, an erm gene was detected using PCR. Eight isolates were positive for erm(B) and one isolate was positive for erm(C). No isolates contained erm(A), erm(D) or erm(F). The positive isolates were identified to genus level. Two erm(B) positive isolates were identified as Enterococcus spp., and the erm(C)-positive isolate as a Streptococcus spp., probably indicating survival from animal waste. The remaining six erm(B) positive isolates all belonged to the Bacillus cereus group. The erm(B) gene has not previously been identified in B. cereus but is frequently found among enterococci. This result could indicate horizontal transfer from bacteria of animal origin to indigenous soil bacteria.

Environmental Mutagenesis and Related Subjects (2)

Bell, D. A., J. G. Levine, et al. (1991). "DNA sequence analysis of revertants of the hisD3052 allele of Salmonella typhimurium TA98 using the polymerase chain reaction and direct sequencing: Application to 1-nitropyrene-induced revertants." Mutation Research/Environmental Mutagenesis and Related Subjects **252**(1): 35.

http://www.sciencedirect.com/science/article/B73H5-47RNW7X-4Y/2/b29592180fa2fd00c8e8df584ca00592

We have used the polymerase chain reaction (PCR) to speed the DNA sequence analysis of revertants of Salmonella typhimurium TA98. Briefly, a crude DNA extract from a single colony was prepared and used in an asymmetric PCR to amplify a 328-bp fragment containing the hisD3052 mutation approximately in the center. Following ultrafiltration, the ssDNA was sequenced using an end-labeled probe and dideoxy sequencing. The most frequent mutation among the revertants was a -2 deletion of GC or CG within the sequence CGCGCGCG, which is upstream of the hisD3052 mutation. This deletion occurred in 38% (6/16) of the spontaneous (-S9) revertants and in 94% (15/16) of a set of 1-nitropyrene-induced revertants. Other mutations, mostly deletions but also some complex mutations (i.e., single mutational events involving a combination of insertions, deletions, and substitutions), occurred within quasipalindromic regions of DNA. Possible mutational mechanisms are discussed, and the results with 1-NP are compared to those obtained in other systems.

Ushijima, T., Y. Hosoya, et al. (1995). "A rapid method for detection of mutations in the lacI gene using PCR-single strand conformation polymorphism analysis: demonstration of its high sensitivity."

Mutation Research/Environmental Mutagenesis and Related Subjects **334**(3): 283.

http://www.sciencedirect.com/science/article/B73H5-4BGYP09-2/2/662569fab04dd8b445da9cfec4cff96e

The lacI gene has been used as a target gene in various mutation assays. We modified single strand conformation polymorphism (SSCP) analysis by introducing restriction digestion to detect mutations in the gene rapidly, and determined the sensitivity of the method. The entire coding sequence and partial promoter region of the lacI gen were amplified by the polymerase chain reaction with [[alpha]-32P]dCTP in a 1247 base pair fragment, digested into eight restriction fragments, and analyzed by SSCP. The sensitivity of the method was assessed using 160 phages with lacI mutations, which were selected by assay of expression of [beta]-galactosidase after their infection into E. coli. Of the 160 mutants, 146 (91.3%) showed shifted bands in the first condition of SSCP analysis (without glycerol, 20[deg]C). The remaining 14 mutants were analyzed in a second condition (with 5% glycerol, 20[deg]C), and eight of them showed shifted bands (cumulatively 96.3% of the 160 mutants). The remaining six mutants were analyzed in a third condition (with 5% glycerol, 10[deg]C), and all of them showed shifted bands (cumulatively 100%). Sequencing of the restriction fragments with mobility shifts in the 160 mutants revealed 108 kinds of mutations, 100 (92.6%) being detected in the first condition, seven (cumulatively 99.1%) in the second condition, and one (cumulatively 100%) in the third condition. This method greatly reduced the time to identify lacI mutations, and allowed the detection of multiple mutations in one lacI mutant. The results also show that in general PCR-SSCP analysis is very sensitive when test fragments are shorter than about 250 base pairs and electrophoresis is performed under at least two conditions.

Environmental Toxicology and Pharmacology (1)

Ito, T., M. Ikeda, et al. (2000). "Peroxynitrite formation by diesel exhaust particles in alveolar cells: Links to pulmonary inflammation." Environmental Toxicology and Pharmacology **9**(1-2): 1.

http://www.sciencedirect.com/science/article/B6T6D-41Y876R-1/2/879c04aec3fc41ddf598e5a2c382b2f1

Diesel exhaust particles (DEP) are assumed to be a causal substance for pulmonary inflammation. As peroxynitrite is recently implicated in inflammation and cytotoxity, the hypothesis was tested that instillation of DEP induces formation of peroxynitrite in cells migrated in lung. Rats were intratracheally instilled with DEP suspension (2 mg/0.5 ml/kg) and killed 24 h later. Alveolar cells were collected by broncho-alveolar lavage. Population of alveolar cells increased more than twice by DEP exposure, mainly due to a large increase of neutrophils. Peroxynitrite formation (NG-nitro--arginine methylester and superoxide dismutase inhibitable chemiluminescence) was detected in alveolar cells from treated rats, and 12-O-tetradecanoylphorbol 13-acetate-stimulation enhanced it. In addition, DEP induced expression of inducible NO synthase mRNA in these cells. But peroxynitrite was not detectable in cells from control. These results indicate that DEP exposure results in peroxynitrite formation in migrated cells, which leads to pulmonary inflammation.

Enzyme and Microbial Technology (2)

Abbad-Andaloussi, S., C. Lagnel, et al. (2003). "Multi-criteria comparison of resting cell activities of bacterial strains selected for biodesulfurization of petroleum compounds." Enzyme and Microbial Technology **32**(3-4): 446.

http://www.sciencedirect.com/science/article/B6TG1-47MJ556-1/2/4019c86313b6741d32f27162e9d9c1ae

Five isolates able to utilize dibenzothiophene (DBT) as a sole sulfur source and to convert it to 2-hydroxybiphenyl (HBP) with high rates were selected to investigate their potentialities as biocatalysts of a diesel oil biodesulfurization process. Conventional and chemotaxonomic analyses and 16S ribosomal DNA (rDNA) sequencing showed that these strains belonged to the Rhodococcus/Gordonia cluster. The desulfurizing activities of resting cells were compared under various conditions to evaluate their stability in both aqueous and organic media, their sensitivity to the presence of hexadecane and their sulfur substrate selectivity. In spite of their taxonomic similarity, the five strains exhibited different properties. This diversity was not confirmed by the analysis of the desulfurizing genes by amplification and sequencing of large fragments of dszA, dszB, dszC, and dszD genes which revealed that four of the five selected strains had a dsz genotype identical to those of the reference strain, Rhodococcus erythropolis IGTS8.

Louwrier, A. and A. van der Valk "Thermally reversible inactivation of Taq polymerase in an organic solvent for application in hot start PCR." Enzyme and Microbial Technology **In Press, Corrected Proof** http://www.sciencedirect.com/science/article/B6TG1-4FHJYCT-1/2/f0aeb94d61d36171d10a2f19ff3dd0e4

0In the past, Taq polymerase was reversibly inactivated by modification with a dicarboxylic acid anhydride in aqueous media, to enable 'hot start PCR'. However, there are various constraints in using such a method including temperature and concentration. Here we describe an alternative method whereby Taq polymerase may be reversibly inactivated following incubation with an excess of citraconic anhydride at elevated temperatures, in an anhydrous non-protic organic solvent - tert-butyl methyl ether - by first drying the enzyme with a salt or carbohydrate excipient to form an amorphous powder. Reactivation of the enzyme is due to the instability of the chemical modification at low pH following a short incubation in a suitable buffer.

European Journal of Soil Biology (1)

Oravecz, O., G. Nyiro, et al. (2002). "A molecular approach in the analysis of the faecal bacterial community in an African millipede belonging to the family Spirostreptidae (Diplopoda)." European Journal of Soil Biology **38**(1): 67.

http://www.sciencedirect.com/science/article/B6VR7-4593YCJ-F/2/df594d8506e8806abdf591459d00b104

Forty selected bacterial isolates of the faecal microbial community in an African millipede

belonging to the family Spirostreptidae were characterized by amplified ribosomal DNA-restriction analysis (ARDRA) and by sequencing either the first 500 bp or almost the entire length of the 16S rRNA gene. Sequence data show that the faecal microbial community investigated is dominated by members of the class Actinobacteria with most of the strains belonging to the genera Rhodococcus, Cellulomonas and Leifsonia. In certain cases, low similarity values to the previously described 16S rDNA sequences suggest that these strains presumably constitute new species within the Actinobacteria.

Experimental and Molecular Pathology (5)

Angeline, T., N. Jeyaraj, et al. (2004). "Prevalence of MTHFR gene polymorphisms (C677T and A1298C) among Tamilians." Experimental and Molecular Pathology **77**(2): 85.

http://www.sciencedirect.com/science/article/B6WFB-4CMHSX3-1/2/8a93d2f30fa40c34bb2a5a0d958c87da

We have investigated the incidence of the C677T and A1298C methylene tetrahydrofolate reductase (MTHFR) gene single nucleotide polymorphisms (SNPs) in the South Indian Tamil Nadu population with a total number of 72 individuals. The MTHFR genotyping was performed using the polymerase chain reaction followed by restriction enzyme analysis. Homozygosity for the MTHFR A1298C SNP was detected in 15.3% (11/72) of the individuals tested, and 47.2% (34/72) were heterozygous for this SNP. Homozygosity for the C677T MTHFR SNP was detected in 1.38%(1/72), and the frequency of the C677T heterozygotes was 18.1%(13/72). When we analyzed the combined frequency of the two SNPs, the frequency of double heterozygosity was19.6%, and the frequency of double homozygosity was completely absent among the study group. The 'C' allele frequency for MTHFR A1298C was 0.389, and the 'T' allele frequency for C677T mutation was 0.104. Out of the 72 individuals included in the study, 52 were acute myocardial infarction (AMI) patients and 20 were healthy individuals with no documented history of heart disease. The results of this study indicate that the MTHFR A1298C SNP is more prevalent among the Tamilians when compared to the MTHFR C677T SNP, suggesting a possible role of MTHFR A1298C in the pathogenesis of heart diseases.

Dilioglou, S., J. M. Cruse, et al. (2003). "Costimulatory function of umbilical cord blood CD14+ and CD34+ derived dendritic cells." Experimental and Molecular Pathology **75**(1): 18.

http://www.sciencedirect.com/science/article/B6WFB-48M7RHB-1/2/4d34d2a03ef15be2d5fa4eadbd19b483

Dendritic cells (DCs) consist of a heterogeneous population of hematopoietic cells characterized by their unique dendritic morphology, their efficient antigen-presenting capability to activate naive CD4+ and CD8+ T cells, and their lack of lineage specific markers. Functional properties comparing umbilical cord blood monocyte-derived and umbilical cord blood stem cell-derived DCs have not yet been investigated. CD14+ monocytes and CD34+ stem cells were isolated from human umbilical cord blood and were induced to differentiate into dendritic cells using 100 ng/mL granulocyte-macrophage colony stimulating factor (GM-CSF), 25 ng/mL IL-4, 2.5 ng/mL tumor necrosis factor-[alpha] (TNF-[alpha]), 100 ng/mL GM-CSF, 25 ng/mL stem cell factor, and 2.5 ng/mL TNF-[alpha], respectively. Flow cytometric analysis revealed that the 14-day-old dendritic

cells were CD80+, CD86+, CD83+, CD54+, CD1a+, CD11b+, CD11c+, HLA-DR+, CD34-, CD3-, CD19-, CD14-, and CD16-. Reverse transcription polymerase chain reaction was employed to detect expression of mRNA for CD80 and CD86. Differentiating monocytes initially expressed CD86 while CD80 appeared on day 2. Differentiating stem cells expressed CD80 and CD86 on day 2 of culture. The surface expression of CD80 and CD86 was studied over the course of differentiation. Mixed lymphocyte reaction was employed to evaluate the two types of lineage-derived DCs. Prior to the functional assay, CD14+ and CD34+ derived DCs were stimulated for 18 h with 0.1 mg/mL and 1.0 mg/mL E. coli lipopolyssacharide, respectively. Monoclonal antibodies (mabs) to CD80 and CD86 were employed to assess their costimulatory roles. A decrease of stimulation as depicted by decreased T cell activation was significant with mabs to both CD80 and CD86 on monocyte-derived DCs while only mabs to CD86 induced decreased T cell activation by stem cell-derived DCs. The varied functional role of CD80 and CD86 costimulatory molecules is associated with DC differentiation from distinct cord blood isolated hematopoietic lineages. These studies demonstrate that DC association with distinct hematopoietic lineages is of relevance in transplantation and vaccine therapies.

Dilioglou, S., J. M. Cruse, et al. (2003). "Function of CD80 and CD86 on monocyte- and stem cell-derived dendritic cells." Experimental and Molecular Pathology **75**(3): 217.

http://www.sciencedirect.com/science/article/B6WFB-49H1JWG-1/2/ef3e5e05b82f674896d346334774ac02

Dendritic cells (DCs) consist of a heterogeneous population of hematopoietic cells characterized by their unique dendritic morphology, their efficient antigen-presenting capability to activate naive CD4+ and CD8+ T cells, as well as their lack of lineage-specific markers. Functional properties comparing umbilical cord blood monocyte-derived and umbilical cord blood stem cell-derived DCs have not yet been investigated. Human umbilical cord blood CD14+ monocytes and CD34+ stem cells were induced to differentiate into dendritic cells using 100 ng/mL granulocyte-macrophage colony-stimulating factor (GM-CSF), 25 ng/mL interleukin (Il)-4, 2.5 ng/mL tumor necrosis factor [alpha] (TNF-[alpha]) and 100 ng/mL GM-CSF, 25 ng/mL stem cell factor, and 2.5 ng/mL TNF-[alpha], respectively. Differentiated dendritic cells were CD80+, CD86+, CD83+, CD54+, CD1a+, CD11b+, CD11c+, HLA-DR+, CD34-, CD3-, CD19-, CD14-, and CD16-. Reverse transcription polymerase chain reaction revealed that differentiating monocytes initially expressed CD86 mRNA while CD80 mRNA appeared on Day 2. Differentiating stem cells expressed both CD80 and CD86 mRNA on Day 2 of culture. Mixed lymphocyte reaction was employed to evaluate the two types of lineage-derived DCs. Monoclonal antibodies (mabs) to CD80 and CD86 were employed to assess their costimulatory roles. CD14 and CD34 derived DCs prior to the functional assay were stimulated for 18 h with 0.1 and 1.0 mg/mL Escherichia coli lipopolyssacharide, respectively. A decrease in stimulation as depicted by decreased T-cell activation was significant with mabs to both CD80 and CD86 on monocyte-derived DCs while only mabs to CD86 induced decreased T-cell activation by stem cell-derived DCs. The varied functional role of CD80 and CD86 costimulatory molecules is associated with DC differentiation from distinct cord blood-isolated hematopoietic lineages. These studies demonstrate that DC association with distinct hematopoietic lineages is of relevance in transplantation and vaccine therapies.

Foldes-Papp, Z., J. M. Costa, et al. (2004). "Specifically associated PCR products probed by coincident detection of two-color cross-correlated fluorescence intensities in human gene polymorphisms of methylene tetrahydrofolate reductase at site C677T: a novel measurement approach without follow-up mathematical analysis." Experimental and Molecular Pathology **76**(3): 212.

http://www.sciencedirect.com/science/article/B6WFB-4BMC81C-5/2/a846c00fba2ee8726e469cc50c5506f3

Whole blood samples of known methylene tetrahydrofolate reductase (MTHFR) genotypes from 24 individuals were examined at site C677T. Their amplified DNA products were assessed by two-color fluorescence cross-correlation measurements and agarose gel electrophoresis/capillary gel electrophoresis. DNA subpopulations were identified which were not associated with the proper genotype by primer combinations and cycling conditions called multiplexes. We confirmed that DNA analysis by two-color fluorescence cross-correlation measurements allowed the detection of fluorescence signals specifically associated with the proper genotypes in a mixture of amplified nontarget DNA molecules without DNA sizing. The measurement approach does not require complex, follow-up mathematical analysis and is applicable to any single nucleotide polymorphisms. The simple immunogenetic model showed how the approach works to reveal specific DNA target by preventing detection of nontarget DNA. Under those experimental conditions, a new ultrasensitive, and specific method for clinical immunologists is born.

Nemoto, T., M. Kitagawa, et al. (2004). "Expression of IAP family proteins in esophageal cancer." Experimental and Molecular Pathology **76**(3): 253.

http://www.sciencedirect.com/science/article/B6WFB-4C47KRR-1/2/d7abc59caf8a861de01d7afcff237ffd

Members of the inhibitor of apoptosis protein (IAP) family, including survivin, have been reported to be expressed in many tumors. However, their expression in esophageal cancer has not been clarified completely. We investigated the expression of mRNA for IAP family proteins in samples from esophageal cancers and their adjacent normal mucosa tissues by real-time quantitative RT-PCR. The survivin expression in esophageal cancer was significantly higher than that in normal mucosa (P P P < 0.05). Immunohistochemical staining demonstrated cytoplasmic as well as nuclear expression of survivin in esophageal cancer, and further, in situ hybridization analysis demonstrated cytoplasmic expression of mRNA for survivin. The results suggest that the expression of IAP family proteins, especially survivin, may be associated with the biological character of esophageal cancer, such as apoptosis.

Experimental and Toxicologic Pathology (1)

Ohtsuka, R., Y. Shutoh, et al. "Changes in histology and expression of cytokines and chemokines in the rat lung following exposure to ovalbumin." Experimental and Toxicologic Pathology **In Press, Corrected Proof** http://www.sciencedirect.com/science/article/B7GJ4-4FSCV70-1/2/09a879a6277e1a766d6780dacc18449e

Brown Norway (BN) and Fischer 344 (F344) rats were exposed to aerosol of 1% ovalbumin (OVA) solution for 30 min at 1 week after the second sensitization with 1 mg of OVA at 2-week intervals. Changes in the histology and expression of cytokines and chemokines in the lung were examined for up to 96 h after the exposure. The lung weight significantly increased in BN rats but not in F344 rats. Histologically, in the lung of BN rats, multiple foci of hemorrhage in the alveolar space with infiltration of eosinophils and macrophages in the surrounding alveolar septa were first observed. Thereafter, granulomatous lesions developed in the preexisting hemorrhagic foci, finally resulting in formation of multiple eosinophilic granulomas. On the other hand, in F344 rats, infiltration of eosinophils and macrophages was observed around the vessels and bronchi. Thereafter it progressed gradually, resulting in mild thickening of alveolar septa. The levels of

Th1- (interferon-[gamma] and interleukin 2 (IL-2)) and Th2-related cytokines (IL-4 and IL-5) and chemokines (eotaxin and monocyte chemoattractant protein-1) mRNAs measured by reverse transcription-polymerase chain reaction method were elevated in the lung of both strains, and the levels were higher in BN rats than in F344 rats. These results suggest that BN rats are more sensitive to OVA-sensitization/inhalation than F344 rats and that the difference in the severity of lung lesions between BN and F344 rats may reflect the difference in the expression levels of cytokines and chemokines between these two strains.

Experimental Biology and Medicine (5)

Embree-Ku, M. and K. Boekelheide (2002). "Absence of p53 and FasL Has Sexually Dimorphic Effects on Both Development and Reproduction." Experimental Biology and Medicine **227**(7): 545-553.

http://www.ebmonline.org/cgi/content/abstract/227/7/545

Reproduction and development are highly dependent on apoptosis to balance the proliferation that necessarily occurs during these processes. How the absence of two apoptotic factors in mice would affect reproduction and development was examined. Given previous reports of increased neural tube defects in p53-/- female fetuses, decreased fertility in gld female mice, and altered spermatogenesis in both p53 and gld male mice, the possibility that these phenotypes might be enhanced by the elimination of a second apoptotic factor was investigated. The reproductive vigor and the health of offspring were monitored during the production of the new double-deficient strain (FasL-/-p53-/-) for any changes from the reported phenotypes. Thus, any unusual phenotypes that could lead to new models for studying mechanisms of health and disease would be identified. Double-deficient male offspring appeared healthy and occurred at expected frequencies. Additionally, spermatogenesis and male fertility were unaffected by the gene deficiencies. On the other hand, FasL+/+p53-/- and FasL-/-p53-/- female mice were susceptible to increased malformations and post-natal death. These abnormalities were consistent with previous reports of neural tube defects in p53-/- female mice. Fertility rates were also significantly decreased in p53-/- female mice that lived to be adults, an observation not previously reported. Finally, the absence of both FasL and p53 led to dystocia in pregnant female mice, suggesting that the two genes play complementary roles in parturition. Therefore, although male mouse development and reproduction remained unaffected by p53 and FasL deficiencies, female mouse development was adversely affected by the absence of p53, and no live litters were born to female mice with the combined absence of both FasL and p53. In this report, we suggest a potential mechanism involving corpora luteal regression to explain this defect in parturition in FasL-/-p53-/- female mice.

Kenny, T. P., C. L. Keen, et al. (2004). "Pentameric Procyanidins Isolated from Theobroma cacao Seeds Selectively Downregulate ErbB2 in Human Aortic Endothelial Cells." Experimental Biology and Medicine **229**(3): 255-263.

http://www.ebmonline.org/cgi/content/abstract/229/3/255

Flavonoids isolated from cocoa have biological activities relevant to oxidant defenses, vascular health, tumor suppression, and immune function. The intake of certain dietary flavonoids, along with other dietary substances such as tocopherols, ascorbate, and carotenoids, is

epidemiologically associated with a reduced risk of cardiovascular disease. Flavonoids have also been shown to modulate tumor pathology in vitro and in animal models. We took advantage of the conserved sequences found in tyrosine kinases to study the influence of cocoa fractions and controls on gene expression. We report that the pentameric procyanidin (molecular weight of 1442 daltons) fraction isolated from cocoa was a potent inhibitor of tyrosine kinase ErbB2 expression, a receptor important in angiogenesis regulation. Consistent with this primary observation, the cocoa flavonoid fraction also suppressed human aortic endothelial cell (HAEC) growth and decreased expression of two tyrosine kinases responsive to ErbB2 modulation, namely VEGFR-2/KDR and MapK 11/p38{beta}2. These inhibitory effects were observed when HAECs were treated with the flavonol fraction (molecular weight 280 daltons) isolated from cocoa, which comprise the structural subunits from which the procyanidin flavonoid subclass is biosynthetically constructed. Down-regulation of ErbB2 and inhibition of HAEC growth by cocoa procyanidins may have several downstream implications, including reduced vascular endothelial growth factor (VEGF) activity and angiogenic activity associated with tumor pathology. These results suggest specific dietary flavonoids are capable of selectively inhibiting ErbB2 and therefore may offer important insight into the design of therapeutic agents that target tumors overexpressing ErbB2.

Redell, J. B., A. N. Moore, et al. (2003). "Expression of the Prodynorphin Gene after Experimental Brain Injury and Its Role in Behavioral Dysfunction." Experimental Biology and Medicine **228**(3): 261-269.

http://www.ebmonline.org/cgi/content/abstract/228/3/261

Traumatic brain injury (TBI) causes excess release of neurotransmitters, such as glutamate, and increases intracellular calcium levels. Elevated levels of calcium, and perhaps other intracellular second messengers, as a result of TBI can alter the expression of many genes. The protein products of some of these genes may be signals for TBI-associated memory dysfunction. Therefore, identification of genes whose expression is altered after TBI in the hippocampus, a structure in the medial temporal lobe that plays a critical role in memory formation and storage, and elucidation of the role(s) of their protein products may shed light on the molecular mechanisms underlying TBI-elicited memory dysfunction. The prodynorphin gene is expressed in hippocampal granule cells, and its expression has been reported to be enhanced as a result of elevated intracellular calcium. The prodynorphin protein is proteolytically cleaved to generate multiple dynorphin peptides, which can modulate neurotransmitter release through the activation of presynaptic {kappa} opioid receptors. In this study, we report that 1) TBI transiently increases prodynorphin mRNA in the hippocampus, 2) dynorphin peptide immunoreactivity is enhanced for up to 24 hr after TBI and 3) intracerebroventricular infusion of the {kappa} receptor antagonist nor-binaltorphimine (nor-BNI) impairs subsequent performance in a spatial memory task. These results suggest that dynorphin action may serve a beneficial role after TBI.

Sharma, R., M. Sharma, et al. (2002). "Induction of Metallothionein-I Protects Glomeruli from Superoxide-Mediated Increase in Albumin Permeability." Experimental Biology and Medicine **227**(1): 26-31.

http://www.ebmonline.org/cgi/content/abstract/227/1/26

Metallothioneins (MT) are low-molecular-weight, heat-stable, cysteine-rich proteins with four isoforms. MT-I and MT-II are ubiquitous and are induced by oxidative, physical, and chemical stress. MT-I is an efficient scavenger of superoxide (*O2) and hydroxyl ion (OH-). We have demonstrated that *O2 and hypohalous acid can cause an increase in glomerular albumin permeability (Palb) in vitro. The purpose of this study was to document the protective effect of MT gene product on the *O2-mediated increase in Palb. Glomeruli from Sprague-Dawley rats in 4%

BSA medium were incubated for 4 hr at 37{degrees}C in duplicate tubes. Each set contained glomeruli alone or with 5 {micro}M Cd++, 0.3 mM Spermine-NONOate (NO donor), 0.3 mM Sulfo-NONOate (nitrous oxide donor), 0.6 mM SNP (nonspecific NO donor) and SNP + carboxy-PTIO (10 mg/ml). After incubation, one set of tubes was used to isolate total RNA for the measurement of the mRNA levels of MT-I by reverse transcriptase polymerase chain reaction (RT-PCR). Duplicate tubes were incubated for an additional 10 min with 10 nM of *O2, and Palb was measured using video microscopy. RT-PCR of total RNA from Cd++ and Spermine-NONOate treated glomeruli revealed a 2-fold induction of MT-I expression at the mRNA level.*O2 caused a significant increase in Palb (0.8 {+/-} 0.06 vs. control 0.0 {+/-} 0.12, P < 0.05) and induction of MT-I in glomeruli by Cd++ or by Spermine-NONOate blocked this effect (0.21 {+/-} 0.12 and 0.24 {+/-} 0.19, respectively, P < 0.05 vs. *O2). In contrast, Sulfo-NONOate and SNP did not induce mRNA for MT-I in glomeruli and did not provide protection against *O2-mediated increase in Palb. We conclude that MT-I gene products may play an important role in protecting the glomerular filtration barrier from the injury induced by reactive oxygen species in immune and/or nonimmune renal diseases.

Yachie, A., T. Toma, et al. (2003). "Heme Oxygenase-1 Production by Peripheral Blood Monocytes During Acute Inflammatory Illnesses of Children." Experimental Biology and Medicine **228**(5): 550-556.

http://www.ebmonline.org/cgi/content/abstract/228/5/550

Monocytes play key roles both in innate and adaptive antigen-specific immunity and they constitute critical components of the immune responses. Although most of the monocyte-derived cytokines exhibit proinflammatory functions in vivo, heme oxygenase-1 (HO-1), an inducible heme-degrading enzyme, exerts potent anti-inflammatory effect through production of carbon monoxide and bilirubin. We compared HO-1 production by monocytes in vivo in various acute inflammatory illnesses and in normal controls. Freshly isolated monocytes produced little HO-1 as detected by immunohistochemistry, but it was rapidly induced in vitro upon stimulation. HO-1 production by monocytes was selective because it was not induced in other leukocyte populations, including granulocytes and lymphocytes. Monocytes from acute inflammatory illnesses, such as Kawasaki disease and acute infectious diseases, viral or bacterial, produced significant levels of HO-1, as detected by flow cytometry, immunohistochemistry, and reverse transcription polymerase chain reaction. Quantitative analysis of HO-1 mRNA expression by real-time polymerase chain reaction revealed that monocytes from controls exhibited low, but significant levels of HO-1 mRNA, indicating that circulating monocytes produce HO-1 constantly, in response to basal level of oxidative stress encountered daily. Significantly elevated HO-1 mRNA levels seen in acute inflammatory illnesses suggest that monocyte HO-1 production serve as potent anti-inflammatory agent to control excessive cell or tissue injury in the presence of oxidative stress and cytokinemia.

Experimental Parasitology (5)

Carlos Polanco, J., J. Antonia Rodriguez, et al. (2002). "Plasmodium vivax: parasitemia determination by real-time quantitative PCR in Aotus monkeys." Experimental Parasitology **100**(2): 131.

http://www.sciencedirect.com/science/article/B6WFH-45TDHV0-

7/2/ce01ef4158c85f4f014650cf6e888693

Nowak, T. S. and E. S. Loker (2005). "Echinostoma paraensei: differential gene transcription in the sporocyst stage." Experimental Parasitology **109**(2): 94.

http://www.sciencedirect.com/science/article/B6WFH-4F94YMS-2/2/f5a217c4e08e43ab497c0c29532b703c

The sporocyst stage of trematode development plays the crucial role of establishing a successful infection in the molluscan intermediate host. Due to the small size and presence of this stage within the tissues of the host, much of our current knowledge of sporocyst biology relies on cultured specimens. To gain insight into the transcriptional patterns of early sporocysts, suppression subtractive hybridization was employed to identify 69 unique expressed sequence tags likely to be upregulated in cultured sporocysts of Echinostoma paraensei, a trematode parasite of the planorbid snail, Biomphalaria glabrata. Upwards of 70% of the unique sequences were not identified by homology to known genes. However, one transcript may encode an inhibitor of nitric oxide synthase, indicating a possible role in protection against host defense mechanisms. An array containing the majority of the sequenced clones was probed with in vivo-derived cDNA, confirming for the first time in vivo expression of putative sporocyst genes. However, qPCR quantification demonstrated significant reductions in transcription rates in cultured versus in vivo sporocysts for three of six transcripts tested. Additionally, five of the six tested transcripts demonstrated significant variation in expression over the entire life cycle, with the significant upregulation occurring during early intramolluscan development or in the free-living stages immediately preceding snail penetration, confirming the efficacy of the SSH technique.

Perez-Rosado, J., G. W. Gervais, et al. (2002). "Plasmodium berghei: analysis of the [gamma]-glutamylcysteine synthetase gene in drug-resistant lines." Experimental Parasitology **101**(4): 175.

http://www.sciencedirect.com/science/article/B6WFH-47T8BJ8-2/2/d51ab84f57e92a2e3b552258e839be2c

The rapid emergence of multidrug-resistant Plasmodium falciparum is a worldwide concern. Despite the magnitude of the problem, the mechanisms involved in this phenomenon are not well understood. One current proposal suggests that toxic heme molecules are degraded by glutathione (GSH), and that anti-malarial drugs, such as chloroquine (CQ), inhibit this degradation, thus implicating GSH in drug resistance. Furthermore, in some strains of Plasmodium berghei and P. falciparum, chloroquine resistance is accompanied by an increase in glutathione levels and increased activity in GSH-related enzymes. We are investigating the relationship between the [gamma]-glutamylcysteine synthetase (ggcs) gene, the rate-limiting enzyme in de novo synthesis of GSH, and drug resistance in P. berghei at the molecular level. In this report, we have demonstrated an increase in pbggcs mRNA levels associated with CQ and mefloquine (MFQ) resistance. In addition, the pbggcs gene locus structure was shown to be similar and localized to chromosome 8 in four parasite lines of P. berghei with different drug resistance profiles. This work suggests a link between increased GSH levels and drug resistance in Plasmodium.Index Descriptors and Abbreviations: Apicomplexa; Malaria; Plasmodium berghei; Drug resistance; [gamma]-Glutamylcysteine synthetase; Glutathione; Chloroquine; MDR, multidrug resistance phenotype; AMO, amodiaquine; MFQ, mefloquine; CQ, chloroquine; GSH, glutathione; ggcs, [gamma]-glutamylcysteine synthetase; GB, GenBank; RBC, red blood cell; BSO, buthionine sulfoximine; pfggcs, Plasmodium falciparum ggcs gene; pbggcs, Plasmodium berghei ggcs gene; RPA, ribonuclease protection assay.

Rafati, S., A. Nakhaee, et al. (2003). "Expression of cysteine proteinase type I and II of Leishmania infantum and their recognition by sera during canine and human visceral leishmaniasis." Experimental Parasitology **103**(3-4): 143.

http://www.sciencedirect.com/science/article/B6WFH-48W2R7B-1/2/74ba10e22ca9bd473693dd797c82dedf

In this study, the mature domains of type I (CPB) and type II (CPA) cysteine proteinases (CPs) of Leishmania infantum were expressed and their immunogenic properties defined using sera from active and recovered cases of human visceral leishmaniasis and sera from infected dogs. Immunoblotting and ELISA analysis indicated that a freeze/thaw extract of parasite antigens showed similar and intensive recognition in both active cases of human and dog sera but lower recognition in recovered human individuals. The total IgG of actively infected human sera was higher than in recovered cases when rCPs were used as antigen. In contrast to dog sera, both active and recovered human cases have higher recognition toward rCPB than rCPA. Furthermore, the asymptomatic dogs in contrast to the symptomatic cases exhibited specific lymphocyte proliferation to both crude antigens and rCPs.

Rosa, R., O. R. Rodrigues, et al. (2005). "Leishmania infantum: soluble proteins released by the parasite exert differential effects on host immune response." Experimental Parasitology **109**(2): 106.

http://www.sciencedirect.com/science/article/B6WFH-4F7YMVY-1/2/81582cf47910cd53467d4bcd56e158be

The objective of this study was to analyse the modulatory effect of proteins released by cultured Leishmania infantum promastigotes on the cellular immune response of infected susceptible (BALB/c) and more resistant (C57BL/6) mice strains after 30 and 45 days of infection. One month after parasite inoculation, L. infantum released protein fractions (High, Inter, and Low according to molecular weight) stimulated C57BL/6 mice spleen cells to proliferate and to express cytokines. Following the decrease of parasite load only the Low protein fraction induced a considerable release of IL-4. In BALB/c mice, specific immune response to protein fractions was only observed at the higher parasitic level, with the fraction Inter promoting the production of IL-4 and fractions High and Low inducing high levels of IL-12. These results point out to a role of these proteins fractions in the modulation of host immunity, that depending on the host genetic background and parasite magnitude, seem to be critical in the control of parasite replication levels, thus avoiding premature host death.

FASEB J (12)

Cellai, C., A. Laurenzana, et al. (2002). "Specific PAF antagonist WEB-2086 induces terminal differentiation of murine and human leukemia cells." FASEB J: 01-0602fje.

http://www.fasebj.org/cgi/content/abstract/01-0602fjev1

A pharmacological approach to neoplasia by differentiation therapy relies on the availability of

cytodifferentiating agents whose antitumor efficacy is usually assayed first on malignant cells in vitro. Using murine erythroleukemia cells (MELCs) as the model, we found that WEB-2086, a triazolobenzodiazepine-derived PAF antagonist originally developed as an anti-inflammatory drug, induces a dose-dependent inhibition of MELC growth and hemoglobin accumulation as a result of a true commitment to differentiation. MELCs treated for 5 days with 1 mM WEB-2086 show greater than or equal to 85% benzidine-positive cells, increased expression of a- and b-globin genes, and down-regulation of c-Myb. This differentiation pattern, which does not involve histone H4 acetylation and is abrogated by the action of phorbol 12-myristate 13-acetate, recalls the pattern induced by hexamethylene bisacetamide (HMBA). In addition to MELCs, human erythroleukemia K562 and HEL and myeloid HL60 cells are massively committed to maturation by WEB-2086 and, with some differences, by its analog, WEB-2170. This suggests that WEB-2086, structurally distant from other known inducers, might be a member of a new class of cytodifferentiation agents active on a broad range of transformed cells in vitro and useful, prospectively, for anticancer therapy due to their high tolerability in vivo. Key words: inducer · PAF receptor · maturation · neoplasia · differentiation therapy

Deplancke, B. and H. R. Gaskins (2003). "Hydrogen sulfide induces serum-independent cell cycle entry in nontransformed rat intestinal epithelial cells." FASEB J: 02-0883fje.

http://www.fasebj.org/cgi/content/abstract/02-0883fjev1

Hydrogen sulfide (H2S), produced by commensal sulfate-reducing bacteria, is an environmental insult that potentially contributes to chronic intestinal epithelial disorders. We tested the hypothesis that exposure of nontransformed intestinal epithelial cells (IEC-18) to the reducing agent sodium hydrogen sulfide (NaHS) activates molecular pathways that underlie epithelial hyperplasia, a phenotype common to both ulcerative colitis (UC) and colorectal cancer. Exposure of IEC-18 cells to NaHS rapidly increased the NADPH/NADP ratio, reduced the intracellular redox environment, and inhibited mitochondrial respiratory activity. The addition of 0.2-5 mM NaHS for 4 h increased the IEC-18 proliferative cell fraction ($P<0.05$), as evidenced by analysis of the cell cycle and proliferating cell nuclear antigen expression, while apoptosis occurred only at the highest concentration of NaHS. Thirty minutes of NaHS exposure increased ($P<0.05$) c-Jun mRNA concentrations, consistent with the observed activation of mitogen activated protein kinases (MAPK). Microarray analysis confirmed an increase ($P<0.05$) in MAPK-mediated proliferative activity, likely reflecting the reduced redox environment of NaHS-treated cells. These data identify functional pathways by which H2S may initiate epithelial dysregulation and thereby contribute to UC or colorectal cancer. Thus, it becomes crucial to understand how genetic background may affect epithelial responsiveness to this bacterial-derived environmental insult. Key words: IEC-18 colorectal cancer ulcerative colitis epithelial hyperproliferation microarray analysis

Fisher, M. C., S. H. Zeisel, et al. (2002). "Perturbations in choline metabolism cause neural tube defects in mouse embryos in vitro." FASEB J: 01-0564fje.

http://www.fasebj.org/cgi/content/abstract/01-0564fjev1

A role for choline during early stages of mammalian embryogenesis has not been established, although recent studies show that inhibitors of choline uptake and metabolism, 2-dimethylaminoethanol (DMAE), and 1-O-octadecyl-2-O-methyl-rac-glycero-3-phosphocholine (ET-18-OCH3), produce neural tube defects in mouse embryos grown in vitro. To determine potential mechanisms responsible for these abnormalities, choline metabolism in the presence or absence of these inhibitors was evaluated in cultured, neurulating mouse embryos by using chromatographic techniques. Results showed that 90%-95% of 14C-choline was incorporated

into phosphocholine and phosphatidylcholine (PtdCho), which was metabolized to sphingomyelin. Choline was oxidized to betaine, and betaine homocysteine methyltransferase was expressed. Acetylcholine was synthesized in yolk sacs, but 70 kDa choline acetyltransferase was undetectable by immunoblot. DMAE reduced embryonic choline uptake and inhibited phosphocholine, PtdCho, phosphatidylethanolamine (PtdEtn), and sphingomyelin synthesis. ET-18-OCH3 also inhibited PtdCho synthesis. In embryos and yolk sacs incubated with 3H-ethanolamine, 95% of recovered label was PtdEtn, but PtdEtn was not converted to PtdCho, which suggested that phosphatidylethanolamine methyltransferase (PeMT) activity was absent. In ET-18-OCH3treated yolk sacs, PtdEtn was increased, but PtdCho was still not generated through PeMT. Results suggest that endogenous PtdCho synthesis is important during neurulation and that perturbed choline metabolism contributes to neural tube defects produced by DMAE and ET-18-OCH3. Key Words: neurulation · dimethylaminoethanol · ET-18-OCH3 · embryo culture

Ho, W.-Z., J.-P. Lai, et al. (2002). "HIV enhances substance P expression in human immune cells." FASEB J: 01-0655fje.

http://www.fasebj.org/cgi/content/abstract/01-0655fjev1

Substance P (SP), a potent modulator of neuroimmunoregulation, is expressed in human immune cells. We observed elevated plasma SP levels in HIV-infected men compared with uninfected subjects. In the present study, we investigated the possible cellular source of the increased SP level caused by HIV infection. Using real-time reverse transcriptase-polymerase chain reaction, we demonstrated that monocyte-derived macrophages (MDM) and lymphocytes from both placental cord blood and adult peripheral blood expressed SP mRNA, which was significantly increased by HIV infection. HIV-induced SP expression was positively related to virus replication in the infected MDM. Purified recombinant HIV envelope glycoprotein 120 (gp120) derived from both the macrophage-tropic strain (MN) and the T lymphocyte-tropic strain (IIIB), when added to MDM cultures, enhanced SP mRNA expression. The gp120-induced SP expression was abrogated by pretreating the cells with soluble CD4. Furthermore, the activation of HIV in the latently infected promonocytic cell line (U1) and T-cell line (ACH-2) up-regulated SP mRNA expression. These data support the hypothesis that interaction of HIV and SP may have significant in vivo relevance to the immunopathogenesis of HIV infection and AIDS. Key words: lymphocytes · gp120 · tachykinin · neuroimmunoregulation · monocyte-derived macrophages

Hong, C.-S., M.-C. Cho, et al. (2002). "Cardiac remodeling and atrial fibrillation in transgenic mice overexpressing junctin." FASEB J: 01-0908fje.

http://www.fasebj.org/cgi/content/abstract/01-0908fjev1

Junctin is a 26-kDa integral membrane protein, colocalized with the ryanodine receptor (RyR) and calsequestrin at the junctional sarcoplasmic reticulum (SR) membrane in cardiac and skeletal muscles. To elucidate the functional role of junctin in heart, transgenic (TG) mice overexpressing canine junctin (24-29 folds) under the control of mouse a-myosin heavy chain promoter were generated. Overexpression of the junctin in mouse heart was associated with heart enlargements, bradycardia, atrial fibrillation, and increased fibrosis. Many ultrastructural alterations were observed in TG atria. The junctional SR cisternae facing transverse-tubules contained a dense matrix of calsequestrin in TG heart. According to echocardiography, TG mice showed enlarged left ventricles, dilated right atriums, and ventricles with paradoxical septal motion and impaired left ventricular systolic function. Overexpression of junctin led to down-regulation of triadin and RyR but to up-regulation of dihydropyridine receptor. The L-type Ca2+ current density and action potential durations increased, which could be the cause for the bradycardia in TG heart. This study provides an important example of pathogenesis leading to

substantial cardiac remodeling and atrial fibrillation, which was caused by overexpression of junctin in heart. Key words: excitation-contraction coupling · sarcoplasmic reticulum · ryanodine receptor · dihydropyridine receptor

Inoh, H., N. Ishiguro, et al. (2002). "Uni-axial cyclic stretch induces the activation of transcription factor nuclear factor kB in human fibroblast cells." FASEB J: 01-0354fje.

http://www.fasebj.org/cgi/content/abstract/01-0354fjev1

The effect of uni-axial cyclic mechanical stretch on the activation of the transcription factor nuclear factor kB (NF-kB) was investigated in a human fibroblast cell line (TIG-1). In response to uni-axial cyclic stretch, NF-kB was found to be translocated into the nucleus. The NF-kB was first detectable 2 min after the onset of stretch and then peaked at 4 min and returned to the basal level within 10 min. To investigate whether NF-kB is activated following the translocation into the nucleus, we measured the luciferase activity in the cells transfected with pNF-kB-luciferase. The activity of luciferase increased 4 min after the initiation of cyclic stretch, peaked at 15 min (6.4-fold increase), and decreased gradually. We examined the involvement of the stretch-activated (SA) channel in the stretch-induced NF-kB activation. The application of Gd3+, a blocker of the SA channel, or the removal of extracellular Ca2+ inhibited both the translocation into the nucleus and the activation of NF-kB, which suggests that NF-kB is activated by uni-axial cyclic stretch via SA channel activation in human lung fibroblasts. Key words: stretch-activated channel · gadolinium · calcium · fibroblast

Kassed, C. A., T. L. Butler, et al. (2004). "Injury-induced NF-κB activation in the hippocampus: implications for neuronal survival." FASEB J: 03-0773fje.

http://www.fasebj.org/cgi/content/abstract/03-0773fjev1

Nuclear factor (NF)-B p50 protein is involved in promoting survival in hippocampal neurons after trimethyltin (TMT)-injury. In the current study, hippocampal NF-B activity was examined and quantitated from transgenic B-lacZ reporter mice after chemical-induced injury. NF-B activity was localized primarily to hippocampal neurons and significantly elevated over that in saline-treated mice between 4 and 21 days after TMT injection. Seven days after TMT injection, a timepoint of elevated NF-B activity, gene expression in the hippocampus was studied by microarray analysis through comparison of expression profiles between treated nontransgenic and p50-null mice with their saline-injected controls. Seventeen genes increased in nontransgenic TMT-treated mice relative to saline-treated as well as showing no increase in p50-null mice, indicating a role for p50 in their regulation. One of these genes, the Na+, K+-ATPase- subunit, was detected in brain for the first time. Several of the genes modulated by NF-B are potentially related to neuroplasticity, providing additional evidence that this transcription factor is a neuroprotective signal in the hippocampus. Key words: signal transduction Na+, K+-ATPase neurodegeneration NF-B p50 transcription factors

Kovaleva, E. S., A. G. Yakovlev, et al. (2002). "Human proprotein convertase 2 homologue from a plant nematode: Cloning, characterization, and comparison with other species." FASEB J: 01-0940fje.

http://www.fasebj.org/cgi/content/abstract/01-0940fjev1

Proprotein convertases (PCs) are evolutionarily conserved enzymes responsible for processing

the precursors of many bioactive peptides in mammals. The invertebrate homologues of PC2 play important roles during development that makes the enzyme a good target for practical applications in pest management. Screening of a plant nematode Heterodera glycines cDNA library resulted in isolation of a full-length clone encoding a PC2-like precursor. The deduced protein (74.2 kD) exhibits strong amino acid homology to all known PC2s, including human, and shares the main structural characteristics: signal peptide; prosegment; catalytic domain, with D/H/S catalytic triad, PC2-specific residues, and 7B2 binding sites; P domain (with RRGDT pentapeptide); and carboxyl terminus. Comparative analysis of PC2s from 15 species discloses the presence of an insert in the catalytic domain unique to nematodes. Expression of PC2-like mRNA found in eggs and juveniles was undetectable in adult stages of H. glycines. Nucleotide analysis reveals distinctive differences in base composition and codon usage between H. glycines and Caenorhabditis elegans PC2s. The H. glycines cDNA clone encoding PC2 is the first one isolated from plant-parasitic nematodes. Key words: cDNA cloning · (G+C) content · plant-parasitic nematode · Heterodera glycines

Lynn, F. C., S. A. Thompson, et al. (2002). "A novel pathway for regulation of glucose-dependent insulinotropic polypeptide receptor expression in b-cells." FASEB J: 02-0243fje.

http://www.fasebj.org/cgi/content/abstract/02-0243fjev1

Glucose-dependent insulinotropic polypeptide (GIP) is secreted postprandially and acts in concert with glucose to stimulate insulin secretion from the pancreas. Here, we describe a novel pathway for the regulation of GIP receptor (GIPR) expression within clonal b-cell lines, pancreatic islets, and in vivo. High (25 mM) glucose was able to significantly reduce GIPR mRNA levels in INS(832/13) cells after only 6 h. In contrast, palmitic acid (2 mM) and WY 14643 (100 mM) stimulated approximate doublings of GIPR expression in INS(832/13) cells under low (5.5 mM), but not high (25 mM), glucose conditons, suggesting that fat can regulate GIPR expression via PPARa in a glucose-dependent manner. Both MK-886, an antagonist of PPARa, and a dominant negative form of PPARa transfected into INS(832/13) cells caused a significant reduction in GIPR expression in low, but not high, glucose conditions. Finally, in hyperglycemic clamped rats, there was a 70% reduction in GIPR expression in the islets and a 71% reduction in GIP-stimulated insulin secretion from the perfused pancreas. Thus, evidence is presented that the GIPR is controlled at normoglycemia by the fatty acid load on the islet; however, when exposed to hyperglycemic conditions, the GIPR is down-regulated, which may contribute to the decreased responsiveness to GIP that is observed in type 2 diabetes. Key words: PPARa · type 2 diabetes · Zucker rats · INS(832/13) cells · gastric inhibitory polypeptide

Nickenig, G., S. Baudler, et al. (2002). "Redox-sensitive vascular smooth muscle cell proliferation is mediated by GKLF and Id3 in vitro and in vivo." FASEB J **16**(9): 1077-1086.

http://www.fasebj.org/cgi/content/abstract/16/9/1077

Reactive oxygen species such as superoxide and hydroxyl radicals have been implicated in the pathogenic growth of various cell types. The molecular mechanisms involved in redox-sensitive cell growth control are poorly understood. Stimulation of cultured vascular smooth muscle cells (VSMC) with xanthin/xanthin oxidase (X/XO) increases proliferation, whereas stimulation with hydrogen peroxide and Fe3+NTA (H-Fe) causes growth arrest of VSMC. Differential Display led to the identification of two novel, differentially regulated redox-sensitive genes. The dominant negative helix-loop-helix protein Id3 is induced by X/XO and down-regulated by H-Fe. The transcription factor gut-enriched Kruppel-like factor (GKLF) is induced by H-Fe but not by X/XO. Induction of GKLF and inhibition of Id3 via transfection experiments leads to growth arrest, whereas overexpression of Id3 and inhibition of GKLF cause cell growth. Id3 down-regulation is

induced via binding of GKLF to the Id3 promotor and concomitantly reduced Id3 gene transcription rate. GKLF induction by H-Fe is mediated through hydroxyl radicals, p38MAP kinase-, calcium-, and protein synthesis-dependent pathways. Id3 is induced by X/XO via superoxide, calcium, p38, and p42/44 MAP kinase. GKLF induces and Id3 depresses expression of p21WAF1/Cip1, p27KIP1, p53. Induction of Id3 is accomplished by angiotensin II via superoxide release. A vascular injury mouse model revealed that Id3 is overexpressed in proliferating vascular tissue in vivo. These findings reveal novel mechanisms of redox-controlled cellular proliferation involving GKLF and Id3 that may have general implications for our understanding of vascular and nonvascular growth control.--Nickenig, G., Baudler, S., Muller, C., Werner, N., Welzel, H., Strehlow, K., Bohm, M. redox-sensitive vascular smooth muscle cell proliferation is mediated by GKLF and Id3 in vitro and in vivo.

Obreja, O., P. K. Rathee, et al. (2002). "IL-1{beta} potentiates heat-activated currents in rat sensory neurons: involvement of IL-1RI, tyrosine kinase, and protein kinase C." FASEB J **16**(12): 1497-1503.

http://www.fasebj.org/cgi/content/abstract/16/12/1497

Interleukin 1{beta} (IL-1{beta}) is a proinflammatory cytokine that maintains thermal hyperalgesia and facilitates the release of calcitonin gene-related peptide from rat cutaneous nociceptors in vivo and in vitro. Brief applications of IL-1{beta} to nociceptive neurons yielded a potentiation of heat-activated inward currents (Iheat) and a shift of activation threshold toward lower temperature without altering intracellular calcium levels. The IL-1{beta}-induced heat sensitization was not dependent on G-protein-coupled receptors but was mediated by activation of protein kinases. The nonspecific protein kinase inhibitor staurosporine, the specific protein kinase C inhibitor bisindolylmaleimide BIM1, and the protein tyrosine kinase inhibitor genistein reduced the sensitizing effect of IL-1{beta} whereas negative controls were ineffective. RT-PCR and in situ hybridization revealed IL-1RI but not RII expression in neurons rather than surrounding satellite cells in rat dorsal root ganglia. IL-1{beta} acts on sensory neurons to increase their susceptibility for noxious heat via an IL-1RI/PTK/PKC-dependent mechanism.--Obreja, O., Rathee, P. K., Lips, K. S., Distler, C., Kress, M. IL-1{beta} potentiates heat-activated currents in rat sensory neurons: involvement of IL-1RI, tyrosine kinase, and protein kinase C.

Van Der Meer-Van Kraaij, C., E. M. M. Van Lieshout, et al. (2003). "Mucosal pentraxin (Mptx), a novel rat gene 10-fold down-regulated in colon by dietary heme." FASEB J **17**(10): 1277-1285.

http://www.fasebj.org/cgi/content/abstract/17/10/1277

Consumption of red meat is associated with increased colon cancer risk. Our previous work indicated that this association might be due to the heme content of red meat. In rat studies, dietary heme increased colonic cytotoxicity and epithelial cell turnover, carcinogenesis biomarkers. Here we apply DNA microarray technology to examine effects of heme on colonic gene expression. A rat colon-specific microarray was constructed and hybridized in duplicate to RNA extracts from colon scrapings of rats fed diets with or without heme (n=6-7). We were able to reproducibly identify changes in colonic mRNA abundance in response to heme. Most striking was a >10-fold down-regulation of a single rat gene, an unprecedented gene-modulating effect of a dietary component. Based on homology, the novel gene encodes a pentraxin, the first identified in colon. Pentraxins are postulated to be involved in dealing with dying cells. Quantitative PCR confirmed the strong heme-induced down-regulation of this gene, which we named mucosal pentraxin (Mptx). Overall, our data support the efficacy of cDNA array expression profiling to investigate effects of specific nutrients in an in vivo system and may provide an approach to establishing markers for diet-induced stress of mammalian colonic mucosa.--van der Meer-van

Kraaij, C., van Lieshout, E. M. M., Kramer, E., van der Meer, R., Keijer, J. Mucosal pentraxin (Mptx), a novel rat gene 10-fold down-regulated in colon by dietary heme.

FEBS J. (2)

Asada, A., H. Orii, et al. (2005). "Planarian peptidylglycine-hydroxylating monooxygenase, a neuropeptide processing enzyme, colocalizes with cytochrome b561 along the central nervous system." FEBS J. **272**(4): 942-955.

http://content.febsjournal.org/cgi/content/abstract/272/4/942

Planarians are one of the simplest animal groups with a central nervous system. Their primitive central nervous system produces large quantities of a variety of neuropeptides, of which many are amidated at their C terminus. In vertebrates, peptide amidation is catalyzed by two enzymes [peptidylglycine {alpha}-hydroxylating monooxygenase (PHM) and peptidyl-{alpha}-hydroxylglycine {alpha}-amidating lyase] acting sequentially. In mammals, both enzymatic activities are contained within a single protein that is encoded by a single gene. By utilizing PCR with degenerate oligonucleotides derived from conserved regions of PHM, we succeeded in cloning a full-length cDNA encoding planarian PHM. The deduced amino acid sequence showed full conservation of five His residues and one Met residue, which bind two Cu atoms that are essential for the activity of PHM. Northern blot analysis confirmed the expression of a PHM mRNA of the expected size. Distribution of the mRNA was analyzed by in situ hybridization, showing specific expression in neurons with two morphologically distinct structures, a pair of the ventral nerve cords and the brain. The distribution of PHM was very similar to that of cytochrome b561. This indicates that the ascorbate-related electron transfer system operates in the planarian central nervous system to support the PHM activity and that it predates the emergence of Plathelminthes in the evolutionary history.

Jensen, K. F., S. Arent, et al. (2005). "Allosteric properties of the GTP activated and CTP inhibited uracil phosphoribosyltransferase from the thermoacidophilic archaeon Sulfolobus solfataricus." FEBS J. **272**(6): 1440-1453.

http://content.febsjournal.org/cgi/content/abstract/272/6/1440

The upp gene, encoding uracil phosphoribosyltransferase (UPRTase) from the thermoacidophilic archaeon Sulfolobus solfataricus, was cloned and expressed in Escherichia coli. The enzyme was purified to homogeneity. It behaved as a tetramer in solution and showed optimal activity at pH 5.5 when assayed at 60 {degrees}C. Enzyme activity was strongly stimulated by GTP and inhibited by CTP. GTP caused an approximately 20-fold increase in the turnover number kcat and raised the Km values for 5-phosphoribosyl-1-diphosphate (PRPP) and uracil by two- and >10-fold, respectively. The inhibition by CTP was complex as it depended on the presence of the reaction product UMP. Neither CTP nor UMP were strong inhibitors of the enzyme, but when present in combination their inhibition was extremely powerful. Ligand binding analyses showed that GTP and PRPP bind cooperatively to the enzyme and that the inhibitors CTP and UMP can be bound simultaneously (KD equal to 2 and 0.5 {micro}M, respectively). The binding of each of the inhibitors was incompatible with binding of PRPP or GTP. The data indicate that UPRTase undergoes a transition from a weakly active or inactive T-state, favored by binding of UMP and

CTP, to an active R-state, favored by binding of GTP and PRPP.

FEBS Letters (157)

Akopov, S. B., L. G. Nikolaev, et al. (1998). "Long terminal repeats of human endogenous retrovirus K family (HERV-K) specifically bind host cell nuclear proteins." FEBS Letters **421**(3): 229.

http://www.sciencedirect.com/science/article/B6T36-3S0F2K8-D/2/8001c6f5ba11652a714af6e2b17cad62

Solitary long terminal repeats (LTRs) of the human endogenous retroviruses, scattered in several thousand copies throughout the human genome, are potentially capable of affecting the expression of closely located genes. To assess their regulatory potential, the LTR sequences of one of the most abundant HERV families (HERV-K) were screened for the presence of binding sites for the host cell nuclear factors using mobility shift and UV-crosslinking assays. It was shown that the LTR sequences of two subfamilies harbor a specific binding site for a complex consisting of at least three proteins, ERF1, ERF2 and ERF3 of 98, 91 and 88 kDa apparent molecular mass, respectively. This binding site is located in the 5' region of the LTR U3 element. The preservation of the specific protein binding site in different HERV-K LTR sequences suggests their possible role in regulation of nearby located genes.

Arand, M., M. Knehr, et al. (1991). "An impaired peroxisomal targeting sequence leading to an unusual bicompartmental distribution of cytosolic epoxide hydrolase." FEBS Letters **294**(1-2): 19.

http://www.sciencedirect.com/science/article/B6T36-45K11DT-12/2/ce2cc1ed36c9c1dad8c5bbc411e03b13

To gain an understanding of the mechanism by which the subcellular distribution of cytosolic epoxide hydrolase (cEH) is directed, we have analyzed the carboxy terminal region of rat liver cEH by means of cDNA cloning to define the structure of its possible peroxisomal targeting sequence (PTS). Purified cEH was subjected to peptide analysis following endoproteinase Glu-C digestion and HPLC-separation of the fragments. The obtained sequence information was used to perform PCR experiments resulting in the isolation of a 680 bp cDNA clone encoding the carboxy terminus of cEH. The deduced amino acid sequence displays a terminal tripeptide Ser-Lys-Ile which is highly homologous to the PTS (Ser-Lys-Leu) found in other peroxisomal enzymes. This slight difference appears to be sufficient to convert the signal sequence into an impaired and therefore ambivalent PTS, directing the enzyme partly to the peroxisomes and allowing part to reside in the cytosol.

Balza, E., A. Siri, et al. (1993). "Production and characterization of monoclonal antibodies specific for different epitopes of human tenascin." FEBS Letters **332**(1-2): 39.

http://www.sciencedirect.com/science/article/B6T36-449TJ2W-58/2/38ccb896beefb57237bb81c097fda5df

We have obtained and characterized 11 monoclonal antibodies (mAbs) specific for different domains of human tenascin (TN). Five of these mAbs reacted with epitopes contained in the TN area that undergoes alternative splicing and are thus able to recognize specific TN isoforms. These mAbs are a useful tool to study the expression and distribution of TN and its different isoforms in normal and pathological tissues.

Barbirato, F., J. C. Verdoes, et al. (1998). "The Rhodococcus erythropolis DCL14 limonene-1,2-epoxide hydrolase gene encodes an enzyme belonging to a novel class of epoxide hydrolases." FEBS Letters **438**(3): 293.

http://www.sciencedirect.com/science/article/B6T36-3V5MRHV-13/2/afe9425316706f02f18c9c5946408a0b

Recently, we reported the purification of the novel enzyme limonene-1,2-epoxide hydrolase involved in limonene degradation by Rhodococcus erythropolis DCL14. The N-terminal amino acid sequence of the purified enzyme was used to design two degenerate primers at the beginning and the end of the 50 amino acids long stretch. Subsequently, the complete limonene-1,2-epoxide hydrolase gene (limA) was isolated from a genomic library of R. erythropolis DCL14 using a combination of PCR and colony hybridization. The limA gene encoded a 149-residue polypeptide with a deduced molecular mass of 16.5 kDa. It was functionally expressed in Escherichia coli. The amino acid sequence of limA contains neither any of the conserved regions of the [alpha],[beta]-hydrolase fold enzymes, to which most of the previously reported epoxide hydrolases belong, nor any of the conserved motifs present in leukotriene A4 hydrolase. The structural data presented in this paper confirm previous physical and biochemical findings [van der Werf et al. (1998) J. Bacteriol. 180, 5052-5057] that limonene-1,2-epoxide hydrolase is the first member of a new class of epoxide hydrolases.

Bardouille, C., D. Vullhorst, et al. (1996). "Expression of chloride channel 1 mRNA in cultured myogenic cells: a marker of myotube maturation." FEBS Letters **396**(2-3): 177.

http://www.sciencedirect.com/science/article/B6T36-3Y158RJ-H/2/cfd81a522b6a0c6d2a2075e475dd8f32

The chloride channel ClC-1 is required to maintain a normal excitability of mature muscle fibers; its blockade leads to hyperexcitability, the hallmark of the disease myotonia. In mouse and rat myotubes, representing the embryonic stage of muscle, ClC-1 mRNA is not detectable by Northern blotting. From neonatal to adult, ClC-1 expression increases at least fourfold. Using RT-PCR and hybridization on cultured myotubes were found ClC-1 mRNA at a level of 0.4-1.1% of that in mature mouse muscle, and <=0.01% in myoblasts, at stages when desmin mRNA levels are already high. The level of ClC-1 mRNA is thus a sensitive and specific indicator of the maturation of skeletal muscle cells.

Bartsch, J. W., M. Jackel, et al. (2000). "Steroid RU 486 inducible myogenesis by 10T1/2 fibroblastic mouse cells." FEBS Letters **467**(1): 123.

http://www.sciencedirect.com/science/article/B6T36-3YGD3FC-W/2/29efc1e06f0ad666d451e1c8e2590661

For reconstruction or repair of damaged tissues, an artificially regulated switch from proliferation

to differentiation would be of great advantage. To achieve conditional myogenesis, we expressed MyoD in mouse C3H 10T1/2 fibroblastic cells, using a gene regulation system based on the synthetic steroid RU 486. By stable co-transfection of a plasmid construct with the RU 486 dependent activator and an integrating inducible MyoD construct, a cell clone, designated 10T-RM, was obtained in which MyoD expression was stringently controlled by RU 486. 12 h after addition of 10 nM RU 486 to 10T-RM cells, saturation levels of MyoD mRNA were observed and >=2 days later, mRNA for embryonal myosin heavy chain (MyHCemb) was abundant and mononucleated cells fused into myotubes.

Berg, L.-P., M. K. Shamsher, et al. (1997). "Expression of human TRPC genes in the megakaryocytic cell lines MEG01, DAMI and HEL." FEBS Letters **403**(1): 83.

http://www.sciencedirect.com/science/article/B6T36-3R7B21X-1H/2/e855be2db900778b1efa1292f7b62000

Store-regulated Ca2+ entry represents a major mechanism for Ca2+ influx in non-excitable cells although many details remain to be evaluated including the identification of cation entry channels. Recently human homologues of the Drosophila proteins TRP and TRPL, have been described (TRPC1, TRPC1A, HTRP1) and suggested as candidate cation channels. In this study we sought to examine if the producers of blood platelets, megakaryocytic cells (using the cell lines MEG01, DAMI, HEL), expressed these genes. RNA was prepared from the cell lines and platelets and converted to cDNA. The cDNA was then subjected to 30-35 cycles of PCR using gene specific primers for TRPC1-3. PCR products of the expected sizes were observed for all three TRPC genes in the three cell lines. Direct sequencing confirmed their identity. Additionally for TRPC1, a larger species, and for TRPC2, a smaller species was detected in all three cell lines with sequencing revealing the fragments to contain TRPC sequence, suggesting that they were either products of alternative splicing events or from closely related genes. These results suggest that TRPC genes are expressed in megakaryocytic cell lines and that the TRPC proteins may play a role in mediating cation influx in both megakaryocytes and platelets.(c) 1997 Federation of European Biochemical Societies.

Betts, A. M., G. P. Collett, et al. (1998). "Paracrine regulation of talin mRNA expression by androgen in human prostate." FEBS Letters **434**(1-2): 66.

http://www.sciencedirect.com/science/article/B6T36-3TMXY8S-G/2/9abcf3825afa2a95e652faaa2cffc902

Androgens are essential for normal prostate physiology and are intimately associated with the growth and progression of prostate cancer. However, few androgen regulated genes in the prostate have been identified. Using the mRNA differential display technique a 164-bp cDNA fragment was identified as being androgen regulated in the human prostate. Nucleotide sequence analysis of this fragment revealed 84% homology with the gene encoding the cytoskeletal protein talin. Confirmation of the androgen regulation of this gene was carried out using Northern analysis. Primary prostatic stromal cells treated with conditioned medium (CM) from androgen-treated primary prostatic epithelial cells showed an approximate 2-fold reduction in talin mRNA levels compared with stromal cells treated with CM from epithelial cells not exposed to androgens. Expression of talin mRNA in human prostatic tissue was confirmed by in situ hybridisation. The highest levels of expression were present in the epithelial cells, with lower levels of expression in the stroma. Thus, androgen regulation of talin expression may play a role in normal and/or aberrant growth and development of the prostate.

Betts, A. M., I. Waite, et al. (1997). "Androgen regulation of ornithine decarboxylase in human prostatic cells identified using differential display." FEBS Letters **405**(3): 328.

http://www.sciencedirect.com/science/article/B6T36-3R85JMS-4T/2/a9bd9c45fdefcf69337d5b6b17282ece

Androgens are essential for normal prostate physiology and have a permissive role in the development and progression of prostate cancer. Using the mRNA differential display technique, ornithine decarboxylase (ODC) was identified to be up-regulated by androgens in human prostatic LNCaP cells. On Northern analysis, the induction of ODC expression by 10 nM androgen was rapid and continued up to 48 h exposure with a maximum 6.3-fold up-regulation. The anti-androgen Casodex inhibited the androgen-induced up-regulation of ODC, whereas the protein synthesis inhibitor cycloheximide did not. Together these data suggest that regulation is mediated through the androgen receptor protein and does require secondary protein synthesis, respectively. The kinetics of induction of ODC were almost identical to those of prostate specific antigen. Taken together these data suggest that ODC is directly regulated by androgens in LNCaP cells.

Bianchi, C., I. Muradore, et al. (2002). "The expression of the non-receptor tyrosine kinases Arg and c-abl is differently modulated in B lymphoid cells at different stages of differentiation." FEBS Letters **527**(1-3): 216.

http://www.sciencedirect.com/science/article/B6T36-46P9TFM-2/2/75cde191a7eef1bd335eddffd8e82e70

The products of the human ARG gene and the human ABL gene characterize the Abelson family of non-receptor tyrosine protein kinases. Both genes are ubiquitously expressed. The interactions of these two similar protein kinases are still not well known, although it has been suggested that they could cooperate, with redundant actions, to provide intracellular signals in the cells. Lymphopenia occurs in mice with homozygous disruption of c-abl, indicating that in certain tissues Arg is unable to substitute c-abl functions. In B and T lymphoid cell lines at different stages of differentiation, we studied, by a reverse transcriptase-competitive polymerase chain reaction and Western blotting, Arg and c-abl in order to evaluate whether the expression pattern of the two genes could give insight as to why they do not exhibit overlapping roles in lymphocytes and whether the product levels of the two genes are related to lymphoid differentiation. The data showed that their expression is differently modified in lymphoid B cell lines. The highest Arg transcript and protein levels are in the mature B cells.

Bishop, K., K. Gough, et al. (1999). "Synechococcus mutants resistant to an enamine mechanism inhibitor of glutamate-1-semialdehyde aminotransferase." FEBS Letters **450**(1-2): 57.

http://www.sciencedirect.com/science/article/B6T36-3WHK65P-D/2/0f079da757bc990004867817b941790f

An enamine mechanism-based inactivator of mammalian [delta]-aminobutyric acid aminotransferase, 4-amino 5-fluoropentanoic acid is a potent inhibitor of cell growth and pigment formation in the cyanobacterium Synechococcus PCC 6301. It was demonstrated that 4-amino 5-fluoropentanoic acid inhibits the aminolaevulinate synthesis at glutamate 1-semialdehyde aminotransferase and that in the mutant obtained by exposing cells to 40 [mu]M 4-amino 5-

fluoropentanoic acid, this enzyme was insensitive to the inhibitor. The specific activity of glutamate 1-semialdehyde aminotransferase in cell extracts was lower in the mutant, although the cell growth rate was unaffected. The decrease in sensitivity to 4-amino 5-fluoropentanoic acid in the mutant is due to a structural gene mutation, a single base change in the hemL gene resulting in a S162T substitution in the gene product.

Bobe, R., C. Lacabaratz-Porret, et al. (1998). "Expression of two isoforms of the third sarco/endoplasmic reticulum Ca2+ATPase (SERCA3) in platelets. Possible recognition of the SERCA3b isoform by the PL/IM430 monoclonal antibody." FEBS Letters **423**(2): 259.

http://www.sciencedirect.com/science/article/B6T36-3S4XR8S-11/2/3b699601d8a57859706d6d6aaedcf709

Human platelets express several sarco/endoplasmic reticulum Ca2+ATPase (SERCA) isoenzymes: SERCA2b of 100 kDa apparent molecular mass and two distinct enzymes of 97 kDa, one of them identified as being the SERCA3a isoform. The molecular identity of the third enzyme specifically recognized by the PL/IM430 monoclonal antibody has remained elusive. First, the study of the 3'-end part of platelet SERCA3 mRNA, by means of RT-PCR amplification using sets of primers covering the N-3 to N (ultimate) exons of the human SERCA3 sequence, revealed the presence of two distinct mRNA sequences, SERCA3a and a longer variant. Second, this additional sequence was identified as SERCA3b and found to refer to the insertion of a new exon of 73 bp, located at bp 349 from the beginning of the intronic sequence, linking the penultimate (N-1) exon to the last exon (N) of the human SERCA3 gene. Third, a relationship between the expression of this SERCA3b mRNA and the PL/IM430 recognizable SERCA protein was observed. SERCA3b mRNA was found to be absent in epithelial HeLa cells not recognized by the PL/IM430 antibody and the expression of this SERCA3b RNA species correlated with that of the SERCA protein recognized by PL/IM430 which was down-modulated in the platelet precursor megakaryocytic CHRF 288-11 cell line as well as upon in vitro lymphocyte activation. Taken together, these results strongly support the notion of the presence of the SERCA3b protein in human cells by showing SERCA3b mRNA in platelets and the fact that the protein corresponding to this mRNA species is very likely the 97 kDa protein recognized by the PL/IM430 antibody.

Braks, J. A. M., K. C. W. Guldemond, et al. (1992). "Structure and expression of Xenopus prohormone convertase PC2." FEBS Letters **305**(1): 45.

http://www.sciencedirect.com/science/article/B6T36-44WKGD7-D/2/27261a0df74f99783a15c7f70bd08648

The multifunctional prohormone, proopiomelanocortin (POMC), is processed in the melanotrope cells of the pituitary pars intermedia at pairs of basic amino acid residues to give a number of peptides, including [alpha]-melanophore-stimulating hormone ([alpha]-MSH). This hormone causes skin darkening in amphibians during background adaptation. Here we report the complete structure of Xenopus laevis prohormone convertase PC2, the enzyme thought to be responsible for processing of POMC to [alpha]-MSH. A comparative structural analysis revealed an overall amino acid sequence identity of 85-87% between Xenopus PC2 and its mammalian counterparts, with the lowest degree of identity in the signal peptide sequence (28-36%) and the region amino-terminal to the catalytic domain (59-60%). The occurrence of a second, structurally different PC2 protein reflects the expression of two Xenopus PC2 genes. The expression pattern of PC2 in the Xenopus pituitary gland of black- and white-adapted animals was found to be similar to that of POMC, namely high expression in active melanotrope cells of black animals. This observation is in line with a physiological role for PC2 in processing POMC to [alpha]-MSH.

Brandle, U., P. Spielmanns, et al. (1997). "Desensitization of the P2X2 receptor controlled by alternative splicing." FEBS Letters **404**(2-3): 294.

http://www.sciencedirect.com/science/article/B6T36-3R85JMS-26/2/464688ac2a75e10079d3906dc4f31c08

P2X receptors are ion channels gated by extracellular ATP. We report here cloning of a P2X2 receptor splice variant (P2X2-2) carrying a 207 bp deletion in the intracellular C-terminus and the analysis of the corresponding genomic structure of the P2X2 gene. P2X2-2 is as highly expressed as the original P2X2 sequence in various tissues. ATP-activated currents mediated by heterologous expressed P2X2 or P2X2-2 receptors showed significant differences in desensitization time constants and steady-state currents in the continuous presence of ATP. These results imply functional differences between cells differentially expressing these P2X2 isoforms.(c) 1997 Federation of European Biochemical Societies.

Brauer, A. U., R. Nitsch, et al. (2004). "Identification of macrophage/microglia activation factor (MAF) associated with late endosomes/lysosomes in microglial cells." FEBS Letters **563**(1-3): 41.

http://www.sciencedirect.com/science/article/B6T36-4BX0GRB-2/2/655e12e7db1415a975c42d4156f5ba15

Damage to the central nervous system triggers rapid activation and specific migration of glial cells towards the lesion site. There, glial cells contribute heavily to secondary neuronal changes that take place after lesion. In an attempt to identify the molecular cues of glial activation following brain trauma we performed differential display reverse transcription-polymerase chain reaction screenings from lesioned and control hippocampus. Here we report on the identification of the macrophage/microglia activation factor (MAF), a new membrane protein with seven putative transmembrane domains. Expression analysis revealed that MAF is predominantly expressed in microglial cells in the brain, and is upregulated following brain lesion. Overexpression of MAF in non-glial cells shows an intracellular codistribution with the lysosomal marker endosome/lysosome-associated membrane protein-1 (lamp-1). Furthermore, MAF-transfected cells show that MAF is primarily associated with late endosomes/lysosomes, and that this association can be disrupted by activation of protein kinase C-dependent pathways. In conclusion, these results imply that MAF is involved in the dynamics of lysosomal membranes associated with microglial activation following brain lesion.

Bucci, C., A. Lutcke, et al. (1995). "Co-operative regulation of endocytosis by three RAB5 isoforms." FEBS Letters **366**(1): 65.

http://www.sciencedirect.com/science/article/B6T36-3YRNY44-CC/2/1cb7bfe6fff21d80b805fcb4c6316749

Rab proteins are small GTPases involved in the regulation of membrane traffic. Rab5a has been shown to regulate transport in the early endocytic pathway. Here we report the isolation of cDNA clones encoding two highly related isoforms, Rab5b and Rab5c. The two proteins share with Rab5a all the structural features required for regulation of endocytosis. Rab5b and Rab5c colocalize with the both transferrin receptor and Rab5a, stimulate the homotypic fusion between early endosomes in vitro and increase the rate of endocytosis when overexpressed in vivo. These data demonstrate that three Rab5 isoforms cooperate in the regulation of endocytosis in

eukaryotic cells.

Carriere, F., K. Thirstrup, et al. (1994). "Cloning of the classical guinea pig pancreatic lipase and comparison with the lipase related protein 2." FEBS Letters **338**(1): 63.

http://www.sciencedirect.com/science/article/B6T36-44DSMYD-G/2/8c943da3fb99724ce1e532f170b03d4e

Starting from total pancreatic mRNAs, the classical guinea pig pancreatic lipase was cloned using rapid amplification of 3' and 5' cDNA ends. Internal oligonucleotide primers were designed from a partial cDNA clone including the region coding for the lid domain. Using this strategy, we did not amplify the cDNA corresponding to the pancreatic lipase related protein 2 in which the lid domain is deleted. Amino acid sequences of the classical guinea pig pancreatic lipase and the related protein 2 were compared based on the primary and tertiary structures of the classical human pancreatic lipase. Their distinct physiological roles are discussed in the light of functional amino acid differences.

Chapelin, C., B. Duriez, et al. (1997). "Isolation of several human axonemal dynein heavy chain genes: genomic structure of the catalytic site, phylogenetic analysis and chromosomal assignment." FEBS Letters **412**(2): 325.

http://www.sciencedirect.com/science/article/B6T36-3R7B21X-5M/2/dbd4b55516ddf718b3fd3739015fbf49

Dynein heavy chains (DHCs) are the main components of multisubunit motor ATPase complexes called dyneins. Axonemal dyneins provide the driving force for ciliary and flagellar motility. Recent molecular studies demonstrated that multiple DHC isoforms are produced by separate genes. We describe the isolation of five human axonemal DHC genes. Analysis of the human genomic clones revealed the existence of intronic sequences that were used to demonstrate that human axonemal DHC genes are located on different chromosomes. The cloned human DHC sequences were integrated into an evolutionary approach based on phylogenetic analysis. Tissue expression studies showed that these human axonemal DHCs are expressed in testis and/or trachea, two tissues with axonemal structures that can be altered in primary ciliary dyskinesia, making DHC genes strong candidates in the genesis of these human diseases.

Chessell, I. P., J. Simon, et al. (1998). "Cloning and functional characterisation of the mouse P2X7 receptor." FEBS Letters **439**(1-2): 26.

http://www.sciencedirect.com/science/article/B6T36-3V7JH3Y-5/2/95992c921456c775f2e80834cf2f4b38

We have isolated a 1785-bp complementary DNA (cDNA) encoding the murine P2X7 receptor subunit from NTW8 mouse microglial cells. The encoded protein has 80% and 85% homology to the human and rat P2X7 subunits, respectively. Functional properties of the heterologously expressed murine P2X7 homomeric receptor broadly resembled those of the P2X7 receptor in the native cell line. However, marked phenotypic differences were observed between the mouse receptor, and the other P2X7 receptor orthologues isolated with respect to agonist and antagonist potencies, and the kinetics of formation of the large aqueous pore.

Conway, S., J. E. Drew, et al. (1997). "Identification of Mel1a melatonin receptors in the human embryonic kidney cell line HEK293: evidence of G protein-coupled melatonin receptors which do not mediate the inhibition of stimulated cyclic AMP levels." FEBS Letters **407**(1): 121.

http://www.sciencedirect.com/science/article/B6T36-3R85JMS-W/2/7bf8fdbbc7a5863d32fe9fe040bc463e

Binding assays using 2-[125I]iodomelatonin revealed high-affinity, guanosine 5'-O-(3-thiotriphosphate) sensitive, melatonin binding sites (Bmax 1.1 fmol/mg protein) in the human embryonic kidney cell line HEK293. Competition studies using the selective melatonin receptor antagonist luzindole and RT-PCR techniques identified these sites as human Mel1a melatonin receptors. Challenge of HEK293 cells with 1 [mu]M melatonin had no effect on forskolin stimulated cyclic AMP levels, whereas in HEK293 cells engineered to stably over-express the human Mel1a melatonin receptor (Bmax>400 fmol/mg protein) melatonin dose-dependently inhibited stimulated cyclic AMP levels (IC50 7.7 pM). These data may indicate that certain tissues, expressing low levels of G protein-coupled melatonin receptors, do not display melatonin mediated inhibition of cAMP.

Corbi, N., M. Perez, et al. (1997). "Synthesis of a new zinc finger peptide; comparison of its 'code' deduced and 'CASTing' derived binding sites." FEBS Letters **417**(1): 71.

http://www.sciencedirect.com/science/article/B6T36-3RD0S7F-3J/2/ae30d3a2d113ad81cbb46408da5931f3

Using two synthetic oligonucleotides, we have constructed a new gene containing three zinc finger motifs of the Cys2-His2 type. We named this artificial gene 'Mago'. The Mago nucleotide triplets encoding the amino acid positions, described to be crucial for DNA binding specificity, have been chosen on the basis of the proposed recognition 'code' that relates the zinc finger's primary structure to the DNA binding target. Here we demonstrate that Mago protein specifically binds the 'code' DNA target, with a dissociation constant (Kd) comparable to the Kd of the well known Zif268 protein with its binding site. Moreover, we show that the deduced Mago 'code' and the 'experimental' selected DNA binding sites are almost identical, differing only in two nucleotides at the side positions.

De Tullio, R., B. Sparatore, et al. (1998). "Rat brain contains multiple mRNAs for calpastatin." FEBS Letters **422**(1): 113.

http://www.sciencedirect.com/science/article/B6T36-3S0FJ2M-1K/2/909c4b9b98eed00395a74f4850d91ecb

This work was undertaken to establish the forms of the calpain inhibitor, calpastatin, expressed in the brain tissue. Five cDNA clones were obtained and the corresponding amino acid sequences were deduced. Three of these proteins contain an N-terminal domain (domain L) and four inhibitory repeats typical of the calpastatin molecule. The other two are truncated forms, containing the domain L, free or associated with a single inhibitory repeat. Other differences, due to exon skipping, produce calpastatin forms with different susceptibility to posttranslational modifications. The more represented mRNA form corresponds to a calpastatin molecule containing the four inhibitory domains. These results may be useful to understand the involvement of calpain in the onset of acute and degenerative disorders of the central nervous

system.

Derst, C., C. Karschin, et al. (2001). "Genetic and functional linkage of Kir5.1 and Kir2.1 channel subunits." FEBS Letters **491**(3): 305.

http://www.sciencedirect.com/science/article/B6T36-42G6KMT-X/2/82b7cd1f871713efcea650d41a3c71cb

We have identified several cDNAs for the human Kir5.1 subunit of inwardly rectifying K+ channels. Alternative splicing of exon 3 and the usage of two alternative polyadenylation sites contribute to cDNA diversity. The hKir5.1 gene KCNJ16 is assigned to chromosomal region 17q23.1-24.2, and is separated by only 34 kb from the hKir2.1 gene (KCNJ2). In the brain, Kir5.1 mRNA is restricted to the evolutionary older parts of the hindbrain, midbrain and diencephalon and overlaps with Kir2.1 in the superior/inferior colliculus and the pontine region. In the kidney Kir5.1 and Kir2.1 are colocalized in the proximal tubule. When expressed in Xenopus oocytes, Kir5.1 is efficiently targeted to the cell surface and forms electrically silent channels together with Kir2.1, thus negatively controlling Kir2.1 channel activity in native cells.

Dijkstra, A. J., F. Hermann, et al. (1995). "Cloning and controlled overexpression of the gene encoding the 35 kDa soluble lytic transglycosylase from Escherichia coli." FEBS Letters **366**(2-3): 115.

http://www.sciencedirect.com/science/article/B6T36-3YRNY51-CP/2/b377eb55bc02de4b01a70b83ededbfa4

The lytic transglycosylases of Escherichia coli are involved in peptidoglycan metabolism and resemble the lysozymes not only in activity, but in the case of the 70 kDa soluble lytic transglycosylase (Slt70), also structurally. Here we report the cloning of the gene that encodes the 35 kDa soluble lytic transglycosylase (Slt35) of E. coli. Based on the sequence of the full-length gene, Slt35 is very likely to be a proteolytically truncated form of a slightly large protein. The homology between Slt35 and Slt70, albelt poor, indicates that the active site architecture of both proteins may be alike. Using the T-7 promoter system, Slt35 was overproduced in large quantities and purified to homogeneity for crystallographic purposes.

Dixon, A. K., A. K. Gubitz, et al. (1995). "Distribution of mRNA encoding the inwardly rectifying K+ channel, BIR1 in rat tissues." FEBS Letters **374**(1): 135.

http://www.sciencedirect.com/science/article/B6T36-3YS2BTR-BS/2/a82315e5067843ebc06baf4349853a03

The distribution of mRNA encoding the inwardly rectifying K+ channel, BIR1 [1] was investigated in rat tissues, and a comparison made with the expression of related genes rcKATP and GIRK1 using the reverse transcription-polymerase chain reaction (RT-PCR). This showed BIR1 to be expressed in all areas of the brain examined, in the eye but not in any other peripheral tissue. This pattern was distinct from rcKATP and GIRK1. Additional in situ hybridisation studies of the central expression of BIR1 demonstrated high levels of BIR1 mRNA in the hippocampus dentate gyrus, taenia tecta and cerebellum and at lower levels in the cortex, habenular nucleus, olfactory bulb, primary olfactory cortex, thalamus, pontine nucleus and amygdaloid nucleus.

Driessen, C. A. G. G., H. J. Winkens, et al. (1998). "The visual cycle retinol dehydrogenase: possible involvement in the 9-cis retinoic acid biosynthetic pathway." FEBS Letters **428**(3): 135.

http://www.sciencedirect.com/science/article/B6T36-3SY8DJK-3/2/1f858d7c977fe47f636009a0c843c694

The 11-cis-retinol dehydrogenase (11-cis-RoDH) gene encodes the short-chain alcohol dehydrogenase responsible for 11-cis-retinol oxidation in the visual cycle. The structure of the murine 11-cis-RoDH gene was used to reinvestigate its transcription pattern. An 11-cis-RoDH gene transcript was detected in several non-ocular tissues. The question regarding the substrate specificity of the enzyme was therefore addressed. Recombinant 11-cis-RoDH was found capable of oxidizing and reducing 9-cis-, 11-cis- and 13-cis-isomers of retinol and retinaldehyde, respectively. Dodecyl-[beta]-1-maltoside used to solubilize the enzyme was found to affect the substrate specificity. This is the first report on a visual cycle enzyme also present in non-retinal ocular and non-ocular tissues. A possible role in addition to its role in the visual cycle is being discussed.

Dzhandzhugazyan, K. N., A. F. Kirkin, et al. (1998). "Ecto-ATP diphosphohydrolase/CD39 is overexpressed in differentiated human melanomas." FEBS Letters **430**(3): 227.

http://www.sciencedirect.com/science/article/B6T36-3T8F754-M/2/a1167a9d0e2a388b34502241365768a2

Ecto-ATPase activities of melanocytes and human melanoma cell lines differing in the stage of progression were compared. A dramatic increase in ecto-ATPase activity above the level of normal melanocytes was demonstrated in the differentiated melanomas and was followed by a gradual decrease with tumor progression. The characteristics of this enzymatic activity were consistent with CD39/ecto-ATP diphosphohydrolase (ATPDase) which was found to be the major ecto-ATP-hydrolysing enzyme in melanomas. Indeed, the expression of CD39 and the level of CD39 mRNA followed a similar pattern. Since CD39 is known to regulate homotypic adhesion and, supposedly, affects the disaggregation step, we suggest that overexpression of CD39 may enable tumor cells to reduce contacts with T-lymphocytes and escape from immunological recognition.

Escoubas, J.-M., L. Briant, et al. (1999). "Oyster IKK-like protein shares structural and functional properties with its mammalian homologues." FEBS Letters **453**(3): 293.

http://www.sciencedirect.com/science/article/B6T36-3WSMG1S-D/2/96a7586d86c612041c66a157c47002f9

In our search for genes involved in oyster immunity we isolated a cDNA encoding a polypeptide closely related to the mammalian I[kappa]B kinase (IKK) family. IKK proteins play a central role in cell signaling by regulating nuclear factor-[kappa]B (NF-[kappa]B) activation. We report here the cloning of an oyster IKK-like protein (oIKK) which possesses the characteristic organization of the mammalian IKK proteins, namely an amino-terminal kinase domain followed by a leucine zipper region and a carboxyl-terminal helix-loop-helix motif. When transfected into human cell lines, oIKK activated the expression of NF-[kappa]B-controlled reporter gene, whereas transfections with mutants of oIKK deleted within the kinase domain or within the helix-loop-helix motif respectively abolished and greatly reduced reporter gene activation. These results indicate that oIKK can replace the hIKK-[alpha] in catalyzing NF-[kappa]B nuclear translocation, and in triggering gene expression. Our results sustain the concept of an evolutionarily conserved

signaling machinery in which IKK plays a major role.

Fajkus, J., R. Kralovics, et al. (1995). "The telomeric sequence is directly attached to the HRS60 subtelomeric tandem repeat in tobacco chromosomes." FEBS Letters **364**(1): 33.

http://www.sciencedirect.com/science/article/B6T36-3YRNY98-H6/2/15424f52fdef41f4eae0887842b933c8

PCR and primers derived from the telomeric repeat (CCCTAAA)n and from the tobacco subtelomeric tandemly repetitive sequence HRS60 (EMBL X12489) were used to amplify the region linking the two loci. A 131 bp PCR product was obtained both from total tobacco DNA and from the DNA fraction enriched for telomeres. Its sequence only consists of the telomeric primer and the attached region of the HRS60 repetitive unit up to the end of the sequence complementary to the HRS60 primer. The site of direct continuity between the two sequences is formed by a (dA)7 tract.

Feild, J. A., J. J. Foley, et al. (1999). "Cloning and characterization of a rabbit ortholog of human G[alpha]16 and mouse G[alpha]15." FEBS Letters **460**(1): 53.

http://www.sciencedirect.com/science/article/B6T36-452YB6S-C/2/56ae20e9596f2d3fa35e85704e9462bd

A cDNA was cloned from a rabbit spleen cDNA library which encoded a G-protein [alpha] subunit peptide of 374 amino acids, that at the peptide level exhibited 86% and 79% identity with human G[alpha]16 and mouse G[alpha]15, respectively. The rabbit G[alpha] subunit cDNA was subcloned into a mammalian expression vector and transiently co-transfected into HEK-293 cells along with cDNAs encoding the human C3a, C5a, or nociceptin/orphanin FQ receptors. In all three cases the rabbit G [alpha] subunit behaved similarly to G[alpha]15 or G[alpha]16 and effectively coupled the transfected receptors to intracellular calcium mobilization pathways. By nucleotide sequence homology and functional activity the rabbit G[alpha] subunit appears to be the ortholog of human G[alpha]16 and mouse G[alpha]15.

Feng, X.-H. and S.-d. Kung (1991). "Diversity of the protein kinase gene family in rice." FEBS Letters **282**(1): 98.

http://www.sciencedirect.com/science/article/B6T36-44M430H-VJ/2/006a87e546cc920d3058628512f46f35

Multiple genes have been found to encode families of protein kinases in animals and yeasts. Little is known of the diversity of protein kinase families in plants. We have used the polymerase chain reaction to identify members of protein kinase gene family in rice. We have cloned eight partial cDNA sequences from which deduced amino acid sequences contained conserved sequences or amino acid residues characteristic of catalytic domains of eukaryotic protein serine/threonine kinases. Our results suggest that there is great complexity in the protein kinase gene family in plants and that protein phophorylation may play an as important role in plants as in other eukaryotes.

Fessing, M. Y., V. M. Belkov, et al. (1998). "Molecular cloning and functional characterization of the cDNA encoding the murine thiopurine S-methyltransferase (TPMT)." FEBS Letters **424**(3): 143.

http://www.sciencedirect.com/science/article/B6T36-3S9674S-6/2/50aa417dcf9c20af220ebbf24f3d1257

Thiopurine S-methyltransferase (TPMT) is a cytosolic enzyme that catalyzes S-methylation of aromatic and heterocyclic sulfhydryl compounds, including anticancer and immunosuppressive thiopurines. Here we report the isolation and functional characterization of the murine TPMT cDNA. The screening of expressed sequence tags database led to isolation of a murine cDNA clone containing an uninterrupted ORF encoding the protein with an amino acid sequence that is 82% similar and 78% identical to the human TPMT. The expression product of the murine cDNA in rabbit reticulocyte and wheat germ lysate coupled transcription-translation systems showed TPMT enzymatic activity. We conclude that the isolated cDNA clone represents the murine TPMT cDNA.

Filipovska, A., M. R. Eccles, et al. (2004). "Delivery of antisense peptide nucleic acids (PNAs) to the cytosol by disulphide conjugation to a lipophilic cation." FEBS Letters **556**(1-3): 180.

http://www.sciencedirect.com/science/article/B6T36-4B664KD-3/2/3f1913f535aa3c55b1b83b2df32085bf

Peptide nucleic acids (PNAs) are effective antisense reagents that bind specific mRNAs preventing their translation. However, PNAs cannot cross cell membranes, hampering delivery to cells. To overcome this problem we made PNAs membrane-permeant by conjugation to the lipophilic triphenylphosphonium (TPP) cation through a disulphide bond. The TPP cation led to efficient PNA uptake into the cytoplasm where the disulphide bond was reduced, releasing the antisense PNA to block expression of its target gene. This method of directing PNAs into cells is a significant improvement on current procedures and will facilitate in vitro and pharmacological applications of PNAs.

Franke, I., G. Meiss, et al. (1998). "Genetic engineering, production and characterisation of monomeric variants of the dimeric Serratia marcescens endonuclease." FEBS Letters **425**(3): 517.

http://www.sciencedirect.com/science/article/B6T36-3SFWBN0-11/2/6839952eda9af06105a3c0282d2faa7a

The Serratia nuclease is a non-specific endonuclease which cleaves single- and double-stranded RNA and DNA. It is a member of a large family of related endonucleases, most of which are dimers of identical subunits, with the notable exception of the Anabaena nuclease which is a monomer. In order to find out whether the dimer state of the Serratia nuclease is essential for its function we have produced variants of this nuclease which based on the crystal structure (Miller, M.D. and Krause, K.L. (1996), Protein Science 5, 24-33) were expected to be unable to dimerise. We demonstrate here that these variants, H184A, H184N, H184T and H184R, are monomers and have the same secondary structure, stability towards chemical denaturation and activity as the wild-type enzyme. This allows to conclude that the dimeric state is not essential for the catalytic function of the Serratia nuclease. In contrast, the S179C variant which is also a monomer shows little activity, presumably because this amino acid substitution changes the structure of the enzyme.

Fukuda, M., H. Kabayama, et al. (2000). "Drosophila AD3 mutation of synaptotagmin impairs calcium-dependent self-oligomerization activity." FEBS Letters **482**(3): 269.

http://www.sciencedirect.com/science/article/B6T36-41BV1R7-R/2/83c5f155f0030628f1ebf4ed35b9f811

Genetic analysis of a Drosophila synaptotagmin (Syt) I mutant (AD3) has revealed that Tyr-334 within the C2B domain is essential for efficient Ca2+-dependent neurotransmitter release. However, little is known as to why a missense mutation (Tyr-334-Asn) disrupts the function of the C2B domain at the molecular level. Here, we present evidence that a Tyr-312 to Asn substitution in mouse Syt II, which corresponds to the Drosophila AD3 mutation, completely impairs Ca2+-dependent self-oligomerization activity mediated by the C2B domain but allows partial interaction with wild-type proteins in a Ca2+-dependent manner. This observation is consistent with the fact that the AD3 allele is homozygous lethal but complements another mutant phenotype. We also showed that the Ca2+-dependent C2B self-oligomerization is inhibited by inositol 1,3,4,5-tetrakisphosphate, a potent inhibitor of neurotransmitter release. All of these findings strongly support the idea that self-oligomerization of Syt I or II is essential for neurotransmitter release in vivo.

G, P. K., M. Laloraya, et al. (1996). "Characterization of a uterine luminal fluid protein ULF-250 using N-terminal microsequencing and RT-PCR identifies a novel estrogen-regulated gene in the rat uterus." FEBS Letters **399**(1-2): 33.

http://www.sciencedirect.com/science/article/B6T36-3Y158VW-2W/2/7d506452e7683dc6f7fa7fd5fcee46f7

We had previously identified an estrogen responsive protein ULF-250, synthesized and secreted by the estrous rat uterus, which is immunologically distinct from complement C3 and [alpha]2-macroglobulin. The N-terminal microsequencing of ULF250 followed by sequence homology analysis showed that this protein is a new member of a class of estrogen responsive proteins in the uterus. Polymerase chain reaction with a ULF-250 specific primer yielded partial sequence information of its message. The observed pattern of ULF-250 message in the uterus during the various stages of the reproductive cycle in the rat suggested a possible regulation of ULF-250 message by 17[beta]-estradiol. Upstream sequencing of ULF-250 message and its promoter domains would provide insight into the mechanism of its regulation by estradiol.

Gaustadnes, M., L. A. J. Kluijtmans, et al. (1998). "Detection of a novel deletion in the cystathionine [beta]-synthase (CBS) gene using an improved genomic DNA based method." FEBS Letters **431**(2): 175.

http://www.sciencedirect.com/science/article/B6T36-3TBCR7V-B/2/4f7fb7581a0de21936ccf34bf96832b5

We elucidated the intron-exon boundaries of the 15 coding exons of the human cystathionine [beta]-synthase (CBS) gene in order to establish an improved method based on PCR and direct sequencing for detection of CBS mutations. Using this method we identified the pathogenic mutations in two Danish siblings with CBS deficiency. Patients were compound heterozygotes: we detected the 833T->C mutation and a novel 22 bp deletion of exon 4 (493-514del) that introduces a frameshift and a stop codon immediately after the deletion. The deletion resulted in no detectable mRNA from this allele, as assessed by sequencing of cDNA. The established method represents an improvement of the existing method based on sequencing of cDNA

because it permits the detection of mutations within the entire coding region of the CBS gene from a peripheral blood sample, including splice mutations and mutations resulting in the lack or a reduced amount of transcript.

Gehwolf, R., M. Griessner, et al. (2002). "First patch, then catch: measuring the activity and the mRNA transcripts of a proton pump in individual Lilium pollen protoplasts." FEBS Letters **512**(1-3): 152.

http://www.sciencedirect.com/science/article/B6T36-44Y0Y4J-1/2/ec388a6d36ab1e67f605b4f8426f4965

Combining the patch-clamp method with single-cell reverse transcription polymerase chain reaction (scRT-PCR) a fusicoccin-induced current reflecting the activity of the plasma membrane H+ ATPase of lily pollen protoplasts was measured and subsequently, the ATPase-encoding mRNAs were collected and amplified. Southern blot signals were observed in all 'patch-catch' experiments and could be detected even in 2560-fold dilutions of the pollen contents. H+ ATPase mRNAs were detectable only in the vegetative but not in the generative cell of pollen as confirmed by immunolocalisation. In 15% of the scRT-PCR experiments, a random non-reproducibility of the PCR was observed, probably caused by varying amounts of ATPase mRNAs in the protoplasts.

Gil-Torregrosa, B., J. L. Urdiales, et al. (1994). "Expression of different mitogen-regulated protein/proliferin mRNAs in Ehrlich carcinoma cells." FEBS Letters **349**(3): 343.

http://www.sciencedirect.com/science/article/B6T36-447G3XB-HT/2/251573f2fafdab235ddc16bb214a1d4a

Results from in vivo and from serum-free primary cultures of Ehrlich cells suggest that the expression of mitogen-regulated protein/proliferin (MRP/PLF) mRNAs is not essential for proliferation of this murine tumor. Two sizes for MRP/PRL-related open reading frames (ORFs) have been detected by reverse transcription/PCR amplification. They are almost identical to that reported for PLF-1; but 20% of the amplified cDNA included a shorter ORF, which lacks the entire sequence corresponding to that of the exon 3 of the mrp/plf genes. Ehrlich carcinoma may represent a good model to study regulation of expression and physiological roles of MRP/PLFs in vivo.

Gromov, P. S., J. E. Celis, et al. (1998). "Human rab11a: transcription, chromosome mapping and effect on the expression levels of host GTP-binding proteins." FEBS Letters **429**(3): 359.

http://www.sciencedirect.com/science/article/B6T36-3T2PCRF-10/2/ea7872d37abf3b0a9cd160a5c6153486

Rab11a is a member of the rab-branch of the ras-like small GTP-binding protein superfamily that is associated with both constitutive and regulated secretory pathways. Using a direct procedure for cDNA cloning of small ras-related GTPases, that is based on the screening of eukaryotic cDNA expression libraries using [[alpha]-32P]GTP as a probe, we have isolated two cDNA clones encoding rab11a. Both clones share identical coding sequences, but differ in the length and sequence of their 3' untranslated regions (3'-UTR). Northern blot hybridisation analysis of various human tissues revealed indeed two mRNA species with lengths of 1.0 and 2.3 kb, respectively. Sequence analysis of the cDNAs identified two different putative polyadenylation signals

(AATAAA) at positions 927 and 2302 of the larger transcript. In addition, the 3'-UTR of the larger transcript exhibited several AU-rich elements (ARE) that are believed to control gene expression by regulating the rate of mRNA degradation. Southern blots of human DNA digested with several rare restriction enzymes, and separated by pulse-field gel electrophoresis, yielded the same macro-restriction fragment pattern when hybridised with probes that discriminate between the two transcripts. Taken together, these findings imply that the two mRNA species originate from a single gene, which we have mapped to 15q21.3-q22.31, by the use of diferent polyadenylation sites. As expected, both rab11a-cDNAs yielded the same protein product when transiently expressed in COS-1 cells, and surprisingly, upregulated the proteome expression profile (de novo synthesis or posttranslational modification of preexisting proteins) of a few other, yet unknown GTP-binding proteins.

Hansen, J., M. Muldbjerg, et al. (1997). "Siroheme biosynthesis in Saccharomyces cerevisiae requires the products of both the MET1 and MET8 genes." FEBS Letters **401**(1): 20.

http://www.sciencedirect.com/science/article/B6T36-3SBNK7R-5/2/3fb726688fdd0253625fedb64233c7f8

Siroheme is a uroporphyrinogen III-derivative used by sulfite reductase as a prosthetic group. We investigated in Saccharomyces cerevisiae the possible involvement in siroheme biosynthesis of three genes, MET1, MET8 and MET20. The MET1 gene from S. cerevisiae was cloned and shown to be the same gene as MET20. Sequence similitudes as well as complementation studies indicate that Met1p and Met8p are both involved in siroheme biosynthesis. In addition, we show formally that S. cerevisiae does not need vitamin B12 for growth.

Harmar, A. J., V. Hyde, et al. (1990). "Identification and cDNA sequence of [delta]-preprotachykinin, a fourth splicing variant of the rat substance P precursor." FEBS Letters **275**(1-2): 22.

http://www.sciencedirect.com/science/article/B6T36-44M4167-8K/2/4c4ba27ad781c2e20e2b385c0e3e5a8d

The neuropeptides substance P and neurokinin A are synthesised from a family of precursor polypeptides encoded by the preprotachykinin A (PFT) gene. In addition to mRNA ([beta]-PPT) containing all 7 exons of the gene, alternatively spliced mRNAs lacking either exon 4 ([gamma]-PPT) or exon 6 ([alpha]-PPT) have been identified. We have determined the sequences of cDNA clones encoding four variants of PPT mRNA from rat dorsal root ganglion (DRG), including a novel mRNA species ([delta]-PPT) in which both exons 4 and 6 are absent. The sequence of [delta]-PPT predicts the existence of a novel tachykinin precursor polypeptide.

Hayakawa, Y., A. Ohnishi, et al. (1995). "Molecular cloning and characterization of cDNA for insect biogenic peptide, growth-blocking peptide." FEBS Letters **376**(3): 185.

http://www.sciencedirect.com/science/article/B6T36-3YS2BCS-1X/2/24b595362cf31f595db54522b38cdb3c

Growth-blocking peptide (GBP) is an insect biogenic peptide that prevents the onset of metamorphosis from larva to pupa. A cDNA coding for GBP is described. Mixed oligonucleotides derived from a GBP peptide sequence were used to generate amplified DNA by the polymerase chain reaction (PCR). Based on the sequence of the amplified DNA, a 41 bases oligonucleotide

was designed for screening a cDNA library which was constructed from the armyworm Pseudaletia separata larvae parasitized with the parasitic wasp Cotesia kariyai. The cloned cDNA for GBP was 809 base pairs in length. An open reading frame of 429 base pairs encodes a pre-pro-peptide of 143 amino acid residues in which GBP is localized at the C-terminal region, and other three peptides including a putative signal peptide and appropriate processing sites for endoproteolytic cleavage precede the GBP sequence. Northern blot analyses demonstrate the presence of a 800-base mRNA transcript in fat body and 2.5-kilobase transcript in brain and nerve cord, suggesting the possibility that the transcription of GBP gene is regulated in a tissue-dependent manner. This interpretation was supported by isolating a GBP cDNA fragment from cDNA pool of brain-nerve cords. GBP mRNA is constantly expressed in both parasitized and non-parasitized last instar larvae and there is no difference in the levels of the mRNA between both larvae, thus indicating that parasitism may effect on translational or posttranslational level to elevate plasma GBP concentration.

Hellmuth, M., J. Paulukat, et al. (2004). "Nitric oxide differentially regulates pro- and anti-angiogenic markers in DLD-1 colon carcinoma cells." FEBS Letters **563**(1-3): 98.

http://www.sciencedirect.com/science/article/B6T36-4BYP29F-5/2/8df39ad81b624a14e737e251c7f50ae2

Inducible nitric oxide (NO) synthase (iNOS) appears to be a marker of tumor progression in colon carcinogenesis. Here we investigated effects of NO on selected chemokines that differentially regulate angiogenesis, namely pro-angiogenic interleukin (IL)-8 as well as tumor-suppressive interferon-inducible protein-10 (IP-10) and monokine induced by interferon-[gamma] (MIG). These chemokines are expressed by DLD-1 colon carcinoma cells after stimulation with IL-1[beta]/interferon-[gamma]. Expression of IL-8 was markedly upregulated by NO. Moreover, NO enhanced expression of vascular endothelial growth factor (VEGF). In contrast, expression of IP-10 and MIG was suppressed by NO. The present data are consistent with previous observations that link NO to enhanced tumor angiogenesis and imply that NO-mediated upregulation of IL-8 and VEGF as well as downregulation of IP-10 and MIG may contribute to this phenomenon.

Heyes, D. J., G. E. M. Martin, et al. (2000). "NADPH:protochlorophyllide oxidoreductase from Synechocystis: overexpression, purification and preliminary characterisation." FEBS Letters **483**(1): 47.

http://www.sciencedirect.com/science/article/B6T36-41CP99F-B/2/5e52bcb3ee2ea53bcde274551991c992

NADPH:protochlorophyllide oxidoreductase (POR) catalyses the light-dependent reduction of protochlorophyllide to chlorophyllide, a key regulatory reaction in the chlorophyll biosynthetic pathway. POR from the cyanobacterium Synechocystis has been overproduced in Escherichia coli with a hexahistidine tag at the N-terminus. This enzyme (His6-POR) has been purified to homogeneity and a preliminary characterisation of its kinetic and substrate binding properties is presented. Chemical modification experiments have been used to demonstrate inhibition of POR activity by the thiol-specific reagent N-ethyl maleimide. Substrate protection experiments reveal that the modified Cys residues are involved in either substrate binding or catalysis.

Hinchigeri, S. B., B. Hundle, et al. (1997). "Demonstration that the BchH protein of Rhodobacter capsulatus activates S-adenosyl--methionine:magnesium protoporphyrin IX methyltransferase."

FEBS Letters **407**(3): 337.

http://www.sciencedirect.com/science/article/B6T36-3R85JMS-B6/2/29658101df0d2c27810564067f3fb297

The bchH gene of Rhodobacter capsulatus has been cloned into an expression strain of Escherichia coli. Following induction of expression of the BchH protein, it was found that the E. coli strain also accumulated porphyrins with the fluorescence properties of protoporphyrin and zinc protoporphyrin. It was also found that the soluble BchH protein increased the activity of S-adenosyl--methionine:magnesium protoporphyrin IX methyltransferase, when mixed with membranes of an expression strain of E. coli into which the bchM gene (which encodes the methyltransferase) had been cloned, as well as membranes of a bchH mutant of R. capsulatus.

Hinshelwood, A., G. McGarvie, et al. (2002). "Characterisation of a novel mouse liver aldo-keto reductase AKR7A5." FEBS Letters **523**(1-3): 213.

http://www.sciencedirect.com/science/article/B6T36-464XYWD-H/2/72cd5cbc2f2229c88d7db7e2aa174e44

We have characterised a novel aldo-keto reductase (AKR7A5) from mouse liver that is 78% identical to rat aflatoxin dialdehyde reductase AKR7A1 and 89% identical to human succinic semialdehyde (SSA) reductase AKR7A2. AKR7A5 can reduce 2-carboxybenzaldehyde (2-CBA) and SSA as well as a range of aldehyde and diketone substrates. Western blots show that it is expressed in liver, kidney, testis and brain, and at lower levels in skeletal muscle, spleen heart and lung. The protein is not inducible in the liver by dietary ethoxyquin. Immunodepletion of AKR7A5 from liver extracts shows that it is one of the major liver 2-CBA reductases but that it is not the main SSA reductase in this tissue.

Hisano, T., M. Ono, et al. (1996). "Increased expression of T-plastin gene in cisplatin-resistant human cancer cells: identification by mRNA differential display." FEBS Letters **397**(1): 101.

http://www.sciencedirect.com/science/article/B6T36-3Y158TN-2F/2/bb91595d6c8e882513fe91fb0b3473b3

The cellular resistance to the potent anticancer agent cis-diamminedichloroplatinum(II) (cisplatin) is thought to be mediated by multiple mechanisms. The technique of differential display of mRNAs was applied to various cisplatin-resistant cell lines and the corresponding parental sensitive human bladder, prostatic, and head and neck cancer cells in order to identify genes that underlie cisplatin resistance. Twenty-four clones were confirmed by Northern blot analysis to be expressed differentially between resistant and the corresponding sensitive cells. Partial DNA sequences of the eight clones that showed a threefold or greater increase in expression in either the resistant cells (seven clones) or sensitive cells (one clone) revealed that two were derived from the T-plastin gene and one from the tissue factor gene. The abundance of T-plastin mRNA in cisplatin-resistant T24/DDP10 cell was ~12 times that in the parental T24 cells. Transfection of T24/DDP10 cells with a vector encoding full-length T-plastin antisense RNA demonstrated that reduced T-plastin expression was associated with increased sensitivity to cisplatin. These results are consistent with the hypothesis that several mechanisms participate cooperatively in the acquisition of cisplatin resistance in human cancer.

Hitoshi, N., K. Ikuko, et al. (1995). "cDNA and deduced amino acid sequence of human PK-120, a plasma kallikrein-sensitive glycoprotein." FEBS Letters **357**(2): 207.

http://www.sciencedirect.com/science/article/B6T36-495NDGJ-R/2/8dbbb3cf9dd28d0be9c60ef397c26f25

PK-120 is a substrate for plasma kallikrein (PK), recently purified from human plasma. Here we have established the cDNA sequence for human PK-120 mRNA. The deduced amino sequence of PK-120 revealed that it consists of 902 amino acid residues with a calculated mass of 116,423 Da. The putative cleavage sites by PK have been proposed, suggesting that PK-120 may be a precursor of a bioactive peptide. Most interestingly, PK-120 showed significant sequence identities to heavy chains (HCs) of the inter-[alpha]-trypsin inhibitor (ITI) superfamily.

Holzinger, A., A. A. Roscher, et al. (1998). "Genomic organization and chromosomal localization of the human peroxisomal membrane protein-1-like protein (PXMP1-L) gene encoding a peroxisomal ABC transporter." FEBS Letters **426**(2): 238.

http://www.sciencedirect.com/science/article/B6T36-3SY3H7M-M/2/3e1cb6af7893d9da1a8a514c8f3a7603

The cDNA of the peroxisomal membrane protein-1-like protein (PXMP1-L, synonyms: PMP69, P70R), a novel peroxisomal ATP binding cassette transporter of yet unknown function, has recently been cloned. The best known peroxisomal member of this protein family is the adrenoleukodystrophy protein, defects of which are the underlying cause of X-linked adrenoleukodystrophy (X-ALD). Here we describe the complete exon-intron structure (19 exons and 18 introns covering 16.0 kb) of the human PXMP1-L gene, transcript variants, the localization on chromosome 14q24 by cytogenetic analysis and sequencing of the putative promoter region. PXMP1-L has been proposed to play a role as a modifier in determining the phenotypic variations observed in X-ALD. The data presented will enable sequence analysis of the PXMP1-L gene in X-ALD patients and facilitate the analysis of PXMP1-L function.

Hoog, J.-O. (1995). "Cloning and characterization of anovel rat alcohol dehydrogenase of class II type." FEBS Letters **368**(3): 445.

http://www.sciencedirect.com/science/article/B6T36-3YRNXMS-D/2/fed2117238298d424dfdbd41c1dd625d

A class II type alcohol dehydrogenase from rat liver was characterized at the cDNA level after screening cDNA libraries in combination with PCR amplification of the 5'-part. The open reading frame translates into a polypeptide of 376 amino acid residues, which show 73% positional identity to the human class II enzyme. This suggests that the class II enzyme is the most variable form of the mammalian alcohol dehydrogenases. A deletion is apparent corresponding to position 294 of the human enzyme and amino acid residues unique to the rat protein of those interacting with the coenzyme NAD+ are found at positions 47, 51, 178, and 271. Position 47 is occupied by Pro instead of Arg or His found in most mammalian alcohol dehydrogenases. This exchanged residue will not hydrogen bond to the pyrophosphate of the coenzyme and will change the local environment around position 47 to strictly hydrophobic.

Huber, S., G. Braun, et al. (1998). "CFTR mRNA and its truncated splice variant (TRN-CFTR) are

differentially expressed during collecting duct ontogeny." FEBS Letters **423**(3): 362.

http://www.sciencedirect.com/science/article/B6T36-3S5BCS2-M/2/de3efd5f2e7c19e035bc3f7a1ec7ba7f

The collecting duct epithelium originates from the embryonic ureter by branching morphogenesis. Ontogeny-dependent changes of CFTR mRNA expression were assessed by quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) in primary monolayer cultures of rat ureteric buds (UB) and cortical collecting ducts, microdissected at different embryonic and postnatal developmental stages. The amount of wild-type CFTR-specific PCR product in UB declined to 20% of the initial value between embryonic gestational day E15 and postnatal day P1. After birth the CFTR product increased transiently between P1 and P7 by a factor of 10 and decreased towards day P14. PCR products specific for TRN-CFTR, a truncated splice variant, however, were low in early embryonic cells, increased markedly between day E17 and P2, and reached a plateau postnatally. Therefore, mRNA encoding TRN-CFTR does not appear to have a specific embryonic-morphogenetic function. By contrast, such function is suggested for wild-type CFTR mRNA as its abundance was high in early embryonic nephrogenesis, as well as during a postnatal period shortly before branching morphogenesis is completed.

Hundle, B. S., D. A. O'Brien, et al. (1993). "In vitro expression and activity of lycopene cyclase and [beta]-carotene hydroxylase from Erwinia herbicola." FEBS Letters **315**(3): 329.

http://www.sciencedirect.com/science/article/B6T36-44F7M5N-50/2/eacb38d4b3d2329c2cc465fb45a7dedf

The cyclisation of lycopene to [beta]-carotene and the hydroxylation of [beta]-carotene to zeaxanthin are common enzymatic steps in the biosynthesis of carotenoids in a wide range of bacteria, fungi, and plants. We have individually expressed in E. coli the two genes coding for these enzymatic steps in Erwinia herbicola. The cyclase and hydroxylase enzymes have apparent molecular weights of 43 kDa and 22 kDa, respectively, as determined by SDS-PAGE. Hydroxylase in vitro activity was obtained only in the cytoplasmic fraction. Cyclase also demonstrated enzyme activity in a crude cell-free lysate, although to a lesser extent.

Ikeuchi, Y., N. A. Katerelos, et al. (1998). "The enhancing of a cysteine proteinase activity at acidic pH by protein engineering, the role of glutamic 50 in the enzyme mechanism of caricain." FEBS Letters **437**(1-2): 91.

http://www.sciencedirect.com/science/article/B6T36-3V79DV9-N/2/fbb96321f2e781e2179ac062ba293637

Carica papaya produces four cysteine proteinases. Calculations show that the Cys25, His159 essential ion pair is fully ionised at pH 2.99, where activity cannot be detected, but apparently an additional ionisation with a pKa of 4 is essential for activity (an electrostatic switch). Caricain (EC 3.4.22.30) wt and D158E genetic backgrounds were used to study the contribution of E50A to activity. E50 or E135 are candidates for the switch, E50A would be expected to reduce activity. However, activity increased at pH 5.0 in both backgrounds and at the pH optimum in D158E E50A but decreased slightly in the wt background. This challenges the hypothesis of an electrostatic switch.

Imhof, M. and B. Trueb (1998). "An alternative insert of three amino acids is incorporated into collagen XIV in a developmentally regulated fashion." FEBS Letters **438**(3): 325.

http://www.sciencedirect.com/science/article/B6T36-3V5MRHV-1B/2/f597d926a2968a5852eb71661f891156

We have identified a novel splice variant of chicken collagen XIV which contains an insert of three amino acids (Val-Arg-Thr) in the sixth fibronectin type III-like (FNIII) domain. The codons for these amino acids are inserted into the mRNA by skipping of a splice donor site and usage of another donor site 9 bp further downstream in the collagen XIV gene. The percentage of the new splice variant in the total collagen XIV mRNA varies between 22 and 46% in different embryonic tissues. After hatching, however, this percentage increases dramatically and reaches 86% in adult skeletal muscle and 58% in adult gizzard, indicating developmental regulation of this splicing event. Computer modeling suggests that the three extra amino acids cause an increase in the size of a flexible loop connecting two [beta]-strands in the sixth FNIII domain. This increase might affect the exact arrangement of the FNIII domain in the collagen XIV molecule, thereby modulating its interactions with other matrix molecules.

Inokuchi, K., A. Kato, et al. (1996). "Increase in activin [beta]A mRNA in rat hippocampus during long-term potentiation." FEBS Letters **382**(1-2): 48.

http://www.sciencedirect.com/science/article/B6T36-3Y0SKJ0-CJ/2/12c2fe30d3286f121270c70d81bdee07

We have used mRNA differential display to isolate genes that are induced by neural activity in rat hippocampus. One of these encodes activin [beta]A subunit. Convulsive seizure caused by kainate significantly induced the expression of activin [beta]A mRNA. Furthermore high frequency stimulation (HFS) of perforant pathway, which produced a persistent long-term potentiation (LTP) (> 10 h), caused a marked increase at 3 h in the level of activin [beta]A mRNA at the dentate gyrus of urethane-anesthetized rat. The increase was NMDA receptor-dependent. By contrast the level of inhibin [alpha] mRNA was not changed following the induction of LTP. The results suggest a role for activin in maintenance of neural plasticity in the adult brain.

Iwasaki, M., H. Igarashi, et al. (1993). "Cloning, characterization and overexpression of a Streptococcus pyogenes gene encoding a new type of mitogenic factor." FEBS Letters **331**(1-2): 187.

http://www.sciencedirect.com/science/article/B6T36-449TM3T-Y3/2/83318442aa9c70f1a18323d4b5554430

A new type of mitogenic factor, termed MF, has been found in the culture supernatant of Streptococcus pyogenes and its N-terminal amino acid sequence has been determined. On the basis of this sequence, an S. pyogenes gene encoding MF was cloned and its nucleotide sequence was determined. The MF gene includes a long, open reading frame with 813 nucleotides capable of encoding the MF precursor protein with 271 amino acids. Removal of the putative 43 residues as a signal peptide results in the mature MF protein with 228 amino acids. The molecular mass of the mature MF is calculated as 25,363 which is consistent with the previously determined value of 25,370 for MF secreted from S. pyogenes. Neither nucleotide nor amino acid sequence homology was found between the mature MF and other streptococcal pyrogenic exotoxins, such as SPE A, SPE B and SPE C. The mature MF was recombinantly overexpressed as a fusion protein with glutathione S-transferase in Escherichia coli. The recombinant protein showed mitogenic activity in rabbit peripheral blood lymphocytes and

immunoreactivity with the rabbit antiserum raised against the secreted MF from S. pyogenes. These data indicate that a unique gene encoding MF was cloned from S. pyogenes.

Jasmin, B. J., H. Alameddine, et al. (1995). "Expression of utrophin and its mRNA in denervated mdx mouse muscle." FEBS Letters **374**(3): 393.

http://www.sciencedirect.com/science/article/B6T36-3YS2BPP-87/2/ea2a941551f9da0b66cc31d8e2d6d643

Utrophin is a large cytoskeletal protein which shows high homology to dystrophin. In contrast to the sarcolemmal distribution of dystrophin. utrophin accumulates at the postsynaptic membrane of the neuromuscular junction. Because of its localization within this compartment of muscle fibers, expression of utrophin may be significantly influenced by the presence of the motor nerve. We tested this hypothesis by denervating muscles of mdx mouse and monitoring levels of utrophin and its mRNA by immunofluorescence, immunoblotting and RT-PCR. A significant increase in the number of utrophin positive fibers was observed by immunofluorescence 3 to 21 days after sectioning of the sciatic nerve. Quantitative analyses of utrophin and its transcripts in hindlimb muscles denervated for two weeks showed only a moderate increase in the levels of both utrophin (~ 2-fold) and its transcript (~60 to 90%). The present data suggest that although utrophin is a component of the postsynaptic membrane, its neural regulation is distinct from that of the acetylcholine receptor.

Jeong, H. G. and J. Y. Kim (2002). "Induction of inducible nitric oxide synthase expression by 18[beta]-glycyrrhetinic acid in macrophages." FEBS Letters **513**(2-3): 208.

http://www.sciencedirect.com/science/article/B6T36-450HHRN-S/2/9dc927abf408a22ded55db5459c8781b

Glycyrrhizin (GL), a triterpenoid saponin fraction of licorice, is reported to have anti-viral and anti-tumor activities and is metabolized to 18[beta]-glycyrrhetinic acid (GA) in the intestine by intestinal bacteria. However, the mechanism underlying its effects is poorly understood. To further elucidate the mechanism of GA, the aglycone of GL, we investigated the effects of GA on the release of nitric oxide (NO) and at the level of inducible NO synthase (iNOS) gene expression in mouse macrophages. We found that GA elicited a dose-dependent increase in NO production and in the level of iNOS mRNA. Since iNOS transcription has been shown to be under the control of the transcription factor nuclear factor [kappa]B (NF-[kappa]B), the effects of GA on NF-[kappa]B activation were examined. Transient expression assays with NF-[kappa]B binding sites linked to the luciferase gene revealed that the increased level of iNOS mRNA, induced by GA, was mediated by the NF-[kappa]B transcription factor complex. By using DNA fragments containing the NF-[kappa]B binding sequence, GA was shown to activate the protein/DNA binding of NF-[kappa]B to its cognate site, as measured by electrophoretic mobility shift assay. These results demonstrate that GA stimulates NO production and is able to up-regulate iNOS expression through NF-[kappa]B transactivation in macrophages.

Jeung, E.-B., J. Krisinger, et al. (1992). "Molecular cloning of the full-length cDNA encoding the human calbindin-D9k." FEBS Letters **307**(2): 224.

http://www.sciencedirect.com/science/article/B6T36-44P8M4P-PH/2/7fd24e1bb24cdfcaacb6871d75bf9ef8

The full-length cDNA encoding the human calbindin-D9k (CaBP-9k) has been cloned using reverse transcription/PCR methodology with rat- and bovine-derived primers and intestinal RNA. A core product, and both a 5'and 3' product encompassing the full-length cDNA were obtained. The clones include coding region for 79 amino acids, 57 nucleotides 5'- and 159 nucleotides 3'-non-coding region, and a poly(A) tail. The deduced protein sequence is homologous Lo other mammalian CaBPs. Northern analysis revealed the mRNA in human duodenum to be about 600 nucleotides in length, Expression levels in adult human tissue were substantially lower than in child, rat or porcine intestine.

Kazumori, H., S. Ishihara, et al. (2001). "Analysis of gastrin receptor gene expression in proliferating cells in the neck zone of gastric fundic glands using laser capture microdissection." FEBS Letters **489**(2-3): 208.

http://www.sciencedirect.com/science/article/B6T36-4281361-N/2/c9221e475c60ea974480f7ba1e2f385d

Gastrin stimulates proliferation of progenitor cells in the neck zone of gastric fundic mucosa. However, whether it directly enhances this proliferation through its receptors remains unclear. We investigated the expression of gastrin receptors in neck zone proliferating cells in rat gastric fundic glands using a reverse transcription polymerase chain reaction (RT-PCR) coupled with laser capture microdissection and in situ RT-PCR. Gastrin receptor expression was identified in c-fos-expressing cells located in the neck zone, and results of the RT-PCR analysis argued against contamination by other cells, such as enterochromaffin-like, parietal or D cells. Supporting this finding, gastrin receptor gene expression was identified in the neck zone as well as base glands by in situ RT-PCR. Therefore, it is suggested that proliferating cells in the neck zone are stimulated directly by gastrin via their gastrin receptors.

Kennedy, C. H., R. Cueto, et al. (1998). "Overexpression of hMTH1 mRNA: a molecular marker of oxidative stress in lung cancer cells." FEBS Letters **429**(1): 17.

http://www.sciencedirect.com/science/article/B6T36-3T1772M-4/2/e981281ba9f8752a1dc14a4139b5b1a6

Human MutT homologue (hMTH1) mRNA was overexpressed in SV-40-transformed non-tumorigenic human bronchial epithelial cells (BEAS-2B cells) and in 11 out of 12 human lung cancer cell lines relative to normal human bronchial epithelial cells. Expression levels of hMTH1 mRNA were inversely proportional to cellular levels of 8-oxo-deoxyguanosine. Together, these results suggest that hMTH1 gene expression may represent a molecular marker of oxidative stress that could ultimately be used to elucidate the temporal relationships between oxidative stress, genomic instability and the development of lung cancer.

Kepplinger, K. J. F., H. Kahr, et al. (2000). "A sequence in the carboxy-terminus of the [alpha]1C subunit important for targeting, conductance and open probability of L-type Ca2+ channels." FEBS Letters **477**(3): 161.

http://www.sciencedirect.com/science/article/B6T36-40S0BDF-3/2/caed52b58d6118fc80b2653de72b2a2c

The role of the 80-amino acid motif 1572-1651 in the C-terminal tail of [alpha]1C Ca2+ channel

subunits was studied by comparing properties of the conventional [alpha]1C,77 channel expressed in HEK-tsA201 cells to three isoforms carrying alterations in this motif. Replacement of amino acids 1572-1651 in [alpha]1C,77 with 81 non-identical residues leading to [alpha]1C,86 impaired membrane targeting and cluster formation of the channel. Similar to [alpha]1C,86, substitution of its 1572-1598 ([alpha]1C,77L) or 1595-1652 ([alpha]1C,77K) segments into the [alpha]1C,77 channel yielded single-channel Ba2+ currents with increased inactivation, reduced open probability and unitary conductance, when compared to the [alpha]1C,77 channel. Thus, the C-terminal sequence 1572-1651 of the [alpha]1C subunit is important for membrane targeting, permeation and open probability of L-type Ca2+ channels.

Ketchum, K. A. and C. W. Slayman (1996). "Isolation of an ion channel gene from Arabidopsis thaliana using the H5 signature sequence from voltage-dependent K+ channels." FEBS Letters **378**(1): 19.

http://www.sciencedirect.com/science/article/B6T36-3Y0SKW2-MW/2/d4fb4c20fa4d1cb1732a8ce7fe7d4dd4

A degenerate oligonucleotide corresponding to the K+ channel signature sequence (TMTTVGYGD) was used to isolate the genomic and cDNA forms of a new channel gene, AKT3, from Arabidopsis thaliana. The deduced protein sequence has a predicted membrane topography similar to Shaker-like K+ channels. Three distinct modules comprise the carboxyl-terminal half: a nucleotide-binding motif, an ankyrin repeat domain, and a polyglutamate track. Xenopus oocytes injected with cRNA exhibited an inward-rectifying K+ current, demonstrating that the AKT3 polypeptide is a functional transport protein. Two other Arabidopsis K+ transporters (AKT1 and KAT1) share 60% homology with AKT3; together these proteins constitute a family of plant inward-rectifying K+ channels.

Kim, H. M., G. T. Oh, et al. (1997). "MDR-1 gene expression is a minor factor in determining the multidrug resistance phenotype of MCF7/ADR and KB-V1 cells." FEBS Letters **412**(1): 201.

http://www.sciencedirect.com/science/article/B6T36-3R7B21X-4P/2/a32d3e6eb3d012b4d65173bb9003d244

The relevance of MDR-1 gene expression to the multidrug resistance phenotype was investigated. Drug-resistant cells, KB-V1 and MCF7/ADR, constantly expressed mRNA of the MDR-1 gene and were more resistant to vinblastine and adriamycin than drug-sensitive cells, KB-3-1 and MCF7. The drug efflux rate of KB-V1 was the same as KB-3-1 although the MDR-1 gene was expressed in only the resistant cell. The higher intracellular drug concentration of KB-3-1 than KB-V1 was due to the large drug influx. In the case of MCF7 and MCF7/ADR, the influx and efflux of the drug had nearly the same pattern and drug efflux was not affected by verapamil. The amount of ATP, cofactor of drug pumping activity of P-glycoprotein, was not changed by the resistance. These observations suggested that drug efflux mediated by MDR-1 gene expression was not a major determining factor of drug resistance in the present cell systems, and that the drug resistance could be derived from the change in drug uptake and other mechanisms.

Kobayashi, K., J. Sasaki, et al. (2001). "Structural organization, complete genomic sequences and mutational analyses of the Fukuyama-type congenital muscular dystrophy gene, fukutin." FEBS Letters **489**(2-3): 192.

http://www.sciencedirect.com/science/article/B6T36-4281361-J/2/dda13153e103bb499571a94096c699e1

Fukuyama-type congenital muscular dystrophy (FCMD) is an autosomal recessive severe muscular dystrophy in combination with cerebral cortical dysplasia. Previously, we identified the gene responsible for FCMD, termed fukutin, through positional cloning. In this study, we have sequenced 131892 bp of genomic DNA in the region of the fukutin gene on chromosome 9q31 and obtained its complete genomic structure. The fukutin genomic sequence spans approximately 100 kb and is organized into 10 exons (41-6067 bp) and nine introns (1841-21460 bp). Using these sequence data, we have identified three novel fukutin mutations in FCMD patients. We have also located a putative TATA box in the flanking 5' region and identified numerous alternatively spliced fukutin mRNA transcripts. Analysis of expressed sequence tag clusters within the region revealed two novel genes upstream of the fukutin gene. These data provide fundamental information to support detailed genetic and functional analyses of the fukutin gene.

Koivula, T., I. Palva, et al. (1991). "Nucleotide sequence of the secY gene from Lactococcus lactis and identification of conserved regions by comparison of four SecY proteins." FEBS Letters **288**(1-2): 114.

http://www.sciencedirect.com/science/article/B6T36-44XMXYH-1S/2/9ae2c091ef773b6e63bbb1ca8a318bb1

SecY is an integral membrane protein which participates in the translocation of proteins through the bacterial cell membrane. We have cloned the secY gene of Lactococcus lactis, and found its deduced protein sequence, 439 amino acids long, to be similar in length to the previously determined SecY proteins of Escherichia coli, Bacillus subtilis and Mycoplasma capricolum. Comparison of the L. lactis SecY to the 3 other SecY proteins revealed 90 conserved amino acid residues (21%). Nearly half of the conserved residues are clustered in 2 of the 10 transmembrane segments, and in 2 of the 6 cytoplasmic regions. Some of the conserved regions are apparently responsible for the interactions of SecY with signal sequences, and the proteins SecE and SecA.

Korner, I., R. Weber-Nordt, et al. (1997). "Analysis of a regulatory element in the 5'-untranslated region of the bcl-2 gene." FEBS Letters **406**(1-2): 31.

http://www.sciencedirect.com/science/article/B6T36-3RC4V01-7/2/7908e46764f4ec9455912c8593fe3380

The bcl-2 gene is an important antagonist of apoptosis, the programmed cell death. Bcl-2 is highly expressed in a variety of lymphomas. Lymphocytes of patients with chronic lymphocytic leukemia (CLL) express high amounts of bcl-2 even in the absence of the t(14;18) translocation, resulting in a strong resistance towards corticosteroid induced apoptosis. Within the 5'-untranslated region of the bcl-2 gene a p53 dependent negative response element has been described. Genetic alterations within this element could lead to uncontrolled overexpression of bcl-2 and subsequent resistance towards apoptosis. We therefore analyzed the mRNA from the 5'-untranslated region -279 to -85 bp of the bcl-2 gene by direct PCR sequencing from peripheral blood derived lymphocytes from patients with CLL and normal healthy donors. Compared to published sequences (Tsujimoto and Croce (1986) Proc. Natl. Acad. Sci. USA 83, 5214), we consistently found an exchange at position 1271 from A to G and at position 1284 from G to A in all CLL as well as normal donor derived samples analyzed. Thus, CLL specific alterations compared to normal cells could not be found and deregulated expression of bcl-2 in CLL cells

does not appear to be due to alterations in the p53 dependent negative response element of the bcl-2 gene. However, our data add information to published sequence data of this region. (c) 1997 Federation of European Biochemical Societies.

Koshikawa, S., R. Cornette, et al. (2005). "Screening of genes expressed in developing mandibles during soldier differentiation in the termite Hodotermopsis sjostedti." FEBS Letters **579**(6): 1365.

http://www.sciencedirect.com/science/article/B6T36-4FBW3PM-4/2/7f75dc4cbe9eab9c296e216702f5d56d

We investigated the morphological changes accompanying soldier differentiation in the damp-wood termite Hodotermopsis sjostedti. Genes expressed in the developing mandibles, which undergo the most remarkable morphological changes during soldier differentiation, were screened using fluorescent differential display. Database searches for sequence similarities were conducted and the relative expression levels were then quantified by real-time polymerase chain reaction. Among the identified candidate genes, 12 genes were upregulated during soldier differentiation. These included genes for cuticle proteins, nucleic acid binding proteins, ribosomal proteins and actin-binding protein, which were inferred to be involved in caste-specific morphogenesis in termites.

Li, C. M., S. J. Campbell, et al. (2002). "Response heterogeneity of human macrophages to ATP is associated with P2X7 receptor expression but not to polymorphisms in the P2RX7 promoter." FEBS Letters **531**(2): 127.

http://www.sciencedirect.com/science/article/B6T36-46X2H60-7/2/dda7a407a177fb573cd09ef8045504ac

A region 2 kb upstream of exon 1 of the P2X7 gene was sequenced using DNA from nine healthy individuals who exhibited three different ATP response phenotypes (i.e. high, low and interferon gamma-inducible). Five single nucleotide polymorphisms were identified within the nine donor promoter sequences but none were associated with a specific ATP response phenotype. A P2X7 loss of function polymorphism (1513 in exon 13) was also screened for within donor DNA but no response associations were identified. ATP response phenotype was positively associated with P2X7 receptor expression, as assessed by flow cytometry, but not with any identified receptor or promoter gene polymorphisms.

Li, N., Z. Wiesman, et al. (1992). "A functional tomato ACC synthase expressed in Escherichia coli demonstrates suicidal inactivation by its substrate S-adenosylmethionine." FEBS Letters **306**(2-3): 103.

http://www.sciencedirect.com/science/article/B6T36-44P8HX6-2H/2/ccf3fa3a69feed6de45497beb6e5c0c2

1-Aminocyclopropane-1-carboxylate (ACC) synthase is a key enzyme in the biosynthesis of the plant hormone, ethylene. We have isolated, sequenced and expressed a functional tomato (cy Pik-Red) ACC synthase gene in Escherichia coli. ACC synthase expressed in E. coli was inactivated by incubation with S-adenosylmethionine (SAM), the half--time of which was concentration dependent. Mixing the tomato fruit protein extract with the cell-free extract from transformed E. coli did not affect SAM-dependent inactivation of ACC synthase activity. Thus,

single isoforms of the ACC synthase enzyme, which demonstrate the biochemical features expected of the tomato fruit enzyme, can be expressed in E. coli and their structure--function relationships investigated.

Londhe, V., N. McNamara, et al. (2003). "Viral dsRNA activates mucin transcription in airway epithelial cells." FEBS Letters **553**(1-2): 33.

http://www.sciencedirect.com/science/article/B6T36-49FXMRW-1/2/11ae1ba116e0b9110f73154f570c7a11

Double-stranded (ds) RNA is a biologically active component of many viruses including rhinoviruses infecting the upper respiratory tract. Mucus production is a common symptom of such infections. Here, we show that mucin, the glycoprotein subunit of mucus gels, is transcriptionally upregulated in an NF-[kappa]B- and p38-dependent manner when homogeneous cultures of epithelial cells are exposed to dsRNA. Furthermore, upstream of p38 in this system, dsRNA stimulates the extracellular release of ATP and activation of cell surface ATP receptors, which are G protein-coupled. This results in the stimulation of phospholipase C and protein kinase C. These findings suggest that ATP receptor antagonists could be used to modulate mucus production induced by virus.

Lovlie, R., A. K. Daly, et al. (1996). "Ultrarapid metabolizers of debrisoquine: Characterization and PCR-based detection of alleles with duplication of the CYP2D6 gene." FEBS Letters **392**(1): 30.

http://www.sciencedirect.com/science/article/B6T36-3Y0SM18-SD/2/603e9f7d99492f509c436bb29b6b85f9

Up to 7% of Caucasians may demonstrate ultrarapid metabolism of debrisoquine due to inheritance of alleles with duplicated functional CYP2D6 genes. Here we describe the genomic organization of the duplicated CYP2D6 genes in the 42 kb XbaI allele. We postulate that this duplication originates from a homologous, unequal cross-over event which involved two 29 kb XbaI wild-type alleles, and had break points within a 2.8 kb direct repeat (CYP-REP) flanking the CYP2D6 gene. Moreover, we have designed two different PCR assays for detection of alleles with duplicated CYP2D6 genes. Both assays correctly identified 29 out of 29 subjects positive for the 42 kb XbaI allele. No false negative or false positive reactions were observed.

Ma, J.-x., D. Zhang, et al. (1999). "Identification of RPE65 in transformed kidney cells." FEBS Letters **452**(3): 199.

http://www.sciencedirect.com/science/article/B6T36-3WP2JNF-K/2/7954a9d9dcec2ec161b1adea345120a9

The protein RPE65 has an important role in retinoid processing and/or retinoid transport in the eye. Retinoids are involved in cell differentiation, embryogenesis and carcinogenesis. Since the kidney is known as an important site for retinoid metabolism, the expression of RPE65 in normal kidney and transformed kidney cells has been examined. The RPE65 mRNA was detected in transformed kidney cell lines including the human embryonic kidney cell line HEK293 and the African green monkey kidney cell lines COS-1 and COS-7 by reverse transcription PCR. In contrast, it was not detected in human primary kidney cells or monkey kidney tissues under the same PCR conditions. The RPE65 protein was also identified in COS-7 and HEK293 cells by

Western blot analysis using a monoclonal antibody to RPE65, but not in the primary kidney cells or kidney tissues. The RPE65 cDNA containing the full-length encoding region was amplified from HEK293 and COS-7 cells. DNA sequencing showed that the RPE65 cDNA from HEK293 cells is identical to the RPE65 cDNA from the human retinal pigment epithelium. The RPE65 from COS-7 cells shares 98 and 99% sequence identity with human RPE65 at the nucleotide and amino acid levels, respectively. Moreover, the RPE65 mRNA was detected in three out of four renal tumor cultures analyzed including congenital mesoblastic nephroma and clear cell sarcoma of the kidney. These results demonstrated that transformed kidney cells express this retinoid processing protein, suggesting that these transformed cells may have an alternative retinoid metabolism not present in normal kidney cells.

Maekawa, K., N. Imagawa, et al. (1994). "Molecular cloning of a novel protein-tyrosine phosphatase containing a membrane-binding domain and GLGF repeats." FEBS Letters **337**(2): 200.

http://www.sciencedirect.com/science/article/B6T36-449TK8V-K7/2/de1d44e5b8a6790fed7a0cdba8b1a065

A full-length cDNA encoding a novel cytosolic protein-tyrosine phosphatase (PTP), PTP-BAS, was cloned from human basophils. Due to in-frame deletions in the coding region, PTP-BAS exists in three isoforms: 7,455 bp (2,485 aa) for type 1, 7,398 bp (2,466 aa) for type 2 and 6,882 bp (2,294 aa) for type 3. All three isoforms contain a single PTP catalytic domain at the carboxyl termini as well as two distinct structural sequences. Amino terminal sequences of 300 amino acids are homologous to membrane-binding domains of eytoskeleton-associated proteins. Three 90 amino acid internal repetitive sequences are homologous to the GLGF repeats found in guanylate kinase proteins. PTP-BAS was expressed in various human tissues, especially highly in the kidney and lung. Interestingly, the BAS mRNA level in the fetal brain was remarkably high.

Maekawa, K., N. Imagawa, et al. (1992). "Determination of the sequence coding for the [beta] subunit of the human high-affinity IgE receptor." FEBS Letters **302**(2): 161.

http://www.sciencedirect.com/science/article/B6T36-44XN071-86/2/f3b78e958a54319fe6257b06f676e2da

The cDNA encoding the [beta] subunit of the human high-affinity IgE receptor was cloned by a combination or various polymerase chain reactions (PCR). A major portion of the [beta] cDNA was amplified using primers homologous within the sequences of rat and mouse. The 3' unknown sequence was preferentially amplified using the RNA template-specific PCR and the improved two-step PCR. The 5' unknown sequence was specifically amplified by our newly developed PCR walking. Random heptanucleotides tagged with a unique sequence at the 5' end were used as the walking primer. Finally, the entire coding region was amplified and sequenced. The two extracellular loops of the human [beta] subunit were the least homologous to those of rat and mouse.

Malmstrom, S., P. Askerlund, et al. (1997). "A calmodulin-stimulated Ca2+-ATPase from plant vacuolar membranes with a putative regulatory domain at its N-terminus." FEBS Letters **400**(3): 324.

http://www.sciencedirect.com/science/article/B6T36-3PNRX2B-29/2/2f42e70af21a74b4f96a974dcbd7ea5b

A cDNA, BCA1, encoding a calmodulin-stimulated Ca2+-ATPase in the vacuolar membrane of cauliflower (Brassica oleracea) was isolated based on the sequence of tryptic peptides derived from the purified protein. The BCA1 cDNA shares sequence identity with animal plasma membrane Ca2+-ATPases and Arabidopsis thaliana ACA1, that encodes a putative Ca2+ pump in the chloroplast envelope. In contrast to the plasma membrane Ca2+-ATPases of animal cells, which have a calmodulin-binding domain situated in the carboxy-terminal end of the molecule, the calmodulin-binding domain of BCA1 is situated at the amino terminus of the enzyme.

Mann, V. M., J. M. Cooper, et al. (1992). "Quantitation of a mitochondrial DNA deletion in Parkinson's disease." FEBS Letters **299**(3): 218.

http://www.sciencedirect.com/science/article/B6T36-44XN30T-S9/2/6d0ec5aaffe3e019b2df9baa5f04ff79

A 5 kilobase deletion in mitochondrial DNA (mtDNA) has been reported to be responsible for the specific complex I deficiency in the substantia nigra (SN) of the Parkinson's disease (PD) brain. We have studied mitochondrial respiratory chain function in the SN from control and PD subjects, and analysed mtDNA, extracted from the same tissues, by Southern biot and the polymerase chain reaction (PCR). Quantitation of the levels of the deletion indicate that it does not contribute to the pathogenesis of PD nor to a complex I deficiency but seems likely to be an age-related observation.

Matoba, N., N. Doyama, et al. (2001). "Design and production of genetically modified soybean protein with anti-hypertensive activity by incorporating potent analogue of ovokinin(2-7)." FEBS Letters **497**(1): 50.

http://www.sciencedirect.com/science/article/B6T36-43N8FNN-9/2/4f7d3730e86138fc542ee942fde7d694

The potent anti-hypertensive peptide, RPLKPW, has been designed based on the structure of ovokinin(2-7). The sequence encoding this peptide was introduced into three homologous sites in the gene for soybean [beta]-conglycinin [alpha]' subunit. The native [alpha]' subunit as well as the modified, RPLKPW-containing [alpha]' subunit were expressed in Escherichia coli, recovered from the soluble fraction and then purified by ion-exchange chromatography. The RPLKPW peptide was released from recombinant RPLKPW-containing [alpha]' subunit after in vitro digestion by trypsin and chymotrypsin. Moreover, the undigested RPLKPW-containing [alpha]' subunit given orally at a dose of 10 mg/kg exerted an anti-hypertensive effect in spontaneously hypertensive rats, unlike the native [alpha]' subunit. These results provide evidence for the first time that a physiologically active peptide introduced into a food protein by site-directed mutagenesis could practically function in vivo even at a low dose.

Matsui, K., C. Ujita, et al. (2000). "Fatty acid 9- and 13-hydroperoxide lyases from cucumber." FEBS Letters **481**(2): 183.

http://www.sciencedirect.com/science/article/B6T36-415PR4T-N/2/9ca242daefb45ae9777d9d4ed1798977

Fatty acid hydroperoxide lyase (HPL) is a novel P-450 enzyme that cleaves fatty acid hydroperoxides to form short-chain aldehydes and oxo-acids. In cucumber seedlings, the

activities of both fatty acid 9HPL and 13HPL could be detected. High 9HPL activity was especially evident in hypocotyls. Using a polymerase chain reaction-based cloning strategy, we isolated two HPL-related cDNAs from cucumber hypocotyls. One of them, C17, had a frameshift and it was apparently expressed from a pseudogene. After repairing the frameshift, the cDNA was successfully expressed in Escherichia coli as an active HPL with specificity for 13-hydroperoxides. The other clone, C15, showed higher sequence similarity to allene oxide synthase (AOS). This cDNA was also expressed in E. coli, and the recombinant enzyme was shown to act both on 9- and 13-hydroperoxides, with a preference for the former. By extensive product analyses, it was determined that the recombinant C15 enzyme has only HPL activity and no AOS activity, in spite of its higher sequence similarity to AOS.

Matsushita, H., V. G. Lelianova, et al. (1999). "The latrophilin family: multiply spliced G protein-coupled receptors with differential tissue distribution." FEBS Letters **443**(3): 348.

http://www.sciencedirect.com/science/article/B6T36-3VS2KSG-S/2/f99e4ee0bcbec3a6e875795d875be99f

Latrophilin is a brain-specific Ca2+-independent receptor of [alpha]-latrotoxin, a potent presynaptic neurotoxin. We now report the finding of two novel latrophilin homologues. All three latrophilins are unusual G protein-coupled receptors. They exhibit strong similarities within their lectin, olfactomedin and transmembrane domains but possess variable C-termini. Latrophilins have up to seven sites of alternative splicing; some splice variants contain an altered third cytoplasmic loop or a truncated cytoplasmic tail. Only latrophilin-1 binds [alpha]-latrotoxin; it is abundant in brain and is present in endocrine cells. Latrophilin-3 is also brain-specific, whereas latrophilin-2 is ubiquitous. Together, latrophilins form a novel family of heterogeneous G protein-coupled receptors with distinct tissue distribution and functions.

Matsuura, T., K. Takahashi, et al. (2002). "Increased expression of vascular endothelial growth factor in placentas of p57Kip2 null embryos." FEBS Letters **532**(3): 283.

http://www.sciencedirect.com/science/article/B6T36-4772S3F-8/2/e504c547d278102a2a83d1f2f49d97fc

Placentas of mice lacking p57Kip2 expression have trophoblastic hyperplasia. To elucidate the mechanism underlying this phenomenon, we studied expression of two angiogenic factors, vascular endothelial growth factor (VEGF) and placenta growth factor (PlGF). Immunohistochemical analysis with anti-VEGF antibodies indicated that VEGF expression was stronger and more clearly detectable in placentas of p57Kip2 null embryos compared to wild-type placentas. PlGF showed no significant differences between placentas of p57Kip2 null and wild-type embryos. In quantitative analysis, placentas of p57Kip2 null embryos showed higher VEGF messenger (m)RNA and protein levels than did wild-type placentas. PlGF mRNA and protein levels were not significantly different. These findings suggest that VEGF is involved in the hyperplasia that occurs in placentas of p57Kip2 null embryos.

McHale, M., M. C. Coldwell, et al. (1994). "Expression and functional characterisation of a synthetic version of the human D4 dopamine receptor in a stable human cell line." FEBS Letters **345**(2-3): 147.

http://www.sciencedirect.com/science/article/B6T36-447G3F5-

B6/2/3dd6349de20b2af92b176c0f9d8b4d1a

A synthetic version of the human D4 (hD4) dopamine receptor was prepared. The G/C content of the natural gene was reduced by 14% without altering the amino acid composition of the corresponding protein sequence. HEK293 cells were transfected with the synthetic hD4 gene and stable clones resistant to G418 selected. The hD4 receptor expressed from the synthetic gene had identical pharmacological characteristics to the native hD4 receptor [(1991) Nature 350, 610-619; (1992) Nature 358, 149-152]. Functional studies with cells expressing the synthetic hD4 gene indicated negative coupling of this receptor to adenylate cyclase.

McNally, E. M., C. T. Ly, et al. (1998). "Human [epsi]-sarcoglycan is highly related to [alpha]-sarcoglycan (adhalin), the limb girdle muscular dystrophy 2D gene." FEBS Letters **422**(1): 27.

http://www.sciencedirect.com/science/article/B6T36-3S0FJ2M-Y/2/29637ff4e27aaf9d5f0e592ac00a1f71

The dystrophin-glycoprotein complex (DGC) is critical for muscle membrane stability. The sarcoglycans are transmembrane proteins within the DGC, and the function of the sarcoglycans is unknown. Mutations in sarcoglycan genes cause autosomal recessive muscular dystrophy. We have identified a new sarcoglycan gene with high homology to [alpha]-sarcoglycan highlighting the redundancy of the DGC. This gene, named [epsi]-sarcoglycan, has an identical intron-exon structure to [alpha]-sarcoglycan, and is more broadly expressed. The characterization of [epsi]-sarcoglycan should make it possible to determine if it, like the other sarcoglycan genes, is mutated in muscular dystrophy.

Menon, R. P., M. Strom, et al. (2000). "Interaction of a novel cysteine and histidine-rich cytoplasmic protein with galectin-3 in a carbohydrate-independent manner." FEBS Letters **470**(3): 227.

http://www.sciencedirect.com/science/article/B6T36-3YWWV7G-1/2/a59f6c251de8d16da2544596975e5be1

We have used the yeast two-hybrid system to search for cytoplasmic proteins that might assist in the intracellular trafficking of the soluble [beta]-galactoside-binding protein, galectin-3. We utilised as bait murine full-length galectin-3 to screen a murine 3T3 cDNA library. Several interacting clones were found to encode a partial open reading frame and a full-length clone was obtained by rapid amplification of cDNA ends methodology. In various assays in vitro the novel protein was shown to bind galectin-3 in a carbohydrate-independent manner. The novel protein contains an unusually high content of cysteine and histidine residues and shows significant sequence homologies with several metal ion-binding motifs present in known proteins. Confocal immunofluorescence microscopy of permeabilised 3T3 cells shows a prominent perinuclear, as well as cytoplasmic, localisation of the novel protein.

Meyer, K., M. Keil, et al. (1999). "A leucine-rich repeat protein of carrot that exhibits antifreeze activity." FEBS Letters **447**(2-3): 171.

http://www.sciencedirect.com/science/article/B6T36-3W4GJS0-B/2/bfc860bf5e381f2ba1342ac5ff1cf620

A gene encoding an antifreeze protein (AFP) was isolated from carrot (Daucus carota) using

sequence information derived from the purified protein. The carrot AFP is highly similar to the polygalacturonase inhibitor protein (PGIP) family of apoplastic plant leucine-rich repeat (LRR) proteins. Expression of the AFP gene is rapidly induced by low temperatures. Furthermore, expression of the AFP gene in transgenic Arabidopsis thaliana plants leads to an accumulation of antifreeze activity. Our findings suggest that a new type of plant antifreeze protein has recently evolved from PGIPs.

Mimura, N., K. Ichikawa, et al. (1996). "A transient increase of snoN transcript by growth arrest upon serum deprivation and cell-to-cell contact." FEBS Letters **397**(2-3): 253.

http://www.sciencedirect.com/science/article/B6T36-3Y15908-69/2/a0a17e9005e149ba9b16bcdfb3693ec1

To analyze the possible involvement of c-ski and c-sno during the course of in vitro myogenesis, expression of their transcripts during differentiation of a murine muscle cell line (C2C12) was monitored by competitive reverse transcription-polymerase chain reaction (RT-PCR). The transcripts of c-snoN were temporarily increased 25-fold above basal level at 12 h prior to the onset of transcription of muscle-specific gene, e.g. myogenin and muscle creatine kinase, whereas c-ski was expressed invariably. The transient increase of c-snoN was blocked when myogenesis was interrupted by the presence of fetal calf serum in culture medium, probably due to growth factors being included; basic fibroblast growth factor (b-FGF) blocked the transient increase whereas epidermal growth factor (EGF) did not, consistent with the inhibitory effect of b-FGF and no effect of EGF on myotube formation of C2C12. In fibroblastic C3H10T1/2 cells, snoN exhibited a similar transient increase of transcript when growth arrested under the same conditions as for in vitro myogenesis, indicating that the expression of snoN is not sufficient to induce the onset of muscle differentiation and an unknown factor involved in myogenic cells is necessary. The transient increase of snoN transcript may represent a common entrance step of cells into the GO phase where muscle differentiation is substantiated, considering that it was observed upon growth arrest of fibroblastic C3H10T1/2 cells and prior to the elevation of MCK in C2C12 but undetected when entry into G0 was blocked by b-FGF.

Miyatake, R., A. Furukawa, et al. (1996). "Tissue-specific alternative splicing of mouse brain type ryanodine receptor/calcium release channel mRNA." FEBS Letters **395**(2-3): 123.

http://www.sciencedirect.com/science/article/B6T36-497C8B7-2V/2/fa1ff088f384738eb681bb894a742c0d

We detected alternative splicing of the mouse brain type ryanodine receptor (RyR3) mRNA. The splicing variant was located in the transmembrane segment. The non-splicing type (RyR3-II) included a stretch of 341 bp, and that of the 13th codon was stop codon TAA. Reverse transcription-polymerase chain reaction (RT-PCR) analysis shows that RyR3-II mRNA was expressed in various peripheral tissues and brain at all developmental stages. However, interestingly, the splicing type (RyR3-I) mRNA was detected only in the cerebrum. These findings suggest that the splicing variants RyR3-I and RyR3-II may generate functional differences of RyR3 in a tissue-specific manner.

Mochizuki, T., Y. Onda, et al. (2004). "Two independent light signals cooperate in the activation of the plastid psbD blue light-responsive promoter in Arabidopsis." FEBS Letters **571**(1-3): 26.

http://www.sciencedirect.com/science/article/B6T36-4CRXYTW-4/2/08c3ad60ca127659ff2091d2b81447a6

The psbD blue light-responsive promoter (BLRP), whose activation has been considered to require strong blue light, is recognized only by SIG5 among six [sigma] factors of plastid RNA polymerase in Arabidopsis. We found SIG5 transcript accumulation was rapidly induced after a 30-min induction time by blue light (470 nm) with an intensity threshold of 5 [mu]mol m-2 s-1 through cryptochromes. Besides this weak blue light, the psbD BLRP activation required the stronger light such as 50 [mu]mol m-2 s-1 irrespective of blue or red light (660 nm). Thus, the two independent light signalings, the cryptochrome-mediated signaling to induce SIG5 transcription and the stronger light-dependent signaling, cooperate to activate the psbD BLRP.

Monstein, H.-J., J. U. Thorup, et al. (1993). "cDNA deduced procionin: Structure and expression in protochordates resemble that of procholecystokinin in mammals." FEBS Letters **331**(1-2): 60.

http://www.sciencedirect.com/science/article/B6T36-449TM3T-X5/2/d526cad6f56cc1069919498bca82a0e5

Using an improved 3' RACE (PCR) amplification system containing oligonucleotide primer with an inosine at ambiguous codon positions and inverse PCR to amplify the 5' ends, we have isolated and characterized cDNA clones which encode cionin, a protochordean homologue of the mammalian hormones, cholecystokinin (CCK) and gastrin. The full-length cloned cDNA of 510 bp encoded a 128 amino acid preprocionin. Reverse transcription-PCR and subsequent cDNA cloning revealed that cionin mRNA is expressed in both the neuronal ganglion and the gut of the protochordate Ciona intestinalis. The primary structure of procionin resembles that of proCCK more than that of progastrin. Sequence-specific immunochemical analysis showed that the cionin gene is expressed also at peptide level in both the gut and the neural ganglion. The neuronal processing of procionin is, however, more complete both with respect to carboxyamidation and tyrosine O-sulfation. Hence, the tissue-specific expression of the cionin gene in Ciona intestinalis resembles that of the CCK gene in mammals.

Morrow, J. A., I. T. Collie, et al. (1998). "Molecular cloning and functional expression of the human glycine transporter GlyT2 and chromosomal localisation of the gene in the human genome." FEBS Letters **439**(3): 334.

http://www.sciencedirect.com/science/article/B6T36-3V7JRTN-10/2/003053f85d567679c6ed0586007e5062

Neurotransmitter transport systems are major targets for therapeutic alterations in synaptic function. We have cloned and sequenced a cDNA encoding the human type 2 glycine transporter GlyT2 from human brain and spinal cord. An open reading frame of 2391 nucleotides encodes a 797 amino acid protein that transports glycine in a Na+/Cl--dependent manner. When stably expressed in CHO cells, human GlyT2 displays a dose-dependent uptake of glycine with an apparent Km of 108 [mu]M. This uptake is not affected by sarcosine at concentrations up to 1 mM. Radiation hybrid analysis mapped the GlyT2 gene to D11S1308 (LOD=8.988) on human chromosome 11p15.1-15.2.

Morrow, J. A., E. M. Lutz, et al. (1993). "Molecular cloning and expression of a cDNA encoding a receptor for pituitary adenylate cyclase activating polypeptide (PACAP)." FEBS Letters **329**(1-2): 99.

http://www.sciencedirect.com/science/article/B6T36-44F7MXT-FB/2/465875585af5f296362c97173507964b

We have cloned and sequenced a novel cDNA (RPR7) encoding a receptor for pituitary adenylate cyclase activating polypeptide (PACAP). RPR7 was identified by PCR of rat pituitary cDNA, and full-length clones were isolated from a rat olfactory bulb cDNA library. When expressed in COS cells, RPR7 was functionally coupled to increases in intracellular cyclic adenosine monophosphate (cAMP) in response to stimulation by PACAP-38, PACAP-27, vasoactive intestinal peptide (VIP) and peptide histidine isoleucine (PHI). The order of potency of these ligands was PACAP-38 ~ PACAP-27> VIP> PHI, suggesting that the receptor corresponds to the pharmacologically characterised PACAP Type I receptor.

Mosselman, S., J. Polman, et al. (1996). "ER[beta]: Identification and characterization of a novel human estrogen receptor." FEBS Letters **392**(1): 49.

http://www.sciencedirect.com/science/article/B6T36-3Y0SM18-SJ/2/ae12915301071173c5d65dc97bbb6621

A novel estrogen receptor (hereinafter referred to as ER[beta]) was cloned using degenerate PCR primers. A comparison of the amino acid sequence of ER[beta] with the 'classical' ER (ER[alpha]) shows a high degree of conservation of the DNA-binding domain (96%), and of the ligand-binding domain (58%). In contrast, the A/B domain, the hinge region and the F-domain are not conserved. Northern blot analysis revealed that ER[beta] is expressed in human thymus, spleen, ovary and testis. Transient transfections of an ER[beta] expression construct together with an ERE-based reporter construct in CHO cells clearly demonstrated transactivation of ER[beta] by 17[beta]-estradiol. In addition, the ER[alpha] antagonist ICI-164384 is a potent antagonist for ER[beta] as well. Interestingly, the level of transactivation by 17[beta]-estradiol is higher for ER[alpha] than for ER[beta], which may reflect suboptimal conditions for ER[beta] at the level of the ligand, responsive element or cellular context.

Motoyama, K., H. Arima, et al. (2005). "Involvement of CD14 in the inhibitory effects of dimethyl-[alpha]-cyclodextrin on lipopolysaccharide signaling in macrophages." FEBS Letters **579**(7): 1707.

http://www.sciencedirect.com/science/article/B6T36-4FH0T2R-6/2/bd1b705f4972eb363f0348bf40c0859f

The potential use of [alpha]-cyclodextrin and its hydrophilic [alpha]-cyclodextrin derivatives ([alpha]-CyDs) as antagonists against lipopolysaccharide (LPS), which stimulates the nitric oxide (NO) and tumor necrosis factor-[alpha] (TNF-[alpha]) production as well as nuclear factor-[kappa]B (NF-[kappa]B) activation in macrophages was examined. Of three [alpha]-CyDs used in the present study, 2,6-di-O-methyl-[alpha]-CyD (DM-[alpha]-CyD) had greater inhibitory activity than did the other CyDs against NO and TNF-[alpha] production through an impairment of gene expression in macrophage cell lines and primary macrophages stimulated with LPS and lipid A in a concentration-dependent manner. Concomitantly, DM-[alpha]-CyD inhibited NF-[kappa]B translocation into nucleus. These inhibitory effects of DM-[alpha]-CyD could be attributed to the release of CD14 from lipid rafts caused by an efflux of phospholipids, but not cholesterol. These results suggest that DM-[alpha]-CyD may have promise as a potent and unique antagonist for excess activation of macrophages stimulated with LPS.

Mukaide, M., M. Mizokami, et al. (1997). "Three different GB virus C/hepatitis G virus genotypes: Phylogenetic analysis and a genotyping assay based on restriction fragment length polymorphism." FEBS Letters **407**(1): 51.

http://www.sciencedirect.com/science/article/B6T36-3R85JMS-C/2/8df5ae47b87c5e53f6eecbd5ab4630db

The 5'-untranslated region (5'-UTR) sequences of 33 GB virus C/hepatitis G virus (GBV-C/HGV) obtained from different geographic areas were determined through reverse-transcription polymerase chain reaction and dideoxy chain termination sequencing, the alignment of sequences, the estimation of the number of nucleotide substitution per site, and construction of phylogenetic trees. The 5'-UTR of GBV-HGV was found to be heterogeneous, with 70.9-99.5% homology. Three distinct phylogenetic branches were observed consistently in all phylogenetic trees. GBV-C is the prototype for one, HGV for another, and there is a new branch which consisted of GBV-C/HGV isolates from Asia. Genotype-specific restriction sites for the restriction enzymes, ScrFI and BsmFI, were identified, and a simple restriction fragment polymorphism analysis was developed for genotyping. These data provide evidence that GBV-C/HGV consists of three different genotypes. Our simple genotyping assay will also provide a tool for epidemiological studies of GBV-C/HGV infection.

Mutoh, H., H. Bito, et al. (1993). "Two different promoters direct expression of two distinct forms of mRNAs of human platelet-activating factor receptor." FEBS Letters **322**(2): 129.

http://www.sciencedirect.com/science/article/B6T36-44G8D5G-6T/2/7907d5978c7aa8f9581289a7be2116dc

The human platelet-activating factor (PAF) receptor gene exists as a single copy on chromosome 1. We identified two 5'-noncoding exons, each of which has distinct transcriptional initiation sites. These exons are alternatively spliced to a common splice acceptor site on a third exon that contains the total open reading frame to yield two different species of functional mRNA (Transcript 1 and 2). Transcript 1 has consensus sequences for transcription factor NF-[kappa]B and Sp-1, and the Initiator (Inr) sequence homologous to the murine terminal deoxynucleotidyltransferase gene. Transcript 2 also contains consensus sequences for transcription factor AP-1, AP-2, and Sp-1. Transcripts 1 and 2 were both detected in heart, lung, spleen, and kidney, whereas only Transcript 1 was found in peripheral leukocytes, a differentiated human eosinophilic cell line (EoL-1 cells), and brain. Existence of distinct promoters was thus suggested to play a role in the regulatory control of PAF receptor gene expression in different human tissues and cells.

Nagase, I., T. Yoshida, et al. (2001). "Up-regulation of uncoupling proteins by [beta]-adrenergic stimulation in L6 myotubes." FEBS Letters **494**(3): 175.

http://www.sciencedirect.com/science/article/B6T36-42SPKS2-9/2/d208ffe398305d6fbb21da0aba2cbb96

Catecholamine-induced and [beta]-adrenergic receptor ([beta]-AR)-mediated thermogenesis in skeletal muscle is a significant component of whole-body energy expenditure. Skeletal muscle expresses uncoupling protein (UCP) 2 and UCP3, which can dissipate the transmitochondrial electrochemical gradient and thereby may be involved in regulation of energy metabolism. We investigated the effects of [beta]-AR stimulation on UCP2 and UCP3 expression in L6 myotubes. Stimulation of the cells with epinephrine increased the UCP3 mRNA level transiently at 6 h, and

also the UCP2 mRNA level at 6-24 h. The stimulatory effects of epinephrine were also observed in the presence of carbacyclin and 9-cis retinoic acid, and mimicked by isoproterenol and salbutamol ([beta]2-AR agonists), but abolished by propranolol and ICI-118,551 ([beta]2-AR antagonists). Pharmacological and mRNA analyses revealed the existence of [beta]2-AR, but not [beta]1- and [beta]3-ARs, in L6 myotubes. These results suggested that catecholamines up-regulate UCP2 and UCP3 expression through direct action on the [beta]2-AR in skeletal muscle.

Namihira, M., K. Nakashima, et al. (2004). "Developmental stage dependent regulation of DNA methylation and chromatin modification in a immature astrocyte specific gene promoter." FEBS Letters **572**(1-3): 184.

http://www.sciencedirect.com/science/article/B6T36-4CXS0R5-2/2/856ed2349a56a582024d9eb658df5ef8

Astrocytes are generated from neuroepithelial cells after neurons during brain development. However, the mechanism of this sequential generation is not fully understood. Here, we show that a particular cytosine residue in the promoter of the gene encoding the immature astrocyte marker, S100[beta], becomes demethylated, correlating with the time when the S100[beta] expression commences at embryonic day (E) 14. In addition, astrocyte-inducing cytokine, BMP2, increased histone acetylation around the CpG site in neuroepithelial cells at E14 but not E11 when S100[beta] expressing astrocytes are absent. Furthermore, binding of a methyl DNA binding protein, MeCP2, to the S100[beta] gene promoter in neuroepithelial cells was reduced at E14 compared to E11. Thus, demethylation of specific CpG site is suggested to be a critical determinant in regulating astrocyte differentiation in the developing brain.

Napolitani, C., L. Mandrich, et al. (2004). "Mutational analysis of GstI protein, a glutamine synthetase translational inhibitor of Rhizobium leguminosarum." FEBS Letters **558**(1-3): 45.

http://www.sciencedirect.com/science/article/B6T36-4BFXHKS-3/2/e7e343e7f96ed9679ba791c736f504c4

The small GstI protein (63 amino acids) of Rhizobium leguminosarum inhibits the expression of the glnII (glutamine synthetase II) gene, thus reducing the bacterial ability to assimilate ammonium. In order to identify the residues essential for its inhibitory activity, all the 53 non-alanine amino acid residues of GstI were individually mutated into alanine. Based on their capacity to inhibit glnII expression (in two genetic backgrounds) three groups of mutants were identified. The first group displayed an inhibitory activity similar to the wild-type; the second and the third ones showed partial and total loss of inhibitory activity, respectively. Several mutations of the latter group concerned residues conserved in two related sequences from Sinorhizobium meliloti and Agrobacterium tumefaciens. Additionally, we performed experiments to exclude a GstI-mediated mechanism of glutamine synthetase II inhibition/degradation. Finally, the protein was over expressed in Escherichia coli, purified and characterised.

Negro, A., M. Onisto, et al. (1995). "Synthesis and refolding of human TIMP-2 from E. coli, with specific activity for MMP-2." FEBS Letters **360**(1): 52.

http://www.sciencedirect.com/science/article/B6T36-3YN9FDJ-23/2/60d01de668ea24b97d454aca6f725b78

Tissue inhibitors of metalloproteinase (TIMPs) are inhibitory counterparts of collagenases, containing 12 cysteine residues paired to six internal disulphide bridges. TIMP-2, an inhibitory protein of 72 kDa gelatinase/type IV collagenase (MMP-2), was expressed in Escherichia coli as a fusion protein with a 34 amino acid NH2-linked tail containing six consecutive histidine residues. The protein was purified in a single-step using an ion metal affinity column (IMAC) in denaturing conditions. The immobilized fusion TIMP-2 protein was refolded at a high concentration in the column, producing about 5 mg of protein per litre of bacterial cells. It shows specific binding and inhibitory activity against MMP-2, but has no effect against 92 and 45 kDa gelatinases.

Nemoto, N., E. Miyamoto-Sato, et al. (1997). "In vitro virus: Bonding of mRNA bearing puromycin at the 3'-terminal end to the C-terminal end of its encoded protein on the ribosome in vitro." FEBS Letters **414**(2): 405.

http://www.sciencedirect.com/science/article/B6T36-4638RRR-1R/2/aec63a590d673ca7fa1f9cee2735748a

Adequate means for genotype assignment to phenotype is essential in evolutionary molecular engineering. In this study, construction of 'in vitro virus' was carried out in which a genotype molecule (mRNA) covalently binds to the phenotype molecule (protein) through puromycin on the ribosome in a cell-free translation system. Bonding efficiency was ~10%, thus indicating a population of the in vitro virus to have ~1012 protein variants, this number being 104 that in the phage display. The in vitro virus is useful for examining protein evolution in a test tube and the results may possibly serve as basis for a general method for selecting proteins possessing the most desirable functions.

Nemoto, N., E. Miyamoto-Sato, et al. (1999). "Fluorescence labeling of the C-terminus of proteins with a puromycin analogue in cell-free translation systems." FEBS Letters **462**(1-2): 43.

http://www.sciencedirect.com/science/article/B6T36-3XY1H1K-8/2/bfa2ba542534a256231e99ffdb2d7125

We have developed a new method for the C-terminus-specific fluorescence labeling of proteins. This method is based on the experimental finding that a fluorescent puromycin analogue at lower concentrations bonds efficiently to the C-terminus of mature proteins in cell-free translation systems using mRNA without a stop codon. This labeling is performed under moderate conditions and its labeling efficiency is in the range of 50-95%. Here we demonstrate a protein-protein interaction assay using fluorescence polarization measurement. This labeling method should also be useful for other rapid molecular interaction assays without purification of the labeled proteins, such as fluorescence correlation spectroscopy.

Newton, P. M., K. Tully, et al. (2005). "Chronic ethanol exposure induces an N-type calcium channel splice variant with altered channel kinetics." FEBS Letters **579**(3): 671.

http://www.sciencedirect.com/science/article/B6T36-4F4H7NX-4/2/b240bc99ee004e05bb07c583be1eb6ad

Chronic ethanol exposure increases the density of N-type calcium channels in brain. We report that ethanol increases levels of mRNA for a splice variant of the N channel specific subunit [alpha]12.2 that lacks exon 31a. Whole cell recordings demonstrated an increase in N-type

current with a faster activation rate and a shift in activation to more negative potentials after chronic alcohol exposure, consistent with increased abundance of channels containing this variant. These results identify a novel mechanism whereby chronic ethanol exposure can increase neuronal excitability by altering levels of channel splice variants.

Nukaya, M., Y. Takahashi, et al. (2004). "Aryl hydrocarbon receptor-mediated suppression of GH receptor and Janus kinase 2 expression in mice." FEBS Letters **558**(1-3): 96.

http://www.sciencedirect.com/science/article/B6T36-4BDW15Y-5/2/941f6d82cbbcc59e493b754a0d550db5

Differential mRNA display revealed that a cDNA encoding the major urinary protein 2 (MUP2) that belongs to the lipocalin superfamily was absent in livers of mice treated with 3-methylcholanthrene (MC). The expression of MUP2 is known to be stimulated by growth hormone (GH), through the GH receptor (GHR), Janus kinase 2 (JAK2) and signal transducer and activator of transcription 5 (STAT5) signal transduction pathway. Since MC is an aryl hydrocarbon receptor (AhR) ligand, the effects of MC treatment on the expression of GHR, JAK2 or STAT5 in the livers of wild-type or AhR-null mice were examined. The result indicated that the expression of GHR and JAK2 mRNA was greatly decreased by MC in wild-type mice but not in AhR-null mice. In addition, the binding activity of STAT5 bound to STAT5-binding element was reduced after MC treatment in wild-type mice but not in AhR-null mice. Based on these results, we conclude that the suppression of MUP2 mRNA expression by MC is caused by the AhR-mediated disruption of the GH signaling pathway. Possible mechanism(s) by which exposure to aromatic hydrocarbons causes a decrease in the body weight of mice, which has been referred to as wasting syndrome, will also be discussed.

Ohya, S., M. Tanaka, et al. (1997). "Molecular cloning and tissue distribution of an alternatively spliced variant of an A-type K+ channel [alpha]-subunit, Kv4.3 in the rat." FEBS Letters **420**(1): 47.

http://www.sciencedirect.com/science/article/B6T36-3RTXVJV-C/2/f8bf7b23a57e238f165f0b9f5f8effee

We describe here (1) the heterogeneous expression of Ca2+-independent transient (A-type) K+ channel [alpha]-subunits (Kv1.4, Kv3.3, Kv3.4, Kv4.2 and Kv4.3) in rat smooth muscle, heart and brain, (2) the molecular cloning and tissue distribution of a novel alternatively spliced variant of an A-type K+ channel [alpha]-subunit, Kv4.3, and (3) the functional expression of A-type K+ channels in HEK293 cells by the transfection with the novel splice variant of Kv4.3. A cDNA encoding this splice variant was identified from rat vas deferens by RT-PCR cloning. This cDNA clone contains a 1965 bp open reading frame that encodes for a protein of 655 amino acids. It has a 19 amino acid insertion in comparison with Kv4.3 previously reported in rat brain. RT-PCR analyses showed that the mRNAs of this longer variant are abundantly expressed in a number of smooth muscles of the rat, and that the mRNAs of the previously reported clones are absent. The longer splice variant is very weakly expressed in brain, but is the major product in heart.

Okano, S., H. Tokushima, et al. (1996). "Cloning of a novel ubiquitin-conjugating enzyme (E2) gene from the ciliate Paramecium tetraurelia." FEBS Letters **391**(1-2): 1.

http://www.sciencedirect.com/science/article/B6T36-3Y0SKX0-NJ/2/74594989c69bd5f090d94b2813346b15

We isolated a 1.7 kb gene (UbcPl) for a ubiquitinconjugating enzyme from a P. tetraurelia cDNA library and sequenced it. Its deduced polypeptide sequence consists of 425 amino acid residues (48 kDa). The UbcP1 protein contains novel N- and C-terminal extensions in addition to a UBC domain, and within the UBC domain it shares low identity with sequences of other known E2s. A constructed phylogenetic tree suggests that the UbcP1 protein may represent a member of a distinct subfamily of E2s. Southern blot analysis showed that the N-terminal extension of the UbcP1 is conserved in P. multimicronucleatum.

Osanai, T., W. Chai, et al. (2001). "Expression of glycoconjugates bearing the Lewis X epitope during neural differentiation of P19 EC cells." FEBS Letters **488**(1-2): 23.

http://www.sciencedirect.com/science/article/B6T36-455691V-6/2/aa12c15b4f5445ce5d8c34a062607584

The Lewis X (Lex) bearing glycolipids were noticeably increased in amounts during the course of neural differentiation of P19 EC cells induced by retinoic acid (RA, all-trans form). Applying neoglycolipid technology and in situ TLC-LSIMS, the oligosaccharide chains of these scarce Lex bearing glycolipids were partially characterized after released by endoglycoceramidase and subsequent conversion into neoglycolipids. In order to understand the enzymatic basis for the expression of Lex bearing glycolipids, we measured glycolipid, glycoprotein and oligosaccharide fucosyltransferase (Fuc-T) activities using appropriate substrates in P19 EC cells with or without RA treatment. All three Fuc-Ts were increased after RA treatment and the highest activity was in the differentiated neural cells. We then investigated the two possible Fuc-T genes that might be responsible for these changes using RT-PCR analysis. Mouse Fuc-TIX (mFuc-TIX) transcript was detected in all cell types but it was only strongly expressed in RA-induced aggregates and neural cells. In the case of mouse Fuc-TIV (mFuc-TIV) gene, its transcript was only detectable in RA-induced aggregates and not found in either undifferentiated or RA-induced neural cells. These results strongly support that RA induces only a transient expression of the mFuc-TIV gene in cell aggregates but a more persistent expression of the mFuc-TIX gene at the transcription level throughout neural cell differentiation. The mFuc-TIX gene is probably the main cause for the increased expression of Lex glycoconjugates during neural differentiation of P19 EC cells.

Ozog, A., B. Pouzet, et al. (1998). "Characterization of the 3' end of the mouse SERCA 3 gene and tissue distribution of mRNA spliced variants." FEBS Letters **427**(3): 349.

http://www.sciencedirect.com/science/article/B6T36-3SXDXVF-8/2/56a7258709787a19202e1d3824c6afaf

The sarco(endo)plasmic reticulum Ca2+ ATPase (SERCA) type 1 and 2 genes are alternatively spliced at their 3' end. We hypothesized that similar mechanism may occur for SERCA 3. Two spliced variants were identified by RNase protection analysis. We then isolated and sequenced the 3' end portion of the mouse SERCA 3 gene, and confirmed the presence of an alternative mRNA transcript by sequencing a cDNA fragment obtained by RT-PCR. Tissue distribution of the alternatively spliced mRNAs was studied by RT-PCR: SERCA 3b was the only isoform expressed in endothelial cells from aorta and heart and also was the major isoform in lung and kidney whereas SERCA 3a and 3b were coexpressed in trachea, intestine, thymus, spleen, and fetal liver.

Pal, G., G. Sprengel, et al. (1994). "Alteration of the specificity of ecotin, an E. coli serine proteinase

inhibitor, by site directed mutagenesis." FEBS Letters **342**(1): 57.

http://www.sciencedirect.com/science/article/B6T36-44KP5R9-4H/2/83c129f0c1dd400f36bf1ed6007446d0

The gene of ecotin, an E. coli proteinase inhibitor, was cloned, and by site-directed mutagenesis the active site residue of the protein, Met84, was mutated to Lys, Arg and Leu. The recombinant wild-type and mutant inhibitors were overexpressed in E. coli, purified to homogeneity and their inhibitory effects on trypsin, chymotrypsin and elastase were compared. Of these serine proteinases trypsin is the most strongly inhibited by wild type ecotin and its mutants. According to our results the character of residue 84 of ecotin significantly but not dramatically modifies the specificity of the inhibitor.

Pal, G., L. Szilagyi, et al. (1996). "Stable monomeric form of an originally dimeric serine proteinase inhibitor, ecotin, was constructed via site directed mutagenesis." FEBS Letters **385**(3): 165.

http://www.sciencedirect.com/science/article/B6T36-3VXNBMH-1B/2/a1b1319497f92ffcaf52102e2bdcd366

Ecotin, a homodimer protein of E. coli, is a unique member of canonical serine proteinase inhibitors, since it is a potent agent against a variety of serine proteinases having different substrate specificity. Monomers of ecotin are held together mostly by their long C-terminal strands that are arranged as a two-stranded antiparallel [beta]-sheet in the functional dimer. One ecotin dimer can chelate two proteinase molecules, each of them bound to both subunits of ecotin at two different sites, namely the specific primary and the non-specific secondary binding sites. In this study the genes of wild type ecotin and its Met84 Arg P1 site mutant were truncated resulting in new forms of ecotin that lack 10 amino acid residues at their C-terminus. These mutants do not dimerize spontaneously, though in combination with trypsin they assemble into the familiar heterotetramer. Our data suggest that this heterotetramer exists even in extremely diluted solutions, and the interaction, which is responsible for the dimerization of ecotin, contributes to the stability of the heterotetrameric complex.

Pan, T.-C., G. Kostka, et al. (1999). "Complete exon-intron organization of the mouse fibulin-1 gene and its comparison with the human fibulin-1 gene." FEBS Letters **444**(1): 38.

http://www.sciencedirect.com/science/article/B6T36-3VTHRB5-8/2/0471c465a13014238231d74e31d34675

Fibulin-1 is a 90 kDa calcium-binding protein present in the extracellular matrix and in the blood. Two major variants, C and D, differ in their C-termini as well as the ability to bind the basement membrane protein nidogen. Here we characterized genomic clones encoding the mouse fibulin-1 gene, which contains 18 exons spanning at least 75 kb of DNA. The two variants are generated by alternative splicing of exons in the 3' end. By searching the database we identified most of the exons encoding the human fibulin-1 gene and showed that its exon-intron organization is similar to that of the mouse gene.

Pengue, G., P. Cannada-Bartoli, et al. (1993). "The ZNF35 human zinc finger gene encodes a sequence-specific DNA-binding protein." FEBS Letters **321**(2-3): 233.

http://www.sciencedirect.com/science/article/B6T36-44BF0K3-10/2/c61bde552ed2c2c9c2624d20e7c78b09

We developed a rapid method to determine DNA-binding sites for putative DNA-binding proteins. This procedure has been successfully used to define a specific consensus site for the human ZNF35 zinc finger gene. ZNF35 encodes a 58-kDA polypeptide containing 11 consecutive finger motifs located at the amino terminus, and an acidic domain located at the carboxy terminus. These features suggest that ZNF35 is a site-specific DNA-binding protein involved in the regulation of gene expression. We have expressed the ZNF35 protein from E. coli and have employed a Southwestem-polymerase chain reaction method using random oligonucleotides to identify its high-affinity binding site. The core sequence for the ZNF35 protein-binding site is 5'-C/GC/GAAG/TA-3'.

Proudfoot, A. E. I., C. A. Power, et al. (1995). "Characterisation of the RANTES/MIP-1[alpha] receptor (CC CKR-1) stably transfected in HEK 293 cells and the recombinant ligands." FEBS Letters **376**(1-2): 19.

http://www.sciencedirect.com/science/article/B6T36-3YS2BF9-2P/2/e1e2991459cc943197362bec7b4be515

The CC chemokines RANTES and MIP-1[alpha] are known to activate certain leucocytes and leucocytic cell lines. We have produced and fully characterised the recombinant proteins expressed in E. coli. They induce chemotaxis of the pro-monocytic cell line, THP-1 and T cells. THP-1 cells express three of the known CC chemokine receptors. In order to study the activation of a single receptor, we have expressed the shared receptor (CC CKR-1) for RANTES and MIP-1[alpha] stably in the HEK 293 cell line. We have examined the effects of RANTES and MIP-1[alpha] on the CC CKR-1 transfectants by equilibrium binding studies and in a chemotaxis assay. RANTES competes for [125I]RANTES with an IC50 of 0.6 +/- 0.23 nM, whereas MIP-1[alpha] competes for its radiolabelled counterpart with an IC50 of 10 +/- 1.6 nM in the transfectants. These affinities are the same as those measured on the THP-1 cell line. The stably transfected HEK 293 cells respond to both these chemokines in the chemotaxis assay with the same EC50 values as those measured for THP-1 cells. This indicates that this cellular response can be mediated through the CC CKR-1 receptor.

Rafaeloff, R., X. F. Qin, et al. (1996). "Identification of differentially expressed genes induced in pancreatic islet neogenesis." FEBS Letters **378**(3): 219.

http://www.sciencedirect.com/science/article/B6T36-3Y0SKV5-M5/2/f22d1c3f657d0c9b91b3efe61a77af7a

Cellophane wrapping of the hamster pancreas induces islet neogenesis. We have used the mRNA differential display technique to select for genes expressed during islet neogenesis but not in control pancreata. Ten candidate clones have been identified. Upon sequencing, 6 clones showed a high degree of homology to known genes, 1 showed some, and 3 showed no homology to genes of known sequence. Thus, mRNA differential display is a useful technique to identify genes induced during islet neogenesis, and in combination with screening hamster pancreatic cDNA libraries for full length clones, will enhance the likelihood of capturing the participants in this process.

Rasmussen, U. B., V. Vouret-Craviari, et al. (1991). "cDNA cloning and expression of a hamster [alpha]-thrombin receptor coupled to Ca2+ mobilization." FEBS Letters **288**(1-2): 123.

http://www.sciencedirect.com/science/article/B6T36-44XMXYH-1V/2/0b83943138a32cf5365b782ede773358

The serine protease [alpha]-thrombin (thrombin) potently stimulates G-protein-coupled signaling pathways and DNA synthesis in CCL39 hamster lung fibroblasts. To clone a thrombin receptor cDNA, selective amplification of mRNA sequences displaying homology to the transmembrane domains of G-protein-coupled receptor genes was performed by polymerase chain reaction. Using reverse transcribed poly(A)+ RNA from CCL39 cells and degenerate primers corresponding to conserved regions of several phospholipase C-coupled receptors, three novel putative receptor sequences were identified. One corresponds to an mRNA transcript of 3.4 kb in CCL39 cells and a relatively abundant cDNA. Microinjection of RNA transcribed in vitro from this cDNA in Xenopus oocytes leads to the expression of a functional thrombin receptor. The hamster thrombin receptor consists of 427 amino acid residues with 8 hydrophobic domains, including one at the extreme N-terminus that is likely to represent a signal peptide. A thrombin consensus cleavage site is present in the N-terminal extracellular region of the receptor sequence followed by a negatively charged cluster of residues present in a number of proteins that interact with the anion-binding exosite of thrombin.

Rea, G., M. Laurenzi, et al. (1998). "Developmentally and wound-regulated expression of the gene encoding a cell wall copper amine oxidase in chickpea seedlings." FEBS Letters **437**(3): 177.

http://www.sciencedirect.com/science/article/B6T36-3V4CRFD-3/2/42d28ac553cef5a88c21210eb0fead3b

A chickpea cDNA encoding a cell wall copper amine oxidase (CuAO) was cloned and characterised. The 2010 bp open reading frame encodes a protein of 76.5 kDa which shares significant primary structure homology with other known CuAOs. Southern blot analysis indicates that in chickpea CuAO is encoded by a single gene or a small gene family. This cDNA was essential for studying the role of CuAO during seedling development and wound healing in chickpea seedlings. CuAO transcript level and activity were modulated during seedling development in parallel with cell maturation. Moreover, mechanical wounding induced a rapid increase of CuAO mRNA accumulation and enzyme activity which remained high during the wound-healing process. Aminoguanidine, a specific CuAO inhibitor, decreased the deposition of lignin-suberin barrier along the lesion. CuAO may be a limiting factor in H2O2 production in the cell wall of chickpea seedlings and its expression seems to integrate with the remodelling of plant cell wall occurring during ontogenesis and wound healing.

Reppert, S. M., D. R. Weaver, et al. (1996). "Cloning of a melatonin-related receptor from human pituitary." FEBS Letters **386**(2-3): 219.

http://www.sciencedirect.com/science/article/B6T36-3XM3KN9-10/2/3193106ac76910f751ba3be28eef7e60

We have cloned an orphan G protein-coupled receptor from a human pituitary cDNA library using a probe generated by PCR. The cDNA, designated H9, encodes a protein of 613 amino acids that is 45% identical at the amino acid level to the recently cloned human Mel1a and Mel1b melatonin receptors. Structural analyses of the encoded protein and its gene, along with phylogenetic analysis, further show that H9 is closely related to the G protein-coupled melatonin receptor

family. Unusual features of the protein encoded by H9 include a lack of N-linked glycosylation sites and a carboxyl tail >300 amino acids long. H9 transiently expressed in COS-1 cells did not bind [125I]melatonin or [3H]melatonin. H9 mRNA is expressed in hypothalamus and pituitary, suggesting that the encoded receptor and its natural ligand are involved in neuroendocrine function.

Sakura, H., C. Bond, et al. (1995). "Characterization and variation of a human inwardly-rectifying K-channel gene (KCNJ6): a putative ATP-sensitive K-channel subunit." FEBS Letters **367**(2): 193.

http://www.sciencedirect.com/science/article/B6T36-3YRNY0T-8Y/2/1f6394c9cfc2a1b10bfb374c57005587

The ATP-sensitive K-channel plays a central role in insulin release from pancreatic [beta]-cells. We report here the cloning of the gene (KCNJ6) encoding a putative subunit of a human ATP-sensitive K-channel expressed in brain and [beta]-cells, and characterisation of its exon-intron structure. Screening of a somatic cell mapping panel and fluorescent in situ hybridization place the gene on chromosome 21 (21q22.1-22.2). Analysis of single-stranded conformational polymorphisms revealed the presence of two silent polymorphisms (Pro-149: CC-CC and Asp328: GA-GA) with similar frequencies in normal and non-insulin-dependent diabetic patients.

Sasabe, M., K. Toyoda, et al. (2002). "cDNA cloning and characterization of tobacco ABC transporter: NtPDR1 is a novel elicitor-responsive gene." FEBS Letters **518**(1-3): 164.

http://www.sciencedirect.com/science/article/B6T36-45M0T3S-8/2/94e4c23de1af4b5a5bad1822a2e658a2

We isolated an INF1 elicitin-inducible cDNA encoding a pleiotropic drug resistance (PDR)-type ATP-binding cassette (ABC) transporter homolog (NtPDR1) in suspension-cultured tobacco Bright Yellow-2 (BY-2) cells by application of differential display PCR. The NtPDR1 (Nicotiana tabacum PDR protein 1) gene also encodes a 162 kDa protein that includes two putative hydrophilic domains containing the ABC signature motif and two putative hydrophobic domains. Expression of the NtPDR1 gene was rapidly and strongly activated by treatment of BY-2 cells with INF1 elicitin. Further, treatment of BY-2 cells with flagellin, a bacterial proteinaceous hypersensitive reaction elicitor, or yeast extract, a general elicitor, also induced NtPDR1 gene expression. These results indicate that NtPDR1 may be involved in the general defense response in tobacco. This is the first report that microbial elicitors induce the expression of a plant ABC transporter gene.

Sasakawa, N., M. Sharif, et al. (1994). "Attenuation of agonist-induced desensitization of the rat substance P receptor by progressive truncation of the C-terminus." FEBS Letters **347**(2-3): 181.

http://www.sciencedirect.com/science/article/B6T36-447G3J0-CJ/2/8ccab0d6efac2b90738658e4f3599b11

We have investigated the C-terminal tail of the rat substance P receptor (SPR) as a domain essential for agonist-induced desensitization. Four progressively shorter mutants, using premature termination in the C-terminus, were constructed and compared with the unaltered SPR using ectopic expression of wild-type and mutant receptors in Xenopus oocytes. These mutants were designated D16, D47, D70 and D96 with 16, 47, 70 and 96 amino acids residues deleted

from the tail, respectively. Wild type SPR, D16 and D47 exhibited normal current responses when challenged with substance P, but D70 and D96 had reduced maximal current responses (70% and 5% of wild type SPR, respectively). D70, however, exhibited substantial resistance to substance P-induced desensitization in that 55%, versus 8% for wild type SPR, of the peak current of the first response was preserved on second challenge with substance P. Therefore, a domain from residues 338 to 360 of the rat SPR, though not necessary for the functional activity of the receptor, plays an essential role in agonist-induced desensitization.

Schaap, D., J. de Widt, et al. (1990). "Purification, cDNA-cloning and expression of human diacylglycerol kinase." FEBS Letters **275**(1-2): 151.

http://www.sciencedirect.com/science/article/B6T36-44M4167-9R/2/1794843b3b3e43e636da85351baa17a2

Diacylglycerol (DG) kinase attenuates the level of the second messenger DG in signal transduction, and therefore possibly modulates protein kinase C (PKC). DG kinase was purified to homogeneity from human white blood cells, showing an M1 of 86 kDa as determined by SDS-PAGE and gel filtration. Two amino acid sequences of tryptic peptides from DG kinase were determined and degenerate oligonucleotides were prepared and used in the polymerase chain reaction. An amplified DNA fragment was subsequently used to clone the full-length human DG kinase cDNA. This sequence is the human homolog of porcine DG kinase cDNA sequence reported recently [1]. The sequence contains a double EF- hand structure typical for Ca2+ binding proteins. DG kinase further contains a double cysteine repeat that is present in all PKC isoforms, where it constitutes the phorbol ester (and most likely diacylglycerol) binding site. Therefore we speculate that the double cysteine repeat in DG kinase is involved in DG binding. DG kinase is transcribed as a single mRNA of 3.2 kb, that is highly expressed in T-lymphocytes. The human DG kinase cDNA when transfected in mammalian cells (COS-7) results in a 6-7-fold increase of DG kinase activity.

Segade, F., B. Hurle, et al. (1996). "Molecular cloning of a mouse homologue for the Drosophila splicing regulator Tra2." FEBS Letters **387**(2-3): 152.

http://www.sciencedirect.com/science/article/B6T36-3Y0SK4Y-2J/2/469306a405ab854a98dd1f4424dda1eb

We report the identification of a mouse cDNA, SIG41, encoding a protein of 288 amino acids that is 45% identical (58% similar) to the Drosophila splicing regulator Tra2. SIG41 cDNA contains four polyadenylation signals whose alternative use gives rise to four types of transcripts (2.1, 2.0, 1.5, and 1.4 kb) in mouse cells. Northern analysis and RT-PCR assays showed that SIG41 mRNA is present in virtually all the cell lines and tissues studied, with remarkable levels of expression in uterus and brain tissues. Differential stability of the SIG41 mRNAs was detected in mouse macrophage cells.

Selden, C., M. Jones, et al. (1990). "Hepatotropin mRNA expression in human foetal liver development and in liver regeneration." FEBS Letters **270**(1-2): 81.

http://www.sciencedirect.com/science/article/B6T36-449T3JV-4R/2/644251b9b2574e4b320389e5aa468c93

A 569 bp probe against the [beta]-chain of hepatotropin was used to examine expression of RNA for this growth factor in human adult and foetal liver, foetal kidney and pancreas, and rat liver after partial hepatectomy. Low level expression of a 6kb RNA occurred in human adult and normal rat liver. 70% hepatectomy increased expression, peaking at 10 h and returning to near normal levels 24 h after resection. The 6 kb band was strongly expressed in human foetal liver, as compared with adult, but not in foetal kidney or pancreas, suggesting a major role for hepatotropin in both foetal development and regeneration of the liver.

Selvapandiyan, A., K. Majumder, et al. (1995). "Point mutation of a conserved arginine (104) to lysine introduces hypersensitivity to inhibition by glyphosate in the 5-enolpyruvylshikimate-3-phosphate synthase of Bacillus subtilis." FEBS Letters **374**(2): 253.

http://www.sciencedirect.com/science/article/B6T36-3YS2BMK-74/2/8067f165a5c0e05d50efba9b8aa882e4

The role of a conserved arginine (R104) in the putative phosphoenol pyruvate binding region of 5-enolpyruvyl shikimate-3-phosphate synthase of Bacillus subtilis has been investigated. Employing site directed mutagenesis arginine was substituted by lysine or glutamine. Native and mutant proteins were expressed and purified to near homogeneity. Estimation of Michaelis and inhibitor constants of the native and mutant proteins exhibited altered substrate--inhibitor binding mode and constants. Mutation R104K hypersensitized the enzyme reaction to inhibition by glyphosate. The role of R104 in discriminating between glyphosate and phosphoenol pyruvate is discussed.

Shimizu-Hirota, R., H. Sasamura, et al. (2004). "Functional characterization of podocan, a member of a new class in the small leucine-rich repeat protein family." FEBS Letters **563**(1-3): 69.

http://www.sciencedirect.com/science/article/B6T36-4BXDMJ5-1/2/9200ba70d0f1af451a636bb6b219378b

An important component of the extracellular matrix is the group of non-collagenous proteins belonging to the small leucine-rich repeat (SLR) protein family. A new SLR protein, podocan, with structural characteristics different from the known classes of the SLR protein family has been identified recently from the kidney. In this study, we examined the functional characteristics of this SLR protein expressed in cultured cells. Podocan was clearly observed intracellularly and was also detectable in the supernatant. Treatment of the expressed protein with various glycoenzymes suggested that podocan is a glycoprotein containing N-linked oligosaccharides but not a classical proteoglycan. Moreover, podocan was found to bind type 1 collagen. Cells transfected with podocan showed reductions in cell growth and migration, concomitant with increased p21 expression. Podocan mRNA was detected by reverse transcription polymerase chain reaction not only in the kidney, but also in other tissues including the heart and vascular smooth muscle cells, suggesting that podocan may have a potential role in growth regulation in cardiovascular tissues.

Singh, M., P. L. Bhalla, et al. (2003). "Isolation and characterization of a flowering plant male gametic cell-specific promoter." FEBS Letters **542**(1-3): 47.

http://www.sciencedirect.com/science/article/B6T36-48B0K9M-8/2/7cba58da35ca0a47d06db3a27d2e3408

Flowering plant male gametic cell-specific gene expression has been reported recently but the regulatory elements controlling specificity of such genes expressed in generative cell and sperm cells have not been identified and studied. Here, we report the 0.8 kb promoter sequence upstream of the start of the transcription site of the generative cell-specific gene, LGC1, sufficient to regulate the expression of reporter genes in a cell-specific manner. In addition, the diphtheria toxin A-chain- (DT-A)-coding region under the control of the LGC1 promoter sequence confirmed unequivocally the lack of LGC1 expression in vegetative tissues. Transgenic tobacco plants carrying the LGC1-DT/A construct showed normal phenotype except for anthers of these plants that contained sterile and aborted pollen. Truncation and internal deletion analysis of the LGC1 promoter identified -242 bp as the minimal sequence necessary for male gametic cell-specific expression. In addition, a regulatory sequence required for determining generative cell-specific expression of LGC1 was identified. Deletion of this regulatory sequence led to loss of the generative cell specificity resulting in activation of this promoter in other tissues where it is normally repressed. Therefore, male gametic cell specificity of the LGC1 gene seems to be regulated by factors that suppress its activation in other plant cells. This is the first report of a male gametic cell-specific promoter, hence can be used as a novel tool in molecular analyses and experimental manipulation of flowering plant spermatogenesis and fertilization.

Small, K. M., C. A. Seman, et al. (2002). "False positive non-synonymous polymorphisms of G-protein coupled receptor genes." FEBS Letters **516**(1-3): 253.

http://www.sciencedirect.com/science/article/B6T36-45BRX23-6/2/1e8f835d76959a47bb4603ec1b766ebc

Polymorphisms of G-protein coupled receptor (GPCR) genes are associated with disease risk and modification, and the response to receptor-directed therapy. Genomic sequencing (~1700 automated runs) from as many as 120 chromosomes from 60 multiethnic individuals was performed to confirm non-synonymous coding polymorphisms reported in the dbSNP database from 25 randomly selected GPCR genes. These polymorphisms were in regions of the receptors responsible for structural integrity, ligand binding, G-protein coupling and phosphoregulation. However, most of these putative polymorphisms could not be confirmed (false positive rate of 68%). Based on these results, we suggest that the variability of the superfamily is not well defined, and we caution against exclusive reliance on databases for selection of candidate GPCR polymorphisms for disease association and pharmacogenetic studies.

Soldatova, L., L. Kochoumian, et al. (1993). "Sequence similarity of a hornet (D. maculata) venom allergen phospholipase A1 with mammalian lipases." FEBS Letters **320**(2): 145.

http://www.sciencedirect.com/science/article/B6T36-44BMWXC-W8/2/847804d2cc740f274ceb81e30d9566f8

We have determined the sequence of a venom allergen phospholipase A1 from white-faced hornet (Dolichovespula maculata) by cDNA and protein sequencings. This protein of 300 amino acid residues (Dol m I) has no sequence similarity with other known phospholipases. But it has sequence similarity with mammalian lipases; about 40% identity in overlaps of 123 residues. Tests suggest that hornet phospholipase has weak lipase activity. Hornet venom has 3 major allergens, and another hornet allergen antigen 5 (Dot m V) was previously found to have sequence similarity with a mammalian testis protein and a plant leaf protein.

Sparatore, B., E. Melloni, et al. (1996). "A 6 kDa protein homologous to the N-terminus of the HMG1 protein promoting stimulation of murine erythroleukemia cell differentiation." FEBS Letters **386**(2-3): 95.

http://www.sciencedirect.com/science/article/B6T36-3XM3KN9-2/2/647cdda3183ab0002a60f91f183f8240

Murine erythroleukemia (MEL) cells, in addition to an mRNA coding for a 30 kDa high mobility group (HMG)-1 protein, contain an mRNA coding for a 6 kDa HMG1 protein having the following structural properties: (1) its primary structure has 90% homology with the N-terminal sequence of the 30 kDa HMG1 protein; (2) it contains a consensus region of the HMG1 protein family; (3) it is deprived of the cluster of acidic amino acids that characterizes the C-terminal region of the 30 kDa HMG1 protein. This novel small Mr HMG1 protein has been expressed in prokaryotic cells and tested to establish similarities and differences in activity compared to the homologous higher Mr HMG1 protein. It has been found that the low Mr HMG1 form is not released from MEL cells following induction to erythroid differentiation, but is still effective, although with much less efficiency, when added to the external medium, in promoting acceleration in the rate of MEL cell differentiation as well as in activation of [alpha]-protein kinase C. Altogether these results provide evidence for the presence in MEL cells of a multigene family that encodes at least two different HMG1-type sequences most presumably involved, at distinct cellular sites, in different functions although commonly related to the promotion of cell differentiation. Additional information can be considered concerning the relationship between the characteristic N-terminal sequence of HMG1 protein and the extracellular activity on MEL cell differentiation.

Stavri, G. T., Y. Hong, et al. (1995). "Hypoxia and platelet-derived growth factor-BB synergistically upregulate the expression of vascular endothelial growth factor in vascular smooth muscle cells." FEBS Letters **358**(3): 311.

http://www.sciencedirect.com/science/article/B6T36-3YMWT7S-5J/2/0993dcf2076c822739e6d54c5b1ddb42

Vascular endothelial growth factor (VEGF) mRNA expression was analysed in rabbit vascular smooth muscle cells following exposure to hypoxia and platelet-derived growth factor-BB (PDGF-BB). Hypoxia potently upregulated VEGF mRNA steady-state levels in a time- and concentration-dependent manner reaching a maximum level (~30-fold increase) after 12-24 h at 0% 02. In contrast, PDGF-BB caused a modest increase in VEGF expression. However, the combination of PDGF-BB and a threshold hypoxic stimulus (2.5% O2 for 4 h) had a marked synergistic effect. Synergy between hypoxia and PDGF-BB was selective for VEGF expression as hypoxia had no effect on the PDGF-induced upregulation of the proto-oncogene c-myc. These results raise the possibility that hypoxia and PDGF-BB may act in concert to induce VEGF expression in the arterial wall during the development of atherosclerosis.

Strocchi, P., X.-M. Tang, et al. (1995). "Molecular diagnosis of transthyretin Met30 mutation in an Italian family with familial amyloidotic polyneuropathy." FEBS Letters **359**(2-3): 203.

http://www.sciencedirect.com/science/article/B6T36-3YMWT11-1P/2/adb569c19ef6c6e93cb82aba5246a32c

We report the molecular analysis of the transthyretin gene in a large Italian pedigree with familial amyloidotic polyneuropathy and demonstrate the presence of a Met30 mutation. The usefulness of the genetic analysis in the identification of presymptomatic persons and the diagnosis of

individuals with partial symptoms is discussed.

Takabatake, T., M. Ogawa, et al. (1997). "Hedgehog and patched gene expression in adult ocular tissues." FEBS Letters **410**(2-3): 485.

http://www.sciencedirect.com/science/article/B6T36-3RB8YT6-4Y/2/c8aada68d62065bd6ce3a02b2a240f38

We analysed the expression of members of the hh gene family in adult ocular tissues of newt, frog and mouse by RT-PCR method. Shh displayed restricted expression in the neural retina that was conserved in each species analyzed. X-bhh, X-chh and mouse Ihh were detected in the iris and in the retinal pigment epithelium, while mouse Dhh was detected additionally in the neural retina and faintly in the cornea. We also found that two types of ptc genes, potential hh targets and receptors, were expressed in these tissues, suggesting the presence of active hh signalling there.

Takeshima, H., M. Nishi, et al. (1994). "Isolation and characterization of a gene for a ryanodine receptor/calcium release channel in Drosophila melanogaster." FEBS Letters **337**(1): 81.

http://www.sciencedirect.com/science/article/B6T36-44G8CWW-45/2/5b70f1f09d78d74388c074a72ef9b4ca

The nucleotide sequence of a 25.7 kilobase Drosophila melanogaster genomic DNA segment containing a gene for a ryanodine receptor/calcium release channel homologue has been determined. Computer analysis and partial cDNA cloning revealed 26 exons comprising the protein-coding sequence in this gene. The predicted protein is homologous in amino acid sequence and shares characteristic structural features with the mammalian ryanodine receptors. In blot hybridization analysis, a ~16 kilobase RNA species was identified abundantly in a 6-12 h embryo as the transcript from this gene. In situ hybridization to polytene chromosomes indicated that this gene locates at band position 44F on the second chromosome.

Taladriz, S., G. Gonzalez-Aseguinolaza, et al. (1999). "Cloning, molecular analysis and differential cell localisation of the p36 RACK analogue antigen from the parasite protozoon Crithidia fasciculata." FEBS Letters **443**(3): 375.

http://www.sciencedirect.com/science/article/B6T36-3VS2KSG-10/2/7c4211853740c53f824df131015dc1ce

The family of the RACK molecules (receptors for activated C kinases) are present in all the species studied so far. In the genus Leishmania, these molecules also induce a strong immune reaction against the infection. We have cloned and characterised the gene that encodes the RACK analogue from the parasite trypanosomatid Crithidia fasciculata (CACK). The molecule seems to be encoded by two genes. The sequence analysis of the cloned open reading frame indicates the existence of a high degree of conservation not only with other members of the Trypanosomatidae but also with mammalians. The study of the protein kinase C phosphorylation sites shows the presence of three of them, shared with the mammalian species, additional to those present in the other protozoa suggesting a certain phylogenetic distance between the protozoon Crithidia fasciculata and the rest of the Trypanosomatidae. The CACK-encoded polypeptide shows an additional sequence of four amino acids at the carboxy-terminal end, which

produces a different folding of the fragment with the presence of an [alpha]-helix instead of the [beta]-sheet usual in all the other species studied. A similar result is elicited at the amino-terminal end by the change of three amino acid residues. The immunolocalisation experiments show that the CACK displays a pattern with a distribution mainly at the plasma membrane, different from that of the related Leishmania species used as control, that displays a distribution close to the nucleus. Altogether, the data suggest that the existence of the structural differences found may have functional consequences.

Tanaka, Y., M. Mizokami, et al. (1998). "New genotypes of TT virus (TTV) and a genotyping assay based on restriction fragment length polymorphism." FEBS Letters **437**(3): 201.

http://www.sciencedirect.com/science/article/B6T36-3V4CRFD-8/2/489193c88161748d07a606a31af5cfc6

A phylogenetic analysis, using the open reading frame 1 sequence of 93 TT viruses (TTV) obtained from various geographical areas, indicated that the virus could be classified into six different genotypes including three hitherto unreported genotypes. The high reliability of the six clusters was confirmed by bootstrap analysis. On the basis of these sequence data, a new simple genotyping assay based on a restriction fragment length polymorphism of TTV was developed. Using the enzymes NdeI and PstI, followed by cleavage with NlaIII or MseI, it was possible to distinguish between the six TTV genotypes. This system will provide the framework for future detailed epidemiological and clinical investigations.

Tarabykina, S., M. Kriajevska, et al. (2000). "Heterocomplex formation between metastasis-related protein S100A4 (Mts1) and S100A1 as revealed by the yeast two-hybrid system." FEBS Letters **475**(3): 187.

http://www.sciencedirect.com/science/article/B6T36-40J72WK-7/2/f8f7dd4a7835ec8eff94326dd944c049

S100A4 (Mts1) is a Ca2+-binding protein of the S100 family. This protein plays an important role in promoting tumor metastasis. In order to identify S100A4 interacting proteins, we have applied the yeast two-hybrid system as an in vivo approach. By screening a mouse mammary adenocarcinoma library, we have demonstrated that S100A4 forms a heterocomplex with S100A1, another member of the S100 family. The non-covalent heterodimerization was confirmed by fluorescence spectroscopy and electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry. Mutational analysis revealed that replacement of Cys76 and/or Cys81 of S100A4 by Ser abolishes the S100A4/S100A1 heterodimerization, but does not affect the S100A4 homodimerization in vivo.

Tatnell, P. J., W. E. Lees, et al. (1997). "Cloning, expression and characterisation of murine procathepsin E." FEBS Letters **408**(1): 62.

http://www.sciencedirect.com/science/article/B6T36-3R7B21X-G/2/f8d7cc6549777f1020bc564303f3debf

The cDNA encoding murine procathepsin E was isolated and sequenced and recombinant enzyme was produced in Escherichia coli. The activity of the purified recombinant mouse cathepsin E was characterised quantitatively using two synthetic peptide substrates and naturally

occurring inhibitors. The majority of the recombinant enzyme was present as a homodimer (Mr ~80) in which the two monomers were linked by an intermolecular disulfide bond. By analogy to previous studies with human cathepsin E, this is most likely a consequence of the presence of a unique cysteine residue near the N-terminus of the mature proteinase. The availability of (i) recombinant murine enzyme in reasonable quantities and (ii) a full-length cDNA now enables structural investigations and attempts to generate 'knock-out' mice deficient in this important aspartic proteinase to be undertaken.

Tatsura, H., H. Nagao, et al. (2001). "Developing germ cells in mouse testis express pheromone receptors." FEBS Letters **488**(3): 139.

http://www.sciencedirect.com/science/article/B6T36-426XTNW-6/2/bcf8713cdc2f60d0aa21e7c665be0cd1

Pheromone receptors are expressed in the accessory olfactory system, which is vital for non-specific chemical communication and for sexual behavior. Under the hypothesis that some of the pheromone molecules released from female reproductive organs might regulate sperm chemotaxis or chemokinesis, we examined whether the V1R type pheromone receptor mRNAs are expressed in developing germ cells. By a reverse transcription-PCR method, we obtained nine kinds of cDNA fragments belonging to the receptor family. In situ hybridization analysis in testicular sections using probes of testicular pheromone receptors (TVRs) revealed that TVR mRNAs were expressed by spermatids. TVRs were also expressed in the accessory olfactory organ. In the testis, hybridization signals were localized in subsets of the seminiferous tubules, suggesting that TVRs were expressed by selective subsets of the spermatids. In situ hybridization study suggests also that each sperm expresses multiple pheromone receptors. The testicular pheromone receptors might have an important role in the maturation and/or migration of sperm.

Tavladoraki, P., M. E. Schinina, et al. (1998). "Maize polyamine oxidase: primary structure from protein and cDNA sequencing." FEBS Letters **426**(1): 62.

http://www.sciencedirect.com/science/article/B6T36-3SHT1NY-F/2/0891fa5218ed5320b7aa76fd6326d851

The first complete amino acid sequence of a flavin-containing polyamine oxidase was solved by a combined approach of nucleotide and peptide sequence analysis. A cDNA of 1737 bp, isolated from maize seedlings by reverse transcription-polymerase chain reaction and rapid amplification of cDNA ends strategies, was cloned and its sequence determined. This cDNA contains information for a polypeptide chain of 500 amino acids. Its amino-terminal sequence shows the typical features of secretion signal peptides. The primary structure of the mature protein was independently confirmed by extensive amino acid sequencing. Structural relationships with flavin-containing monoamine oxidases are also discussed.

Thai, T.-P., H. Heid, et al. (1997). "Ether lipid biosynthesis: isolation and molecular characterization of human dihydroxyacetonephosphate acyltransferase." FEBS Letters **420**(2-3): 205.

http://www.sciencedirect.com/science/article/B6T36-3RSNBYV-P/2/6ade010c18d51d50516670418256d03d

In this paper we describe isolation and molecular characterization of human

dihydroxyacetonephosphate acyltransferase (DAP-AT). The enzyme was extracted from rabbit Harderian gland peroxisomes and isolated as a trimeric complex by sucrose density gradient centrifugation. From peptide sequences matching EST-clones were obtained which allowed cloning and sequencing of the cDNA from a human cDNA library. The nucleotide-derived amino acid sequence revealed a protein consisting of 680 amino acid residues of molecular mass 77187 containing a C-terminal type 1 peroxisomal targeting signal. Monospecific antibodies raised against this polypeptide efficiently immunoprecipitated DAP-AT activity from solubilized peroxisomal preparations, thus demonstrating that the cloned cDNA codes for DAP-AT.

Thummler, F., A. Beetz, et al. (1990). "Phytochrome in lower plants Detection and partial sequence of a phytochrome gene in the moss Ceratodon purpureus using the polymerase chain reaction." FEBS Letters **275**(1-2): 125.

http://www.sciencedirect.com/science/article/B6T36-44M4167-9H/2/a475fa032649338aab4919b71434f25d

The polymerase chain reaction was carried out with primers hybridizing to conserved regions of the phytochrome genes. With DNA from the moss Ceratodon purpureus 5 overlapping fragments were obtained resulting in a continuous nucleotide sequence of 1474 bp. The deduced amino acid sequence showed homology of around 60% with all known phytochrome sequences. The sequences contained a conserved chromophore attachment site. In light-grown Ceratodon protonemata the phytochrome mRNA with the size of about 4.5 kb was detected.

Toma, S., M. Nakamura, et al. (1997). "Cloning and recombinant expression of rat and human kynureninase." FEBS Letters **408**(1): 5.

http://www.sciencedirect.com/science/article/B6T36-3R7B21X-2/2/d3f6127d9eb733933132aea726b72d28

Kynureninase [E.C.3.7.1.3.] is one of the enzymes involved in the biosynthesis of NAD cofactors from tryptophan through the kynurenine pathway. By tryptic and CNBr digestion of purified rat liver kynureninase, we obtained about 28% of the amino acid sequence of the enzyme. The rat kynureninase cDNA, isolated by means of reverse-transcribed polymerase chain reaction and hybridization screening, codes for a polypeptide of 464 amino acids. Northern blot analysis revealed the synthesis of a 2.0 kb rat kynureninase mRNA. A cDNA encoding human liver kynureninase was also isolated. The deduced amino acid sequence is 85% identical to that of the rat protein. COS-1 cells were transfected with both cDNAs. The Km values of the rat enzyme, for -kynurenine and -3-hydroxykynurenine, were 440+/-20 [mu]M and 32+/-5 [mu]M and of the human enzyme 440+/-20 [mu]M and 49+/-6 [mu]M, respectively. Interestingly, COS-1 cells transfected with the cDNA coding for rat kynureninase also display cysteine-conjugate [beta]-lyase activity.

Tsuboi, R., C. Sato, et al. (1995). "Ultraviolet B irradiation increases endothelin-1 and endothelin receptor expression in cultured human keratinocytes." FEBS Letters **371**(2): 188.

http://www.sciencedirect.com/science/article/B6T36-3YS2C5M-K2/2/05082533c4b4a25376f88d5ccabfcc4a

The effect of ultraviolet B (UVB) irradiation on endothelin-1 (ET-1) and ET receptor expression

was examined using cultured normal human keratinocytes. Keratinocytes secreted ET-1 in the medium at a level of 2.1 pg/day/105 cells. UVB irradiation up to 10 mJ/cm2 increased ET-1 secretion 3-fold, and potentiated expression of mRNA for ET-1. Both ETA and ETB receptor mRNAs were detected in keratinocytes, and their expression was up-regulated by 5 mJ/cm2 UVB irradiation.

Tsuji, L., T. Takumi, et al. (1997). "Identification of novel homologues of mouse importin [alpha], the [alpha] subunit of the nuclear pore-targeting complex, and their tissue-specific expression." FEBS Letters **416**(1): 30.

http://www.sciencedirect.com/science/article/B6T36-3RD0S7F-8/2/7fa805b9621a309d2c967c2e3035bf30

Transport of karyophilic proteins into the nucleus is mediated by nuclear localization signals (NLSs) via a multistep process. The karyophiles are recognized by the importin [alpha] subunit in the cytoplasm to form a stable complex, termed the nuclear pore-targeting complex (PTAC). To date, three different mammalian [alpha] subunits (mSRP1/NPI-1, PTAC58/mPendulin/Rch1 and Qip1) have been identified. In this study, we report the identification of three additional mouse genes homologous to the known [alpha] subunits using RT-PCR methodology and show that the mouse [alpha] subunits can be classified into at least three subfamilies, [alpha]-P, [alpha]-Q and [alpha]-S families, each composed of closely related members (more than 80% amino acid sequence identity). These three subfamilies, however, have ~50% amino acid identity to one another. Northern blot analysis showed that all were differentially expressed in various mouse tissues. These results suggest that the function of these proteins may be controlled in a tissue-specific manner and that their combinatorial expression may play a role in differentiation and organogenesis.

Tygesen, C. K., M. Jorgensen, et al. (1995). "The importance of two specific domains in ligand binding to the AMPA/kainate glutamate receptors GluR2 and GluR6." FEBS Letters **363**(1-2): 184.

http://www.sciencedirect.com/science/article/B6T36-3YRNYDD-MT/2/ceb889ac1b29983c4f26e6af7f62272d

Chimeric receptor subunits of the AMPA receptor subunit GluR2 and the kainate receptor subunit GluR6 were constructed and stably expressed in baby hamster kidney cells. By using ca 2+ imaging and radioligand binding, we demonstrated that substitution of a specific domain showing homology to a bacterial leucine-isoleucine-valine binding protein (LIVBP) had no effect on the affinities of the tested agonists, but decreased the affinities of the antagonists CNQX, DNQX, and NBQX. On the other hand, when the first of two domains showing homology to a bacterial glutamine binding protein (QBP) in GluR2 was substituted with the corresponding region from GluR6, the affinity of AMPA decreased sevenfold and the affinity of kainate increased fourfold, indicating the importance of this domain in binding of these agonists. In contrast to this, the affinities of quisqualate and domoate, two other agonists, were unchanged, indicating that a region located C-terminal to the QBP domain is also involved in agonist binding.

Vilain, A., N. Vogt, et al. (1999). "DNA methylation and chromosome instability in breast cancer cell lines." FEBS Letters **460**(2): 231.

http://www.sciencedirect.com/science/article/B6T36-3XR2GT0-

9/2/1ea22c1b102db257595a0744cf8cfdab

We show that in a series of eight breast cancer cell lines, a direct relationship exists between the overall DNA demethylation and the percentage of rearranged chromosomes, except for cell lines with a highly rearranged genome which can be weakly demethylated. A real time fluorescent detection method was used to quantify by reverse transcription-PCR the expression of the DNA methyltransferase 1 and of the newly discovered DNA demethylase. The overall DNA methylation status seems to result from a complex interplay between the expression of these two genes. Our results suggest that in these tumor cells, the overall DNA demethylation is implicated in one of the mechanisms at the origin of the genome instability and that besides the role of the DNA methyltransferase 1, that of the DNA demethylase may be essential in the control of DNA methylation.

Vorgias, C. E., A. J. Kingswell, et al. (1991). "Cloning, overexpression, purification and crystallisation of ribosomal protein L9 from Bacillus stearothermophilus." FEBS Letters **286**(1-2): 204.

http://www.sciencedirect.com/science/article/B6T36-44XN362-W5/2/a251806c35e0642bfc6e692d066b904c

The cloning, sequencing and overexpression of the gene coding for Bacillus stearothermophilus ribosomal protein L9 is described. The sequence corresponds directly to that presented for the protein itself by classical methods, differing at only a few amino acid positions. The purification and crystallisation of the corresponding L9 protein is presented. The crystals are isomorphous to those described for L9 obtained by conventional methods.

Vullhorst, D., R. Klocke, et al. (1998). "Expression of the potassium channel KV3.4 in mouse skeletal muscle parallels fiber type maturation and depends on excitation pattern." FEBS Letters **421**(3): 259.

http://www.sciencedirect.com/science/article/B6T36-3S0F2K8-M/2/74c0616a14872802ed8c36bdb3c19275

We report the detailed expression pattern of the voltage-dependent potassium channel KV3.4 (rat homologue, Raw3) in mouse skeletal muscle. Using semi-quantitative RT-PCR, we show that its expression is detectable at embryonic day 17 and rises to adult levels within 2 weeks after birth. Expression is fiber type-dependent, with mRNA levels being 5-6-fold lower in the mixed slow/fast soleus muscle than in the fast tibialis anterior and extensor digitorum longus muscles. Fast muscles from myotonic mice exhibit low KV3.4 mRNA levels similar to those of wild-type soleus. In denervated extensor digitorum longus, KV3.4 expression declines to perinatal levels. We conclude that KV3.4 expression in mouse skeletal muscle is regulated by the pattern of excitation.

Wagener, R., B. Kobbe, et al. (1998). "Genomic organisation, alternative splicing and primary structure of human matrilin-4." FEBS Letters **438**(3): 165.

http://www.sciencedirect.com/science/article/B6T36-3V5MRHV-6/2/494fcbcd31d26f12f4b130b32f360ce0

We have recently cloned a cDNA for mouse matrilin-4. By sequence comparison we identified the

12 kb long human matrilin-4 gene as a part of a high-throughput genomic sequence (HS453C12) in the databases. Additionally we found a human matrilin-4 expressed sequence tag (H54037) in the database that had been mapped to chromosome 20q13.1-2. The gene contains 10 exons and, like the matrilin-1 gene, the human matrilin-4 gene contains an AT-AC intron between the two exons en coding the coiled-coil domain. The cDNA sequence of human matrilin-4 was determined by sequencing of RT-PCR products obtained from mRNA of the human embryonic kidney cell line HEK 293. At the amino acid level it showed an overall sequence identity to the mature mouse matrilin-4 of 91% with a maximum of 97% in the second vWFA-like module. Alternative splicing leads to three different mRNAs. They all encode the putative signal peptide, the two vWFA-like domains and the potential coiled-coil [alpha]-helical oligomerisation domain but differ in that either one, two or three EGF-like domains are retained in the mature mRNA. Due to a G to A mutation at the splice donor site of intron C, the third exon encodes an untranslated pseudo-exon specifying the first EGF-like domain when compared to mouse matrilin-4.

Wagener, R., B. Kobbe, et al. (1998). "Matrilin-4, a new member of the matrilin family of extracellular matrix proteins." FEBS Letters **436**(1): 123.

http://www.sciencedirect.com/science/article/B6T36-3TX4VC2-V/2/1195056d594f414cf6bff1d0d4d8f963

Mouse cDNA encoding for matrilin-4 was cloned and the primary structure of this fourth member of the matrilin family was deduced from the nucleotide sequence. The protein precursor of 624 amino acids consists of a putative signal peptide, two vWFA-like domains linked by four epidermal growth factor-like modules and a potential coiled-coil [alpha]-helical oligomerization domain at the C-terminus. The predicted Mr of the mature protein is 66442. Expression in lung, brain, sternum, kidney and heart was detected by Northern blot analysis of mouse mRNA. Additionally an alternatively spliced mRNA lacking the sequence coding for the first vWFA domain was found in 7 weeks old mice leading to a protein precursor of 434 amino acids and a predicted Mr of the mature protein of 45468.

Wenk, J., H.-I. Trompeter, et al. (1992). "Molecular cloning and primary structure of a protein phosphatase 2C isoform." FEBS Letters **297**(1-2): 135.

http://www.sciencedirect.com/science/article/B6T36-44XN0CY-9M/2/6f0e716298055555a78ad8c22f96cd1f

Complementary DNA encoding the isoform of protein phosphatase 2C, termed PP2C2, has been isolated. The cDNA predicts a protein of 390 amino acid residues with a molecular mass of 42,888 Da. The protein displays 76% identity to the PP2C1 isoform.

Xie, Y., T. Sun, et al. (2005). "Acquisition of essential somatic cell cycle regulatory protein expression and implied activity occurs at the second to third cell division in mouse preimplantation embryos." FEBS Letters **579**(2): 398.

http://www.sciencedirect.com/science/article/B6T36-4F19SSK-4/2/fe53d9426754224ccc5bcf11178e774a

It is clear that G1-S phase control is exerted after the mouse embryo implants into the uterus 4.5 days after fertilization (E4.5); null mutants of genes that control cell cycle commitment such as

max, rb (retinoblastoma), and dp1 are embryonic lethal after implantation with proliferation phenotypes. But, a number of studies of genes mediating proliferation control in the embryo after fertilization-implantation have yielded confusing results. In order to understand when embryos might first exert G1-S phase regulatory control, we assayed preimplantation mouse embryos for the acquisition of expression of mRNA, protein, and phospho-protein for max, Rb, and DP-1, and for the proliferation-promoting phospho-protein forms of mycC (thr58/ser62) and Rb (ser795). The key findings are that: (1) DP-1 protein was present in the nucleus as early as the four-cell stage onwards, (2) max protein was in the nucleus, suggesting function from the four-cell stage onwards, (3) both mycC and Rb all form protein was present at increasing quantities in the cytoplasm from the 2 cell and 4/8 cell stage, respectively, (4) the phosphorylated form of mycC phospho was present in the nucleus at high levels from the two-cell stage through blastocyst-stage, and (5) the phosphorylated form of Rb was detected at low levels in the two-cell stage embryo and was highly expressed at the 4/8-cell stage through the blastocyst stage. Taken together, these data suggest that activation of mycC phospho/max dimer pairs, (E2F)/DP-1 dimer pairs, and repression of Rb inhibition of cell cycle progression via phosphorylation at ser795 occurs at the earliest stages of embryonic development. In addition, the presence of max, mycC phospho, DP-1, and Rb phospho in the nuclei of embryonic and placental lineage cells in the blastocyst and in trophoblast stem cells suggests that a similar type of cell cycle regulation is present throughout preimplantation development and in both embryonic and extra-embryonic cell lineages.

Xu, X.-M., X. Zhou, et al. (1999). "The zebrafish genome contains two distinct selenocysteine tRNA[Ser]Sec genes." FEBS Letters **454**(1-2): 16.

http://www.sciencedirect.com/science/article/B6T36-3WWDHMS-4/2/7346cda740af6d06c4c3652609dcf5b3

The zebrafish is widely used as a model system for studying mammalian developmental genetics and more recently, as a model system for carcinogenesis. Since there is mounting evidence that selenium can prevent cancer in mammals, including humans, we characterized the selenocysteine tRNA[Ser]Sec gene and its product in zebrafish. Two genes for this tRNA were isolated and sequenced and were found to map at different loci within the zebrafish genome. The encoding sequences of both are identical and their flanking sequences are highly homologous for several hundred bases in both directions. The two genes likely arose from gene duplication which is a common phenomenon among many genes in this species. In addition, zebrafish tRNA[Ser]Sec was isolated from the total tRNA population and shown to decode UGA in a ribosomal binding assay.

Yang, W., A. A. Reyes, et al. (1994). "Identification of the GABAA receptor subtype mRNA in human pancreatic tissue." FEBS Letters **346**(2-3): 257.

http://www.sciencedirect.com/science/article/B6T36-44W4WMV-54/2/7f5aa319e6a9f0fd63ddf4e334ca8465

Evidence suggests a physiological role of the GABAA receptor in the pancreas. Clinically, an autoimmune reaction involving the GABA biosynthesizing enzyme, glutamic acid decarboxylase has been implicated in the development of insulin-dependent diabetes mellitus. To determine the subtypes of GABAA receptor expressed in human pancreas, we analyzed, with the use of the reverse-transcription/polymerase chain reaction technique human pancreatic tissue for the presence of GABAA receptor subunits [alpha]1-6, [beta]1-3, and [gamma]1-2 transcripts. Unlike brain tissue, pancreatic tissue only expresses the [alpha]2, [beta]3 and [gamma]1 subunits. Our results provide evidence of a specific GABAA receptor subtype expressed in human pancreatic

tissue.

Yoshikawa, M., T. Nakajima, et al. (2004). "TNF-[alpha] and IL-4 regulate expression of fractalkine (CX3CL1) as a membrane-anchored proadhesive protein and soluble chemotactic peptide on human fibroblasts." FEBS Letters **561**(1-3): 105.

http://www.sciencedirect.com/science/article/B6T36-4BVPCF7-5/2/1d64fe6db56a339ddcfaddb149a3d31d

The CX3C chemokine, fractalkine (FKN, CX3CL1), has multiple functions and exists as two distinct forms, a membrane-anchored protein and a soluble chemotactic peptide that cleaves from the cell surface FKN. In this study, we first demonstrated the expression of FKN in tumor necrosis factor (TNF)-[alpha]- and interleukin (IL)-4-stimulated human fibroblasts. The induction of FKN was observed for both forms. We also demonstrated monocyte chemotactic activity in the culture supernatant from the fibroblasts stimulated with these cytokines. These results suggest that TNF-[alpha]- and IL-4-stimulated fibroblasts may play an important role in accumulation of monocytes at inflammatory sites.

Yoshikawa, T., T. Okano, et al. (1998). "A deep brain photoreceptive molecule in the toad hypothalamus." FEBS Letters **424**(1-2): 69.

http://www.sciencedirect.com/science/article/B6T36-3S7X4JD-H/2/2ceb746a5e445cee172a9959f902efeb

We have isolated a cDNA clone encoding a deep brain photoreceptive molecule from the hypothalamic cDNA library of the toad, Bufo japonicus. The deduced amino acid sequence showed the highest similarity to that of pinopsin (75-76%) among vertebrate retinal opsins, indicating the expression of toad pinopsin in the deep brain. Antibodies raised against the C-terminal tail of toad pinopsin stained cell bodies and the knob-like structures of the cerebrospinal fluid-contacting neurons in the anterior preoptic nucleus. This region is known to play an important role in breeding behavior, suggesting that toad pinopsin acts as a photosensor for the photoperiodic gonadal response.

Zhao, C., L. Liu, et al. (1994). "Identification of a new member of the protegrin family by cDNA cloning." FEBS Letters **346**(2-3): 285.

http://www.sciencedirect.com/science/article/B6T36-44W4WMV-59/2/27de7cfc44722fac66f61a3f18bc9e74

The porcine leukocyte protegrins are a family of cysteine-rich antimicrobial peptides the primary structures of which combine features of defensins and tachyplesins. We cloned three protegrins from porcine bone marrow mRNA by PCR, including one (PG-4) that was previously unknown. The 691 bp protegrin cDNAs were> 98.8% identical, and each was surrounded by highly conserved 5' and (in some instances) 3' sequences present in structurally dissimilar antimicrobial and LPS-binding peptides of animal leukocytes.

FEMS Immunology and Medical Microbiology (28)

Al-Soud, W. A., I.-S. Ouis, et al. "Characterization of the PCR inhibitory effect of bile to optimize real-time PCR detection of Helicobacter species." FEMS Immunology and Medical Microbiology **In Press, Corrected Proof** http://www.sciencedirect.com/science/article/B6T2T-4F31MB1-1/2/b63cfb213e49797a35b4a09d91551627

The inhibitory effect of human and porcine bile samples to detect Helicobacter DNA was studied by adding different concentrations of bile samples to PCR mixtures of six thermostable DNA polymerases containing cagA specific primers and Helicobacter pylori DNA. PCR products were amplified by using the Rotorgene system and SYBR Green I. Among the six DNA polymerases tested, rTth had the lowest sensitivity to bile inhibitors, whereas Taq and Tfl had the highest sensitivity. Bile proteins did not inhibit AmpliTaq DNA polymerase, whereas the fraction containing mainly bile acids and their salts inhibited the amplification capacity of AmpliTaq. Heating human bile at 98 [deg]C and adding casein and formamide to the reaction mixture reduced the PCR inhibitory effect of bile. Therefore, a pre-PCR treatment based on dilution and heating of bile, adding casein and formamide to the reaction mixture of rTth DNA polymerase was found efficient to amplify DNA directly in bile.

Denis-Mize, K. S., B. M. Price, et al. (2000). "Analysis of immunization with DNA encoding Pseudomonas aeruginosa exotoxin A." FEMS Immunology and Medical Microbiology **27**(2): 147.

http://www.sciencedirect.com/science/article/B6T2T-3YB4D8T-9/2/96b489eee9c3532825cb28b5c4bd3aab

The promising arena of DNA-based vaccines has led us to investigate possible candidates for immunization against bacterial pathogens. One such target is the opportunistic pathogen Pseudomonas aeruginosa which produces exotoxin A (PE), a well-characterized virulence factor encoded by the toxA gene. In its native protein form, PE is highly cytotoxic for susceptible eukaryotic cells through ADP-ribosylation of elongation factor-2 following internalization and processing of the toxin. To study the biologic and immunological effects of PE following in situ expression, we have constructed eukaryotic plasmid expression vectors containing either the wild-type or a mutated, non-cytotoxic toxA gene. In vitro analysis by transfection of UM449 cells suggests that expression of the wild-type toxA gene is lethal for transfected cells whereas transfection with a mutated toxA gene results in the production of inactive PE which can be readily detected by immunoblot analysis of cell lysates. To investigate the effects resulting from the intracellular expression of potentially cytotoxic gene products in DNA vaccine constructs, we immunized mice with both the wild-type and mutant toxA plasmid constructs and analyzed the resulting humoral and cellular immune responses. Immunization with the mutated toxA gene results in production of neutralizing antibodies against native PE and potentiates a TH1-type response, whereas only a minimal humoral response can be detected in mice immunized with wild-type toxA. DNA-based vaccination with the non-cytotoxic toxAmut gene confers complete protection against challenge with the wild-type PE. Therefore, genetic immunization with genes encoding potentially cytotoxic gene products raises concern with regard to the selection of feasible gene targets for DNA vaccine development.

Fournier, S., S. Dubrou, et al. (2002). "Detection of microsporidia, cryptosporidia and giardia in swimming pools: a one-year prospective study." FEMS Immunology and Medical Microbiology **33**(3): 209.

http://www.sciencedirect.com/science/article/B6T2T-45XRJ79-2/2/200df3f11a690e5abaec319f9daf68eb

In order to estimate the rate of microsporidia, cryptosporidia and giardia contamination of swimming pools, sequential samples of water were collected during a one-year period in six different swimming pools in Paris, France. Fourty-eight samples were submitted to filtrations. Eluates were examined for microsporidia using polymerase chain reaction (PCR) and for cryptosporidia and giardia using immunofluorescence staining. One of 48 specimens was positive for microsporidia. Using DNA sequence analysis, unknown microsporidia species were identified, which were close to an insect microsporidia Endoreticulatus schubergi. One sample was positive for cryptosporidia and none were positive for giardia. This study shows a low level of swimming pool water contamination by microsporidia, cryptosporidia or giardia, demonstrating the efficacy of cleaning filtration and disinfection procedures used in French swimming pools.

Fournier, S., O. Liguory, et al. (2000). "Detection of microsporidia in surface water: a one-year follow-up study." FEMS Immunology and Medical Microbiology **29**(2): 95.

http://www.sciencedirect.com/science/article/B6T2T-41BV7S6-3/2/bc784576f28feea5b9964074de3cc205

In order to estimate the rate and seasonal variation of Enterocytozoon bieneusi contamination of surface water, sequential samples of water from the River Seine in France were collected during a 1-year period. Each sample (300-600 l) was submitted to sequential filtrations, and the filters were then examined for microsporidia using light microscopy and nested polymerase chain reaction (PCR) for E. bieneusi. Amplified products were hybridized with a E. bieneusi-specific probe. Twenty-five samples of water were analyzed during 1 year. Microscopic examination of stained filters proved unreliable for the identification of spores. Using nested PCR, 16 of 25 specimens were positive (64%). Unexpectedly, E. bieneusi was identified in only one sample by specific hybridization underlining the lack of specificity of ours primers. Nevertheless, using DNA sequence analysis, unknown microsporidia species were identified in eight cases, which had highest scores of homology with Vittaforma corneae or Pleistophora sp. This study shows a low rate of water contamination by E. bieneusi suggesting that the risk of waterborne transmission to humans is limited.

Francki, K. T., B. J. Chang, et al. (2000). "Identification of genes associated with copper tolerance in an adhesion-defective mutant of Aeromonas veronii biovar sobria." FEMS Immunology and Medical Microbiology **29**(2): 115.

http://www.sciencedirect.com/science/article/B6T2T-41BV7S6-6/2/4b1731aa13d48354e8267cc317ac764b

TnphoA mutagenesis was used to identify adhesins of Aeromonas veronii biovar sobria 3767, a strain isolated from a diarrhoeal stool specimen. Six mutants, from a library of 154, exhibited significantly reduced levels of adhesion to HEp-2 cells. Primers to the terminal regions of TnphoA were used for inverse PCR and the product from one mutant was cloned into pBluescript and partial sequence data obtained. Scanning GenBank and EMBL data bases revealed DNA sequence similarity to the copA gene of Pseudomonas syringae pv. tomato which confers resistance to copper and other heavy metals. The transposon was located within the copA gene and the mutant exhibited a reduced tolerance to copper. Primer walking, using the inverse PCR product as a template, revealed three open reading frames (ORFs) copA, B and C in A. veronii biovar sobria 3767. The predicted amino acid sequences of ORFs A and B had significant

homology (55 and 34% respectively) to the copA and B proteins of P. syringae. No amino acid or DNA sequence homology existed between ORF C of strain 3767 and any other gene in the data bases scanned. Further analysis of the nucleotide sequence failed to reveal the presence of typical copper regulatory genes within the vicinity of the Aeromonas sequence. The association between copper tolerance and adhesion in A. veronii biovar sobria requires further study.

Geyer, A., A. Roth, et al. (1999). "M protein of a Streptococcus dysgalactiae human wound isolate shows multiple binding to different plasma proteins and shares epitopes with keratin and human cartilage." FEMS Immunology and Medical Microbiology **26**(1): 11.

http://www.sciencedirect.com/science/article/B6T2T-3XK6TGS-2/2/aab5b51fe49971f953db6e3e6a1ebc1e

Besides group A (GAS), Lancefield group C [beta]-haemolytic streptococci (GCS) have been implicated as a causative agent in outbreaks of purulent pharyngitis. In this study we have investigated a class CI M protein of a Streptococcus dysgalactiae human wound isolate designated MC. MC shares similar properties with M proteins of GAS. It contributes to the virulence of the investigated GCS strain as revealed by in vivo phagocytosis in chicken embryos. Further, MC showed multiple binding to the human plasma proteins fibrinogen, albumin, plasminogen, IgA and all subclasses of IgG. Until now, an M protein, especially from a group C strain, with such a multiple binding behaviour has not been described. Immunoblot experiments with 150 patient sera, having a rheumatoid factor titre >1:256, revealed that 26% of these sera showed serological cross-reactivity between a 68-kDa cartilage protein and the N-terminal part of MC. Only 8% of the sera of healthy patients showed this property. In additional, MC also cross-reacted with antibodies recognising epidermal keratins. The cross-reacting 68-kDa protein from cartilage was different from human serum albumin, but was recognised with anti-vimentin immune serum. The MC was cloned and the gene sequenced. By using PCR, recombinant gene fragments encoding characteristic peptide fragments of MC were expressed in Escherichia coli. The peptides were used to map the binding sites for plasma proteins and to locate the cross-reacting epitopes on the MC molecule. In consequence, sequence alignments revealed that MC shared homologous regions with vimentin and different keratins. Our data, obtained with MC, suggest that not only infections with GAS but also infections with GCS and possibly GGS (the latter species can also produce class CI M-like proteins) may be responsible for the formation of streptococcal-associated sequel diseases.

Gonzalez-Rey, C., S. B. Svenson, et al. (2000). "Specific detection of Plesiomonas shigelloides isolated from aquatic environments, animals and human diarrhoeal cases by PCR based on 23S rRNA gene." FEMS Immunology and Medical Microbiology **29**(2): 107.

http://www.sciencedirect.com/science/article/B6T2T-41BV7S6-5/2/31af0f8a6480395605681192a235a513

Twenty-five strains of Plesiomonas shigelloides isolated from aquatic environment, 10 strains from human cases of diarrhoea and five strains from animals were identified by the polymerase chain reaction technique based on 23S rRNA gene. For this purpose, two primers targeted against part of the 5' half of the 23S rRNA gene of P. shigelloides (Escherichia coli number C-912, G-1195; Plesiomonas number C-906, G-1189) were designed. Results from our study indicated that this method might serve as a tool for a rapid and sensitive identification of P. shigelloides from different environmental and clinical sources.

Guiver, M., R. Borrow, et al. (2000). "Evaluation of the Applied Biosystems automated Taqman polymerase chain reaction system for the detection of meningococcal DNA." FEMS Immunology and Medical Microbiology **28**(2): 173.

http://www.sciencedirect.com/science/article/B6T2T-405KDGT-D/2/96d5ea12798ad7c921e9405037ce089c

In a period where the proportion of culture confirmed cases in the UK has been steadily declining, diagnosis by PCR has been used to increase the number of confirmed cases and provide additional epidemiological data. This report presents a comparative evaluation of the fluorogenic probe-based 5' exonuclease assay (Taqman) using the Perkin-Elmer Applied Biosystems automated sequence detection system 7700 with previously reported polymerase chain reaction enzyme-linked immunosorbent (PCR ELISA) assays for the detection of meningococcal DNA in CSF, plasma and serum samples. Taqman assays developed were based on the detection of a meningococcal capsular transfer gene (ctrA), the insertion sequence IS1106 and the sialytransferase gene (siaD) for serogroup B and C determination and compared with similar assays in a PCR ELISA format. The Taqman ctrA assay was specific for Neisseria meningitidis, however the IS1106 assay gave false positive reactions with a number of non-meningococcal isolates. Sensitivity of the Taqman ctrA, IS1106 and siaD assays testing samples from culture-confirmed cases were 64, 69 and 50%, respectively, compared with 26, 67 and 43% for the corresponding PCR ELISA assays. Improvements to the DNA extraction procedure has increased the sensitivity to 93 and 91% for the TaqMan(TM) ctrA and siaD assays, respectively, compared to culture confirmed cases. Since the introduction of Taqman PCR a 56% increase in laboratory confirmed cases of meningococcal disease has been observed compared to culture only confirmed cases. The developed Taqman assays for the diagnosis of meningococcal disease enables a high throughput, rapid turnaround of samples with considerable reduced risk of contamination.

Guo, M., H. Reynolds, et al. (2000). "Isolation and characterization of a human neutrophil aggregation defective mutant of Fusobacterium nucleatum." FEMS Immunology and Medical Microbiology **27**(3): 241.

http://www.sciencedirect.com/science/article/B6T2T-3YKC6HH-8/2/76bac56992f4b4b8d5fcca07ba0c046c

Fusobacterium nucleatum is known to adhere to human polymorphonuclear neutrophils (PMNs) and cause them to aggregate. In this study, we isolated a spontaneously occurring aggregation defective (AGG-) mutant and this mutant will be used for future study of the interactions between this bacterium and human PMN. Genomic DNA fingerprinting by random-primed polymerase chain reaction method revealed a difference between the parent strain and the AGG- mutant. This mutant also showed an altered phenotype in both microbicidal and phagocytic assays, suggesting that the bacterial factor involved in the aggregation may also be very important for the phagocytosis and, subsequently, the killing by human PMNs. Further study of this mutant may help to clarify the molecular mechanisms of the interaction between this pathogen and human PMNs.

Huijsdens, X. W., R. K. Linskens, et al. (2004). "Detection of Helicobacter species DNA by quantitative PCR in the gastrointestinal tract of healthy individuals and of patients with inflammatory bowel disease." FEMS Immunology and Medical Microbiology **41**(1): 79.

http://www.sciencedirect.com/science/article/B6T2T-4BRSXJ6-

1/2/4b6a36f994e043d0cab8cf73ebfc586c

In many animal species different intestinal Helicobacter species have been described and a few species are associated with intestinal infection. In humans, the only member of the Helicobacter family which is well described in literature is Helicobacter pylori. No other Helicobacter-associated diseases have definitely been shown in humans. We developed a sensitive quantitative PCR to investigate whether Helicobacter species DNA can be detected in the human gastrointestinal tract. We tested gastric biopsies (including biopsies from H. pylori positive persons), intestinal mucosal biopsies and fecal samples from healthy persons, and intestinal mucosal biopsies from patients with inflammatory bowel disease (IBD) for the presence of Helicobacter species. All gastric biopsies, positive for H. pylori by culture, were also positive in our newly developed PCR. No Helicobacter species were found in the mucosal biopsies from patients with IBD (n=56) nor from healthy controls (n=25). All fecal samples were negative. Our study suggests that Helicobacter species, other than H. pylori, are not present in the normal human gastrointestinal flora and our results do not support a role of Helicobacter species in IBD.

Ikehata, M., K. Numazaki, et al. (2000). "Analysis of Chlamydia trachomatis serovars in endocervical specimens derived from pregnant Japanese women." FEMS Immunology and Medical Microbiology **27**(1): 35.

http://www.sciencedirect.com/science/article/B6T2T-3Y6RF1B-6/2/accc6359e29aea3fdf0a13fe05bf9537

The polymerase chain reaction (PCR) method has been employed to amplify a chlamydial genome encoding four variable segments of the major outer membrane protein and genotyping of different Chlamydia trachomatis serovars was successfully achieved by means of restriction fragment length polymorphism (RFLP) analysis and sequencing of amplified DNA. These methods were applied to identify the serotypes of C. trachomatis in endocervical specimens obtained from asymptomatic pregnant Japanese women at 28-30 weeks of gestation. Among the 218 specimens, 207 were serotyped 43 (19.3%) as serovar D, 53 (24.3%) as E, 24 (11.0%) as F, 39 (17.9%) as G, 15 (6.9%) as H, 15 (6.9%) as I, five (2.3%) as J, nine (4.1%) as K and four (1.8%) as mixed. Among the 11 unclassified strains by RFLP, six (2.8%) were identified as serovar B variants and five (2.3%) were identified as D/IC-Cal-8. It was suggested that variants of endemic trachoma serovars also have affinity for the urogenital tract of Japanese pregnant women.

Iredell, J. R., U. H. Stroeher, et al. (1998). "Lipopolysaccharide O-antigen expression and the effect of its absence on virulence in rfb mutants of Vibrio cholerae O1." FEMS Immunology and Medical Microbiology **20**(1): 45.

http://www.sciencedirect.com/science/article/B6T2T-3S1PY2B-6/2/59f57d49a729dcb5d3cdbd15c05c83d2

Using defined rfb mutants, defective in the biosynthesis of the O-antigen of the lipopolysaccharide (LPS), and monoclonal antibodies (MAbs) to the A, B and C LPS antigens, we have examined the distribution of the antigens and the effects of their loss. By immunogold electron microscopy, it has been possible to determine the relative amounts of the A, B and C antigens on Inaba and Ogawa cells, confirming previous studies based upon bacterial agglutination and hemagglutination inhibitions. These antigens are absent from rfb:Tn mutants selected as resistant to phages which have been shown to use the O-antigen as their receptor. These mutants were severely attenuated as measured by both LD50 and their ability to compete with the wild-type

parents when analyzed in the infant mouse cholera model. These mutants were unchanged in the export of cholera toxin or other secreted proteins but revealed an altered outer membrane protein profile. The competition defect suggested an effect on TCP (toxin-coregulated pilus). An analysis of the rfb:Tn mutants revealed that they were unable to assemble TCP on their surface, but the major subunit, TcpA, could be found as an intracellular pool. These mutants could be complemented back to wild-type using the cloned rfb region, implying that functional TCP assembly is dependent upon an intact LPS.

Kawakami, K., M. H. Qureshi, et al. (2000). "Involvement of endogenously synthesized interleukin (IL)-18 in the protective effects of IL-12 against pulmonary infection with Cryptococcus neoformans in mice." FEMS Immunology and Medical Microbiology **27**(3): 191.

http://www.sciencedirect.com/science/article/B6T2T-3YKC6HH-2/2/b1002971eec533e02d18630cc36da211

We previously demonstrated that interleukin (IL)-12 protected mice against fatal pulmonary infection with a highly virulent strain of Cryptococcus neoformans, which correlated well with the production of interferon (IFN)-[gamma] as well as IL-18 in the primary infected site. In the present study, we examined the role of endogenously synthesized IL-18 in IL-12-induced host resistance to this pathogen. There was little or no production of IFN-[gamma] and IL-18 both at mRNA and protein levels in lungs of mice infected with C. neoformans, while treatment with IL-12 induced a marked production of these cytokines. Caspase-1 mRNA was expressed in infected mice even without IL-12 treatment. Administration of neutralizing anti-IFN-[gamma] monoclonal antibody (mAb) clearly inhibited production of IFN-[gamma] and IL-18 induced by IL-12, while control IgG did not show such an effect. However, administration of IFN-[gamma] did not induce the production of both cytokines in infected mice, although tumor necrosis factor (TNF)-[alpha] and IFN-[gamma]-inducible protein (IP)-10 were synthesized by the same treatment. Finally, neutralizing anti-IL-18 antibody (Ab) significantly interfered with the production of IFN-[gamma] and elimination of the microorganism from the lung induced by IL-12 treatment. Furthermore, both IFN-[gamma] synthesis and host protection caused by IL-12 were profoundly diminished in IL-18 gene-disrupted mice. Considered collectively, our results indicated that host protection against C. neoformans induced by IL-12 involved endogenously synthesized IL-18 and that the production of IL-18 was mediated at least in part by endogenous IFN-[gamma].

Kawakami, K., K. Shibuya, et al. (1999). "Chemokine responses and accumulation of inflammatory cells in the lungs of mice infected with highly virulent Cryptococcus neoformans: effects of interleukin-12." FEMS Immunology and Medical Microbiology **25**(4): 391.

http://www.sciencedirect.com/science/article/B6T2T-3X942P1-9/2/d9929032264fb5bf75eab038831eef95

We examined the mechanisms involved in the development of lung lesions after infection with Cryptococcus neoformans by comparing the histopathological findings and chemokine responses in the lungs of mice infected with C. neoformans and assessed the effect of interleukin (IL) 12 which protects mice from lethal infection. In mice infected intratracheally with a highly virulent strain of C. neoformans, the yeast cells multiplied quickly in the alveolar spaces but only a poor cellular inflammatory response was observed throughout the course of infection. Very little or no production of chemokines, including MCP-1, RANTES, MIP-1[alpha], MIP-1[beta] and IP-10, was detected at the mRNA level using RT-PCR as well as at a protein level in MCP-1, RANTES and MIP-1[alpha]. In contrast, intraperitoneal administration of IL-12 induced the synthesis of these chemokines and a marked cellular inflammatory response involving histiocytes and lymphocytes in infected mice. Our findings were confirmed by flow cytometry of intraparenchymal leukocytes

obtained from lung homogenates which showed IL-12-induced accumulation of inflammatory cells consisting mostly of macrophages and CD4+ [alpha][beta] T cells. On the other hand, C-X-C chemokines including MIP-2 and KC, which attract neutrophils, were produced in infected and PBS-treated mice but treatment with IL-12 showed a marginal effect on their level, and neutrophil accumulation was similar in PBS- and IL-12-treated mice infected with C. neoformans. Our results demonstrate a close correlation between chemokine levels and development of lung lesions, and suggest that the induction of chemokine synthesis may be one of the mechanisms of IL-12-induced protection against cryptococcal infection.

Koskiniemi, S., M. Sellin, et al. (1998). "Identification of two genes, cpsX and cpsY, with putative regulatory function on capsule expression in group B streptococci." FEMS Immunology and Medical Microbiology **21**(2): 159.

http://www.sciencedirect.com/science/article/B6T2T-3VWP3TW-B/2/6b070aa427b8f8be2c2cf0fa32025907

Two divergently transcribed open reading frames: cpsX and cpsY separated by a common regulatory region was identified upstream of the cpsA-D genes involved in polysaccharide capsule biosynthesis in group B streptococci (GBS). We suggest that these genes are involved in the regulation of capsule expression in GBS, since the CpsX protein shares sequence similarities with LytR of Bacillus subtilis, an attenuator of transcription while CpsY has similarity to a wide variety of members of the LysR family of transcriptional regulators. No deletions, insertions, DNA rearrangements, or apparent differences were discovered in the postulated regulatory genes when the gene region was compared in GBS with different capsule phenotypes. Thus, other yet unidentified gene loci may control capsule phase variation in GBS.

Mitsumori, K., A. Terai, et al. (1998). "Identification of S, F1C and three PapG fimbrial adhesins in uropathogenic Escherichia coli by polymerase chain reaction." FEMS Immunology and Medical Microbiology **21**(4): 261.

http://www.sciencedirect.com/science/article/B6T2T-3TMXY36-3/2/3e9a3f9571085280fe98567790736eae

S and F1C fimbrial adhesins often expressed by uropathogenic Escherichia coli are genetically homologous. A multiply primed polymerase chain reaction (PCR) was developed for discriminating the S (sfa) and F1C (foc) fimbrial operons. A total of 270 uropathogenic E. coli strains and 80 fecal isolates were examined. PCR specifically detected the sfa and foc alleles in 105 (93%) of 113 sfa/foc+ strains by DNA hybridization. Furthermore, 87% of sfa+ uropathogenic E. coli simultaneously possessed the genes encoding the class III P fimbrial adhesin (prsGJ96), [alpha]-hemolysin and cytotoxic necrotizing factor 1. Statistical analysis showed the class II P fimbrial adhesin (papGIA2) and F1C fimbria to be associated with high relative virulence in pyelonephritis and cystitis, respectively. The multiply primed PCR developed should be useful for assessing the contribution of the S and F1C fimbriae in the pathogenesis of urinary tract infections.

Prins, J. M., C. Schultsz, et al. (1996). "Known bacterial virulence factors do not explain the variation in urinary cytokine levels in patients with urosepsis." FEMS Immunology and Medical Microbiology **16**(3-4): 283.

http://www.sciencedirect.com/science/article/B6T2T-3W32484-J/2/0196da240bd9b42c5e5ac58ddf42d8b6

We measured urinary endotoxin, IL-6 and IL-8 levels in 23 patients with gram-negative urosepsis. The endotoxin and cytokine levels showed a 100-1000 fold range. No correlation was found between levels of urinary endotoxin, and IL-6 or IL-8 levels. In all cases bacterial numbers were >= 105 CFU ml-1 urine. The endotoxin content of the isolated microorganisms neither correlated with the urinary cytokine levels, nor with IL-6 and IL-8 levels obtained in vitro when 103 log-phase CFU of each of the bacteria were incubated with heparinized whole blood of three healthy donors. Neither the haemolysin phenotype of the bacteria, nor the presence of the P-pili gene was correlated with the cytokine response in vivo or in vitro. Other factors than known bacterial virulence factors apparently contribute to the wide variation in urinary cytokine levels in urinary tract infection.

Rinne, M. M., M. Gueimonde, et al. (2005). "Similar bifidogenic effects of prebiotic-supplemented partially hydrolyzed infant formula and breastfeeding on infant gut microbiota." FEMS Immunology and Medical Microbiology **43**(1): 59.

http://www.sciencedirect.com/science/article/B6T2T-4CYPVKP-1/2/f1c106edac1f2b8c3da53b7e016fd1e3

The aim of the study was to assess the quantitative and qualitative differences of the gut microbiota in infants. We evaluated gut microbiota at the age of 6 months in 32 infants who were either exclusively breast-fed, formula-fed, nursed by a formula supplemented with prebiotics (a mixture of fructo- and galacto-oligosaccharides) or breast-fed by mothers who had been given probiotics. The Bifidobacterium, Bacteroides, Clostridium and Lactobacillus/Enterococcus microbiota were assessed by the fluorescence in situ hybridization, and Bifidobacterium species were further characterized by PCR. Total number of bifidobacteria was lower among the formula-fed group than in other groups (P = 0.044). Total amounts of the other bacteria were comparable between the groups. The specific Bifidobacterium microbiota composition of the breast-fed infants was achieved in infants receiving prebiotic supplemented formula. This would suggest that early gut Bifidobacterium microbiota can be modified by special diets up to the age of 6 months.

Saruta, K., T. Matsunaga, et al. (1997). "Simultaneous detection of Streptococcus pneumoniae and Haemophilus influenzae by nested PCR amplification from cerebrospinal fluid samples." FEMS Immunology and Medical Microbiology **19**(2): 151.

http://www.sciencedirect.com/science/article/B6T2T-3SCJ0T1-7/2/8cec095b460538d33232f9ae0737c15a

Haemophilus influenzae and Streptococcus pneumoniae are often the cause of serious diseases such as meningitis. We designed a nested PCR assay to identify these pathogens from cerebrospinal fluid samples. The first-step PCR was able to detect eubacterial rRNA genes with a unified set of universal primers. In the second-step PCR, the identification primers, HI I and II and SP I and II, could detect H. influenzae and S. pneumoniae respectively through amplification of the rRNA spacer between the 16S and 23S rRNA genes. We suggest that the two-step PCR assay can be used as a novel method for the immediate and retrospective diagnosis of bacterial meningitis caused by H. influenzae and S. pneumoniae.

Seib, K. L., I. R. A. Peak, et al. (2002). "Phase variable restriction-modification systems in Moraxella catarrhalis." FEMS Immunology and Medical Microbiology **32**(2): 159.

http://www.sciencedirect.com/science/article/B6T2T-44JD8N7-1/2/2d1281ebfc2e1051fb0032ae19a6d4d9

A repetitive DNA motif was used as a marker to identify novel genes in the mucosal pathogen Moraxella catarrhalis. There is a high prevalence of such repetitive motifs in virulence genes that display phase variable expression. Two repeat containing loci were identified using a digoxigenin-labelled 5'-(CAAC)6-3' oligonucleotide probe. The repeats are located in the methylase components of two distinct type III restriction-modification (R-M) systems. We suggest that the phase variable nature of these R-M systems indicates that they have an important role in the biology of M. catarrhalis.

Sjostedt, A., I. Goransson, et al. (1998). "Genotypic and phenotypic characterization of two Swedish isolates and two prototypic strains of Coxiella burnetii." FEMS Immunology and Medical Microbiology **20**(2): 165.

http://www.sciencedirect.com/science/article/B6T2T-3S6TYBD-C/2/445f1dcef9fe5583544c58e13df1a224

Two Swedish isolates of Coxiella burnetii and the two prototype strains of the species, Nine Mile and Priscilla, were characterized with regard to their multiplication and cytopathic effect on BGM cells and by PCR-based amplification of repetitive element DNA and the C. burnetii-specific plasmids QpH1 and QpRS. Moreover, 1330 bp of each 16S rRNA gene were sequence-determined. All four strains multiplied at virtually the same rate and displayed the same type of vacuoles in the BGM cells. Genetic homogeneity was observed inasmuch as the 16S rDNA sequences were identical and the strains showed identical PCR amplification patterns using primers specific to enterobacterial repetitive intragenic consensus DNA sequences. The two Swedish strains and the Priscilla strain also showed identical patterns after PCR amplification of repetitive extragenic palindromic DNA sequences, whereas the Nine Mile strain demonstrated a similar, but not identical pattern. Thus, the investigated strains demonstrated very similar phenotypic and genotypic characteristics. This finding is discussed in view of the very rare occurrence of domestic Q fever in Sweden.

Tewodros, W., I. Karlsson, et al. (1996). "Allelic variation of the streptokinase gene in [beta]-hemolytic streptococci group C and G isolates of human origin." FEMS Immunology and Medical Microbiology **13**(1): 29.

http://www.sciencedirect.com/science/article/B6T2T-463NNRG-1D/2/00faa2e15bec91717ab0fdb533c16ae6

Genetic diversity of the streptokinase gene (sk) from 36 strains of S. equisimilis and 54 strains of group G streptococci was examined. The strains were isolated from patients with various streptococcal disease manifestations and healthy carriers. The region of the gene that corresponds to amino acid residues 174-244, was PCR amplified. The amplified product was subjected to MluI, PvuII, DraI and DdeI digestion. Based on the restriction enzyme digestion patterns nine sk alleles were recognized. There was no correlation between the various sk gene alleles and streptococcal disease manifestations. Three of the nine sk gene alleles, sk4, sk7, and sk8, were detected earlier among group A streptococci. The other six alleles were unique to S. equisimilis and group G streptococci. The most common alleles were sk5, found in 21/90 (23%)

and sk10 detected in 43/90 (47%) of the strains. Alleles sk1 and sk2, the most frequent among group A streptococci, were not found among the strains in the present investigation. Thus, it appears that the sk gene has been evolving in line with other species distinguishing features of the streptococci.

Thoreson, A. C. E., M. Borre, et al. (1999). "Helicobacter pylori detection in human biopsies: a competitive PCR assay with internal control reveals false results." FEMS Immunology and Medical Microbiology **24**(2): 201.

http://www.sciencedirect.com/science/article/B6T2T-3WG32M9-C/2/c941b3dcd90610501d00472670fbd0f3

A polymerase chain reaction assay (PCR) for the diagnosis of Helicobacter pylori in human gastric biopsies was developed. To prevent false-negative results while performing PCR on human tissues, an internal control is necessary. Primer set ACT1-ACT2 which specifically amplifies a 542-bp fragment of the 16S rRNA gene of H. pylori was used. dUTP and hot-start were used to prevent false-positives from carryover of previous products and avoid non-specific extension products. A competitive internal control DNA fragment was constructed to detect the presence of inhibitors. Biopsies from 101 unselected patients with gastric symptoms were tested. PCR results were compared with results from microscopy of histological sections and conventional culturing for H. pylori. Forty-two percent of the biopsies were found to contain compounds inhibiting the PCR. The addition of the internal control assures the performance of the PCR assay and is an important quality control parameter.

Thoreson, A. C. E., M. B. Borre, et al. (1995). "Development of a PCR-based technique for detection of Helicobacter pylori." FEMS Immunology and Medical Microbiology **10**(3-4): 325.

http://www.sciencedirect.com/science/article/B6T2T-41F73GN-R/2/ca49eb7c1dcc2ab605ff26f532b1f90a

A primer-set was designed for specific detection of genes that encode for 16S rRNA of Helicobacter pylori, using direct polymerase chain reaction (PCR). The primers were selected in the hypervariable regions, derived from a complete small subunit 16S rRNA sequence of the reference strain H. pylori CCUG 17874. The primer-set amplified a 537 base pair (bp) sequence specifically from chromosomal H. pylori DNA. Amplification of purified chromosomal H. pylori DNA was achieved at concentrations as low as 1 femto gram (fg), equivalent to 5 bacteria. Furthermore, as few as 1 lysed H. pylori cell was detected by this PCR technique. The specificity of the primers was 100%, since purified chromosomal DNA was detected from all 32 various H. pylori isolates, whereas no other bacteria species were detected, whether related to Helicobacter or not. The 16S rDNA primers successfully detected H. pylori in antral biopsy specimens collected from infected patients.

Wang, R. Y. H., T. Grandinetti, et al. (1997). "Mycoplasma genitalium infection and host antibody immune response in patients infected by HIV, patients attending STD clinics and in healthy blood donors." FEMS Immunology and Medical Microbiology **19**(3): 237.

http://www.sciencedirect.com/science/article/B6T2T-3RHN702-7/2/62cbf3584c8f34a0f55f52294d702e60

Prevalence of Mycoplasma genitalium in humans is still not clear. We have developed a sensitive and specific serological assay for M. genitalium using lipid-associated membrane proteins (LAMPs) as antigens. Antibodies to LAMPs from M. genitalium showed little cross-reactivity to LAMPs from antigenically similar M. pneumoniae. For validity testing, urines from 104 patients were tested by PCR for M. genitalium. All 15 PCR+ patients had M. genitalium-LAMPs antibodies. Moreover, none of 64 antibody-negative patients were PCR+. Serological study of 1800 patients of various diseased groups and healthy blood donors showed M. genitalium was primarily a sexually transmitted microbe that infected patients with AIDS (44.0%), intravenous drugs users with or without HIV infection (42.5%), and also HIV- patients attending STD clinics (42.6%). Only 5.5% HIV- healthy blood donors and 1.3% HIV+ hemophiliacs tested positive. M. genitalium has been associated with acute non-gonococcal urethritis in male patients. However, many sexually active men and women appear to be chronically infected or colonized by the microbe without apparent clinical symptoms and may continue to transmit the organism through sexual contacts.

Yang, C., Y. Jiang, et al. (2003). "Application of real-time PCR for quantitative detection of Campylobacter jejuni in poultry, milk and environmental water." FEMS Immunology and Medical Microbiology **38**(3): 265.

http://www.sciencedirect.com/science/article/B6T2T-48SBNC1-1/2/c0228c25f43d9b9ae99bcbf41938d001

Campylobacter jejuni is a leading human food-borne pathogen. The rapid and sensitive detection of C. jejuni is necessary for the maintenance of a safe food/water supply. In this article, we present a real-time polymerase chain reaction (PCR) assay for quantitative detection of C. jejuni in naturally contaminated poultry, milk and environmental samples without an enrichment step. The whole assay can be completed in 60 min with a detection limit of approximately 1 CFU. The standard curve correlation coefficient for the threshold cycle versus the copy number of initial C. jejuni cells was 0.988. To test the PCR system, a set of 300 frozen chicken meat samples, 300 milk samples and 300 water samples were screened for the presence of C. jejuni. 30.6% (92/300) of chicken meat samples, 27.3% (82/300) of milk samples, and 13.6% (41/300) of water samples tested positive for C. jejuni. This result indicated that the real-time PCR assay provides a specific, sensitive and rapid method for quantitative detection of C. jejuni. Moreover, it is concluded that retail chicken meat, raw milk and environmental water are commonly contaminated with C. jejuni and could serve as a potential risk for consumers in eastern China, especially if proper hygienic and cooking conditions are not maintained.

Youn, J. H., H.-J. Myung, et al. (2004). "Production and characterization of peptide mimotopes of phenolic glycolipid-I of Mycobacterium leprae." FEMS Immunology and Medical Microbiology **41**(1): 51.

http://www.sciencedirect.com/science/article/B6T2T-4BKDRSC-1/2/683fc1eef00e7478a93a8788c981e409

Phenolic glycolipid-I (PGL-I), a Mycobacterium leprae-specific antigen, has been widely used for the serodiagnosis of leprosy and has been implicated in the pathogenesis of leprosy. In an effort to produce an alternate antigen of PGL-I, the mimotope peptides of PGL-I, W(T/R)LGPY(V/M), were obtained using a monoclonal antibody, III603.8, specific to PGL-I by a phage library. The biotin-labeled predominant mimotope peptide of PGLP1, WTLGPYV, bound to III603.8 in a dose-dependent manner in an immunoassay. However, PGLP1 did not bind to anti-PGL-I antibodies in the serum samples from leprosy patients that were reactive to PGL-I. Although the mimotope peptide of WTLGPYV was not effective as an alternate antigen of PGL-I for the serodiagnosis of leprosy, but it would be of interest to know how the mimotope peptides mimic the role of PGL-I

antigen in the pathogenesis of M. leprae infection.

Zhu, P., C.-M. Tsai, et al. (2002). "Immunologic and genetic characterization of lipooligosaccharide variants in a Neisseria meningitidis serogroup C strain." FEMS Immunology and Medical Microbiology **34**(3): 193.

http://www.sciencedirect.com/science/article/B6T2T-46VJGKR-1/2/89e30a15479a581872e32a6d617cc82b

Neisseria meningitidis shows great variation in expression of structurally different lipooligosaccharides (LOS) on its cell surface. To better understand the LOS diversity that may occur within an individual strain, a group C wild-type strain, BB305-Tr4, and two stable isogenic LOS variants, Tr5 and Tr7, were selected for this study. SDS-PAGE analysis showed a size reduction of Tr5 and Tr7 LOS compared to that of Tr4. Immunoblotting showed that parental Tr4 LOS reacted with L1, L2 and L3,7 antibodies, variant Tr5 LOS with L1 and L6 antibodies, while Tr7 LOS was non-typeable. Genetic analysis showed that the gene organization at the lgt-1 locus in the three strains was lgtZ,C,A,B,H4 in Tr4, lgtZ,C,A,H4 in Tr5 and lgtZ,C,A,H9 in Tr7. The genetic differences in the three strains were consistent with their phenotypic changes. Sequence comparison revealed two independent recombination events. The first was the recombination of repeated DNA fragments in the flanking regions to delete lgtB in Tr5. The second was the recombination of a fragment of two genes, lgtB and lgtH4, to create an inactive lgtH9 allele with a mosaic structure in Tr7. These findings suggest that besides phase variation, homologous recombination can contribute to the genetic diversity of the lgt locus and to the generation of LOS variation in N. meningitidis.

FEMS Microbiology Ecology (49)

Araya, R., K. Tani, et al. (2003). "Bacterial activity and community composition in stream water and biofilm from an urban river determined by fluorescent in situ hybridization and DGGE analysis." FEMS Microbiology Ecology **43**(1): 111.

http://www.sciencedirect.com/science/article/B6T2V-47184Y7-1/2/7ef34ff30805aedd8b9e5bcb0d3c5ada

Physiologic activity and community structure of planktonic and biofilm microbial communities in an urban river were analyzed using 5-cyano-2,3-ditolyl tetrazolium chloride (CTC) staining, fluorescent in situ hybridization (FISH) and denaturing gradient gel electrophoresis (DGGE) analysis of polymerase chain reaction (PCR)-amplified 16S rDNA fragments. Respiring bacteria estimated by CTC reduction were higher in biofilms (20%) than in stream water samples (12%). FISH analysis revealed that bacterial populations in both stream water and biofilms were dominated by [beta]-Proteobacteria and Cytophaga-Flavobacterium cluster. Microbial community changes determined by multidimensional scaling analysis from DGGE patterns showed that microbial community structures in biofilms matured within 3-7 days of their formation and did not change further, while those in stream water changed continuously.

Baker, P. W., H. Futamata, et al. (2001). "Molecular diversity of pMMO and sMMO in a TCE-contaminated aquifer during bioremediation." FEMS Microbiology Ecology **38**(2-3): 161.

http://www.sciencedirect.com/science/article/B6T2V-44HXHYT-9/2/06fd908d3c16d01e4efadf0f21366c63

The particulate methane monooxygenase genes (pMMO) were amplified from methane-biostimulated aquifer samples using two sets of primers, A189 plus A682 and pmof2 plus pmor, and the products were cloned and sequenced. The analysis of the sequences revealed a high diversity of pMMO genes in the aquifers. Some of the pMMO gene sequences (LP20 and LP21) isolated in this study were unique and unrelated to methanotrophs previously isolated from this site. Throughout all the samples collected from the different methane biostimulation trials, competitive PCR amplification using specific pMMO primers revealed that type I methanotrophs predominated over type II methanotrophs. The partial soluble methane monooxygenase genes were also amplified from the aquifer samples using specially designed primers but sequencing revealed a lower diversity.

Bergsma-Vlami, M., M. E. Prins, et al. (2005). "Influence of plant species on population dynamics, genotypic diversity and antibiotic production in the rhizosphere by indigenous Pseudomonas spp." FEMS Microbiology Ecology **52**(1): 59.

http://www.sciencedirect.com/science/article/B6T2V-4DS3Y2Y-1/2/70149334125d623296fe84ef88cd5889

The population dynamics, genotypic diversity and activity of naturally-occurring 2,4-diacetylphloroglucinol (DAPG)-producing Pseudomonas spp. was investigated for four plant species (wheat, sugar beet, potato, lily) grown in two different soils. All four plant species tested, except lily and in some cases wheat, supported relatively high rhizosphere populations (5 X 104 to 1 X 106 CFU/g root) of indigenous DAPG-producing Pseudomonas spp. during successive cultivation in both a take-all suppressive and a take-all conducive soil. Although lily supported on average the highest population densities of fluorescent Pseudomonas spp., it was the least supportive of DAPG-producing Pseudomonas spp. of all four plant species. The genotypic diversity of 492 DAPG-producing Pseudomonas isolates, assessed by Denaturing Gradient Gel Electrophoresis (DGGE) analysis of the phlD gene, revealed a total of 7 genotypes. Some of the genotypes were found only in the rhizosphere of a specific plant, whereas the predominant genotypes were found at significantly higher frequencies in the rhizosphere of three plant species (wheat, sugar beet and potato). Statistical analysis of the phlD+ genotype frequencies showed that the diversity of the phlD+ isolates from lily was significantly lower than the diversity of phlD+ isolates found on wheat, sugar beet or potato. Additionally, soil type had a significant effect on both the phlD+ population density and the phlD+ genotype frequencies, with the take-all suppressive soil being the most supportive. HPLC analysis further showed that the plant species had a significant effect on DAPG-production by the indigenous phlD+ population: the wheat and potato rhizospheres supported significantly higher amounts of DAPG produced per cell basis than the rhizospheres of sugar beet and lily. Collectively, the results of this study showed that the host plant species has a significant influence on the dynamics, composition and activity of specific indigenous antagonistic Pseudomonas spp.

Britschgi, T. B. and R. D. Fallon (1994). "PCR-amplification of mixed 16S rRNA genes from an anaerobic, cyanide-degrading consortium." FEMS Microbiology Ecology **13**(3): 225.

http://www.sciencedirect.com/science/article/B6T2V-476VSWV-

1D/2/b0d418d167cab8713d47ba16ef7886bc

The microorganisms participating in the anaerobic biodegradation of cyanide were characterized using 16S rRNA genes as genetic markers of diversity. Segments of mixed population 16S rRNA genes were amplified using the polymerase chain reaction (PCR) and prokaryote-specific amplification primers. Restriction fragment length polymorphism (RFLPs) and screening with the 926f universal sequencing primer were used to categorized the cloned PCR products. Six unique prokaryote sequence were obtained, including four similar to methanogens and two similar to Gram-positive eubacteria.

Clegg, C. D., J. D. van Elsas, et al. (1994). "Assessment of the role of a terrestrial isopod in the survival of a genetically modified pseudomonad and its detection using the polymerase chain reaction." FEMS Microbiology Ecology **15**(1-2): 161.

http://www.sciencedirect.com/science/article/B6T2V-476YGFF-N/2/cea0637cf21254e1fc736220c5e8fbd2

The effects of a terrestrial isopod, Porcellio scaber, on the survival of a genetically modified pseudomonad were studied. Pseudomonas fluorescens KTG was inoculated onto ash leaf litter and supplied to populations of P. scaber. Plate counts were lower in fresh faeces than the ash leaf litter for P. fluorescens KTG, and higher counts were detected in the faeces for the total bacterial population. When faeces were aged by incubation for up to 7 days at 15-17[deg]C, plate counts for P. fluorescens KTG increased during the first day to a level similar to those in the corresponding ash leaf litter, and remained relatively constant thereafter. The total bacterial population in the faeces continued to increase steadily over the 7 days, whilst remaining at a constant level in the ash leaf litter during the same period. Counts of bacteria in faecal material showed that P. fluorescens KTG was present for 6 days after the isopods had fed on inoculated litter although transit times of food through the gut were as little as 5 h. The implications for GEMMO dispersal of bacterial retention in the gut is considered. The polymerase chain reaction was utilised in the detection of the inserted DNA. Positive amplification of the inserted DNA sequence of P. fluorescens KTG was achieved in ash leaf litter, fresh faeces, and faeces from animal which were supplied uninoculated litter for one day after feeding on the inoculated litter. However, plate counts were more sensitive than the polymerase chain reaction in detecting P. fluorescens KTG in the faeces. Our findings suggest that when the GEMMO is ingested by the woodlouse it can survive within the guts and faeces. This has implications for risk assessment of genetically modified bacteria in terrestrial environments.

Coleman, S. S. and J. D. Oliver (1996). "Optimization of conditions for the polymerase chain reaction amplification of DNA from culturable and nonculturable cells of Vibrio vulnificus." FEMS Microbiology Ecology **19**(2): 127.

http://www.sciencedirect.com/science/article/B6T2V-3VWPBY1-6/2/9bec8bf4bdbf2f3dc04ea4bfe83c265f

A series of 16 buffers, differing in pH and MgCl2 concentration, were used to optimize the polymerase chain reaction (PCR) amplification of a 388 bp region of the hemolysin / cytolysin gene from cells of Vibrio vulnificus present in both the culturable and nonculturable states. Both the opaque and translucent morphotypes were examined. Using whole cell lysates, we were able to obtain amplification of DNA from as few as 28.5 cells present in the viable but nonculturable state. With one exception, all buffers that produced amplification using culturable cells also produced amplification using nonculturable cells. However, regardless of the buffer employed,

100 times more nonculturable cells than culturable cells were required to obtain a PCR product. Our data suggest that caution should be exercised when employing PCR optimized against culturable cells when this method is employed for the detection of nonculturable cells.

Daniell, T. J., R. Husband, et al. (2001). "Molecular diversity of arbuscular mycorrhizal fungi colonising arable crops." FEMS Microbiology Ecology **36**(2-3): 203.

http://www.sciencedirect.com/science/article/B6T2V-43F78DM-G/2/dd0d9899f7692e793f7a5f2f5706ddc2

We used differences in small subunit ribosomal RNA genes to identify groups of arbuscular mycorrhizal fungi that are active in the colonisation of plant roots growing in arable fields around North Yorkshire, UK. Root samples were collected from four arable fields and four crop species, fungal sequences were amplified from individual plants by the polymerase chain reaction using primers NS31 and AM1. The products were cloned and 303 clones were classified by their restriction pattern with HinfI or RsaI; 72 were subsequently sequenced. Colonisation was dominated by Glomus species with a preponderance of only two sequence types, which are closely related. There is evidence for seasonal variation in colonisation in terms of both level of colonisation and sequence types present. Fungal diversity was much lower than that previously reported for a nearby woodland.

de Lipthay, J. R., J. Aamand, et al. (2002). "Expression of tfdA genes in aquatic microbial communities during acclimation to 2,4-dichlorophenoxyacetic acid." FEMS Microbiology Ecology **40**(3): 205.

http://www.sciencedirect.com/science/article/B6T2V-45M0SXK-1/2/dba814ab5a1e5b3fe933bfa2a6cd53c5

The role of gene expression during acclimation of aquatic microbial communities was examined by relating transcription of tfdA to the degradation of the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D). The tfdA gene encodes for a 2,4-D/2-oxoglutarate dioxygenase that transforms 2,4-D to 2,4-dichlorophenol. Transcription of tfdA, the abundance of tfdA genes and 2,4-D degrading populations, and the rate of 2,4-D disappearance were followed in laboratory incubations of two pond water samples that were exposed to 0.11 mM 2,4-D. Both communities responded to 2,4-D exposure by induction of tfdA transcription but the dynamics of transcript abundance and the homology to the tfdA riboprobe suggested different populations of 2,4-D degraders in the two ponds. In one community, where tfdA transcripts were highly homologous to the tfdA gene of Ralstonia eutropha JMP134, transcription of tfdA was transient and dropped while 2,4-D degradation continued. In the other freshwater community, where tfdA genes with a lower similarity to the tfdA gene of strain JMP134 were transcribed, transcript levels remained high although 2,4-D degradation had ceased. Restriction fragment length polymorphism analysis of tfdA amplicons similarly demonstrated the presence of different tfdA loci in the two freshwater communities, and this difference in populations of tfdA genes probably explains the observed difference in dynamics of catabolic gene transcription.

de Lipthay, J. R., K. Johnsen, et al. (2004). "Bacterial diversity and community structure of a sub-surface aquifer exposed to realistic low herbicide concentrations." FEMS Microbiology Ecology **49**(1): 59.

http://www.sciencedirect.com/science/article/B6T2V-4C0HSVD-1/2/2dfe75f02be9bf493adbdb8bcea4ba7d

An increasing number of herbicides are found in our groundwater environments. This underlines the need for examining the effects of herbicide exposure on the indigenous groundwater microbial communities, as microbial degradation is the major process responsible for the complete removal of most contaminants. We examined the effect of in situ exposure to realistic low concentrations of herbicides on the microbial diversity and community structure of sub-surface sediments from a shallow aquifer near Vejen (Denmark). Three different community analyses were performed: colony morphology typing, sole-carbon source utilisation in Biolog(R)EcoPlates, and denaturing gradient gel electrophoresis. Cluster analysis demonstrated that the microbial communities of those aquifer sediments that acclimated to the herbicide exposure also had similar community structure. This observation was concurrent for all three community analyses. In contrast, no significant effect was found on the bacterial diversity, except for the culturable fraction where a significantly increased richness and Shannon index was found in the herbicide acclimated sediments. The results of this study show that in situ exposure of sub-surface aquifers to realistic low concentrations of herbicides may alter the overall structure of a natural bacterial community, although significant effects on the genetic diversity and carbon substrate usage cannot be detected. The observed impact was probably due to indirect effects. In future investigations, the inclusion of methods that specifically detect relevant microbial sub-populations and functional genes is therefore recommended.

de Souza, J. T. and J. M. Raaijmakers (2003). "Polymorphisms within the prnD and pltC genes from pyrrolnitrin and pyoluteorin-producing Pseudomonas and Burkholderia spp." FEMS Microbiology Ecology **43**(1): 21.

http://www.sciencedirect.com/science/article/B6T2V-47787FM-1/2/a6ff0485174057c06d7d6d9cc4642683

Pyrrolnitrin (PRN) and pyoluteorin (PLT) are broad-spectrum antibiotics produced by several strains of Pseudomonas and Burkholderia species. Both antibiotics play an important role in the suppression of multiple plant pathogenic fungi. Primers were developed from conserved sequences and amplified prnD and pltC fragments from 18 Pseudomonas and four Burkholderia spp. of worldwide origin that produce either PRN or PLT or both. Subsequent RFLP (restriction fragment length polymorphisms) analysis of the 438-bp pltC fragment showed no polymorphisms among PLT-producing Pseudomonas strains. Polymorphisms within the 786-bp prnD fragment, however, allowed the assessment of the diversity among PRN-producing Pseudomonas and Burkholderia spp. to a level similar to that obtained by three 10-mer primers in random amplified polymorphic DNA analysis. Phylogenetic analysis of 16S rDNA sequences of strains representative of PRN-producing Pseudomonas and Burkholderia species correlated well with their taxonomic status. Phylogenetic relationships inferred from each of the four prn genes and from the complete sequence of the prn biosynthetic locus were similar to 16S rDNA-based phylogeny for most strains, except for Burkholderia pyrrocinia DSM 10685. Both RFLP analysis and comparison of the prn gene sequences showed that B. pyrrocinia DSM 10685 was more closely related to PRN-producing Pseudomonas strains, suggesting that lateral gene transfer may have occurred. Colony hybridization and PCR with PRN- and PLT-specific probes and primers showed that Pseudomonas and Burkholderia spp. harboring the prnD and pltC gene were not present at detectable levels on roots of wheat grown in five agricultural soils collected in The Netherlands, two of them being naturally suppressive to Gaeumannomyces graminis var. tritici. These results suggest that PRN- and PLT-producing Pseudomonas and Burkholderia sp. do not contribute to the natural suppressiveness found in these Dutch take-all decline soils.

Del Panno, M. T., I. S. Morelli, et al. "Effect of petrochemical sludge concentrations on microbial communities during soil bioremediation." FEMS Microbiology Ecology **In Press, Corrected Proof**
http://www.sciencedirect.com/science/article/B6T2V-4FJD7NT-

1/2/9a79dc01f0c8b88a4a821c4bf67aa536

Qualitative and quantitative changes of microbial communities in soil microcosms during bioremediation were determined throughout one year. The soil was contaminated with 0%, 2.5%, 5%, 10% (wt/wt) of petrochemical sludge containing polynuclear aromatic hydrocarbons. We analyzed the hydrocarbon concentration in the microcosms, the number of cultivable bacteria using CFU and most probable number assays, the community structure using denaturing gradient gel electrophoresis, and the metabolic activity of soil using dehydrogenase activity and substrate-induced respiration assays. After one year of treatment, the chemical analysis suggested that the hydrocarbon elimination process was over. The biological analysis, however, showed that the contaminated microcosms suffered under long-term disturbance. The number of heterotrophic bacteria that increased after sludge addition (up to 108-109 cells ml-1) has not returned to the level of the control soil (2-6 X 107 cells ml-1). The community structure in the contaminated soils differed considerably from that in the control. The substrate-induced respiration of the contaminated soils was significantly lower ([approximate]10-fold) and the dehydrogenase activity was significantly higher (20-40-fold) compared to the control. Changes in the community structure of soils depended on the amount of added sludge. The species, which were predominant in the sludge community, could not be detected in the contaminated soils.

der Gucht, K. V., T. Vandekerckhove, et al. "Characterization of bacterial communities in four freshwater lakes differing in nutrient load and food web structure." FEMS Microbiology Ecology **In Press, Corrected Proof** http://www.sciencedirect.com/science/article/B6T2V-4F4WXPM-1/2/ce7d5e1f6ece0da63e8afe858493fef6

The phylogenetic composition of bacterioplankton communities in the water column of four shallow eutrophic lakes was analyzed by partially sequencing cloned 16S rRNA genes and by PCR-DGGE analysis. The four lakes differed in nutrient load and food web structure: two were in a clearwater state and had dense stands of submerged macrophytes, while two others were in a turbid state characterized by the occurrence of phytoplankton blooms. One turbid and one clearwater lake had very high nutrient levels (total phosphorus > 100 [mu]g/l), while the other lakes were less nutrient rich (total phosphorus Our results show that each lake has its own distinct bacterioplankton community. The samples of lake Blankaart differed substantially from those of the other lakes; this pattern was consistent throughout the year of study. The bacterioplankton community composition in lake Blankaart seems to be less diverse and less stable than in the other three lakes. Clone library results reveal that Actinobacteria strongly dominated the bacterial community in lake Blankaart. The relative abundance of Betaproteobacteria was low, whereas this group was dominant in the other three lakes. Turbid lakes had a higher representation of Cyanobacteria, while clearwater lakes were characterized by more representatives of the Bacteroidetes. Correlating our DGGE data with environmental parameters, using the BIOENV procedure, suggests that differences are partly related to the equilibrium state of the lake.

Duckworth, A. W., W. D. Grant, et al. (1996). "Phylogenetic diversity of soda lake alkaliphiles." FEMS Microbiology Ecology **19**(3): 181.

http://www.sciencedirect.com/science/article/B6T2V-3VXNKDM-5/2/80c4a278b1cfcdc8822111c5cb2d3653

16S rRNA genes from a range of aerobic chemoorganotrophic, alkaliphilic soda lake Bacteria and Archaea have been sequenced and subjected to phylogenetic analysis. Gram-negative alkaliphiles were found to be confined to the [gamma]3 subdivision of the Proteobacteria, with many isolates related to the Halomonas/Deleya group. Gram-positive alkaliphiles were found in

both high % G + C and low % G + C divisions of the Gram-positive lineage, with many isolates being related to the Bacillus group, others to Arthrobacter spp. Alkaliphilic Archaea were relatively closely related to members of the genera Natronococcus and Natronobacterium. An anaerobic, thermophilic isolate has been assigned to a new genus within the Thermotogales.

Egert, M., S. Marhan, et al. (2004). "Molecular profiling of 16S rRNA genes reveals diet-related differences of microbial communities in soil, gut, and casts of Lumbricus terrestris L. (Oligochaeta: Lumbricidae)." FEMS Microbiology Ecology **48**(2): 187.

http://www.sciencedirect.com/science/article/B6T2V-4BS08RG-1/2/39ffb14322f1df78bd65f07536fbd58f

Earthworms are important members of the soil macrofauna. They modify soil physical properties, soil organic matter decomposition, and thus regulate carbon and nitrogen cycling in soil. However, their interactions with soil microorganisms are still poorly understood, in particular the effect of gut passage on the community structure of ingested microorganisms. Moreover, it is still unsolved, if earthworms, like many other soil-feeding invertebrates, possess an indigenous gut microbial community. Therefore, we investigated the bacterial and archaeal community structure in soil (with and without additional beech litter), gut, and fresh casts of Lumbricus terrestris, an anecic litter-feeding earthworm, by means of terminal-restriction fragment length polymorphism (T-RFLP) analysis of 16S rRNA gene fragments. Ecological indices of community diversity and similarity, calculated from the T-RFLP profiles, revealed only small differences between the bacterial and archaeal communities in soil, gut, and fresh casts under both feeding conditions, especially in comparison to other soil-feeding invertebrates. However, multivariate statistical analysis combining multidimensional scaling and discriminant function analysis proved that these differences were highly significant, in particular when the earthworms were fed beech litter in addition. Because there were no dominant gut-specific OTUs detectable, the existence of an abundant indigenous earthworm microbial community appears unlikely, at least in the midgut region of L. terrestris.

Engel, A. S., M. L. Porter, et al. (2004). "Bacterial diversity and ecosystem function of filamentous microbial mats from aphotic (cave) sulfidic springs dominated by chemolithoautotrophic "Epsilonproteobacteria"." FEMS Microbiology Ecology **51**(1): 31.

http://www.sciencedirect.com/science/article/B6T2V-4D1V363-1/2/35fb7ba3eae4bf63333d7217ae039979

Filamentous microbial mats from three aphotic sulfidic springs in Lower Kane Cave, Wyoming, were assessed with regard to bacterial diversity, community structure, and ecosystem function using a 16S rDNA-based phylogenetic approach combined with elemental content and stable carbon isotope ratio analyses. The most prevalent mat morphotype consisted of white filament bundles, with low C:N ratios (3.5-5.4) and high sulfur content (16.1-51.2%). White filament bundles and two other mat morphotypes had organic carbon isotope values (mean [delta]13C = -34.7[per mille sign], 1[sigma] = 3.6) consistent with chemolithoautotrophic carbon fixation from a dissolved inorganic carbon reservoir (cave water, mean [delta]13C = -7.4[per mille sign] for two springs, n = 8). Bacterial diversity was low overall in the clone libraries, and the most abundant taxonomic group was affiliated with the "Epsilonproteobacteria" (68%), with other bacterial sequences affiliated with Gammaproteobacteria (12.2%), Betaproteobacteria (11.7%), Deltaproteobacteria (0.8%), and the Acidobacterium (5.6%) and Bacteriodetes/Chlorobi (1.7%) divisions. Six distinct epsilonproteobacterial taxonomic groups were identified from the microbial mats. Epsilonproteobacterial and bacterial group abundances and community structure shifted from the spring orifices downstream, corresponding to changes in dissolved sulfide and oxygen

concentrations and metabolic requirements of certain bacterial groups. Most of the clone sequences for epsilonproteobacterial groups were retrieved from areas with high sulfide and low oxygen concentrations, whereas Thiothrix spp. and Thiobacillus spp. had higher retrieved clone abundances where conditions of low sulfide and high oxygen concentrations were measured. Genetic and metabolic diversity among the "Epsilonproteobacteria" maximizes overall cave ecosystem function, and these organisms play a significant role in providing chemolithoautotrophic energy to the otherwise nutrient-poor cave habitat. Our results demonstrate that sulfur cycling supports subsurface ecosystems through chemolithoautotrophy and expand the evolutionary and ecological views of "Epsilonproteobacteria" in terrestrial habitats.

Etchebehere, C., I. Errazquin, et al. (2001). "Evaluation of the denitrifying microbiota of anoxic reactors." FEMS Microbiology Ecology **35**(3): 259.

http://www.sciencedirect.com/science/article/B6T2V-42SPNHY-5/2/6f0005c26b4ecdd7049b303c5793c76c

Removal of inorganic nitrogen compounds from wastewaters can be accomplished by a combination of the biological processes of nitrification and denitrification. The information on the microbiota present in denitrifying reactors is still scarce. In the present work the evaluation of the denitrifying microbiota of different reactor sludges was performed by specific activity measurements and MPN count of denitrifiers. We also present the isolation and physiological and phylogenetic characterisation of denitrifying bacteria from the anoxic reactor of a combined system treating landfill leachate. Specific denitrifying activity measurements were faster to perform and more reliable than MPN enumerations. 16S rDNA characterisation of the isolates showed that they belonged to the genera Thauera, Acidovorax and Alcaligenes and were closely related to microorganisms retrieved from ecosystems rich in recalcitrant compounds. Two of the isolates could grow on aromatic compounds as sole carbon source.

Etchebehere, C., M. I. Errazquin, et al. (2002). "Community analysis of a denitrifying reactor treating landfill leachate." FEMS Microbiology Ecology **40**(2): 97.

http://www.sciencedirect.com/science/article/B6T2V-45J9482-3/2/2ee742b54e357443880897bd9dc95815

The bacterial community of a denitrifying reactor from a system for landfill leachate decontamination was studied applying cultivation methods, denitrifying activity measurements and characterisation of community 16S rDNA. The sludge presented a high denitrifying activity but a relatively low number of denitrifying bacteria as determined by most probable number. Over 50% of the sequences retrieved in the molecular analysis were related to genera with the capacity to denitrify in the [alpha]- and [beta]-subdivisions of the Proteobacteria. Fifteen percent of the DNAs were related to not yet cultured organisms belonging to the green non-sulphur phylum. High similarity values between sequences from isolates and clones were observed.

Harder, T., S. C. K. Lau, et al. (2003). "A distinctive epibiotic bacterial community on the soft coral Dendronephthya sp. and antibacterial activity of coral tissue extracts suggest a chemical mechanism against bacterial epibiosis." FEMS Microbiology Ecology **43**(3): 337.

http://www.sciencedirect.com/science/article/B6T2V-47787FM-

2/2/ff12e1a81659781481ea4059bce66883

Different bacterial community profiles were observed on the soft coral Dendronephthya sp. and an inanimate reference site using terminal restriction fragment length polymorphism analysis of bacterial community DNA. To correlate the observation with a chemical defense mechanism against bacterial epibiosis, antibacterial effects of coral tissue extracts and waterborne products of coral-associated bacterial isolates (11 morphotypes) were tested against indigenous benthic bacterial isolates (33 morphotypes) obtained in the vicinity of the coral colonies. The coral tissue extracts and waterborne products of coral-associated bacteria inhibited growth and attachment of indigenous bacterial isolates, suggesting an endogenous chemical and an exogenous biological mechanism against bacterial epibiosis in this soft coral.

Henckel, T., U. Jackel, et al. (2001). "Vertical distribution of the methanotrophic community after drainage of rice field soil." FEMS Microbiology Ecology **34**(3): 279.

http://www.sciencedirect.com/science/article/B6T2V-41Y1YF9-C/2/e8a8fecb07bc4219f9e01c1d635e4be8

Anoxic soils, such as flooded rice fields, are major sources of the greenhouse gas CH4 while oxic upland soils are major sinks of atmospheric CH4. Nevertheless, CH4 is also consumed in rice fields where up to 90% of the produced CH4 is oxidized in a narrow oxic zone around the rice roots and in the soil surface layer before it escapes into the atmosphere. After 1 day drainage of rice field soil, CH4 oxidation was detected in the top 2-mm soil layers, but after 8 days drainage the zone of CH4 oxidation extended to 8 mm depth. Simultaneously, the potential for CH4 production decreased, but some production was still detectable after 8 days drainage throughout the soil profile. The vertical distribution of the methanotrophic community was also monitored after 1 and 8 days drainage using denaturing gradient gel electrophoresis after PCR amplification with primer sets targeting two regions on the 16S rRNA gene that are relatively specific for methylotrophic [alpha]- and [gamma]-Proteobacteria, and targeting two functional genes encoding subunits of key enzymes in all methanotrophs, i.e. the genes for the particulate methane monooxygenase (pmoA) and the methanol dehydrogenase (mxaF). Drainage stimulated the methanotrophic community. Eight days after drainage, new methanotrophic populations appeared and a distinct methanotrophic community developed. The population structure of type I and II methanotrophs was differently affected by drainage. Type II methanotrophs ([alpha]-Proteobacteria) were present throughout the soil core directly after drainage (1 day), and the community composition remained largely unchanged with depth. Only two new type II populations appeared after 8 days of drainage. Drainage had a more pronounced impact on the type I methanotrophic community ([gamma]-Proteobacteria). Type I populations were not or only weakly detected 1 day after drainage. However, after 8 days of drainage, a large diversity of type I methanotrophs were detected, altough they were not evenly distributed throughout the soil core but dominated at different depths. A distinct type I community structure had developed within each soil section between 0 and 20 mm soil depth, indicating the widening of suitable habitats for methanotrophs in the rice field soil within 1 week of drainage.

Heuer, H., E. Krogerrecklenfort, et al. (2002). "Gentamicin resistance genes in environmental bacteria: prevalence and transfer." FEMS Microbiology Ecology **42**(2): 289.

http://www.sciencedirect.com/science/article/B6T2V-46MC7G7-3/2/34e0815a463326331cecee2985eccca9

A comprehensive multiphasic survey of the prevalence and transfer of gentamicin resistance

(Gmr) genes in different non-clinical environments has been performed. We were interested to find out whether Gmr genes described from clinical isolates can be detected in different environmental habitats and whether hot spots can be identified. Furthermore, this study aimed to evaluate the impact of selective pressure on the abundance and mobility of resistance genes. The study included samples from soils, rhizospheres, piggery manure, faeces from cattle, laying and broiler chickens, municipal and hospital sewage water, and coastal water. Six clusters of genes coding for Gm-modifying enzymes (aac(3)-I, aac(3)-II/VI, aac(3)-III/IV, aac(6')-II/Ib, ant(2'')-I, aph(2'')-I) were identified based on a database comparison and primer systems for each gene cluster were developed. Gm-resistant bacteria isolated from the different environments had a different taxonomic composition. In only 34 of 207 isolates, mainly originating from sewage, faeces and coastal water polluted with wastewater, were known Gmr genes corresponding to five of the six clusters detected. The strains belonged to genera in which the genes had previously been detected (Enterobacteriaceae, Pseudomonas, Acinetobacter) but also to phylogenetically distant bacteria, such as members of the CFB group, [alpha]- and [beta]-Proteobacteria. Gmr genes located on mobile genetic elements (MGE) could be captured in exogenous isolations into recipients belonging to [alpha]-, [beta]- and [gamma]-Proteobacteria from all environments except for soil. A high proportion of the MGE, conferring Gm resistance isolated from sewage, were identified as IncP[beta] plasmids. Molecular detection of Gmr genes, and broad host range plasmid-specific sequences (IncP-1, IncN, IncW and IncQ) in environmental DNA indicated a habitat-specific dissemination. A high abundance and diversity of Gmr genes could be shown for samples from faeces (broilers, layers, cattle), from sewage, from seawater, collected close to a wastewater outflow, and from piggery manure. In the latter samples all six clusters of Gmr genes could be detected. The different kinds of selective pressure studied here seemed to enhance the abundance of MGE, while an effect on Gmr genes was not obvious.

Husband, R., E. A. Herre, et al. (2002). "Temporal variation in the arbuscular mycorrhizal communities colonising seedlings in a tropical forest." FEMS Microbiology Ecology **42**(1): 131.

http://www.sciencedirect.com/science/article/B6T2V-46HNNV2-1/2/b354537226d3452859e7c0216373d07f

In order to investigate temporal variation in the arbuscular mycorrhizal (AM) fungal community in a tropical forest in the Republic of Panama, seedlings of the canopy emergent Tetragastris panamensis were sampled three times over a period of 3 years. We used AM-specific primers to amplify and clone partial small subunit ribosomal RNA gene sequences. Over 550 clones were classified into 18 AM fungal types. As the seedlings matured, the fungal diversity decreased and there was a significant shift so that previously rare types replaced formerly dominant fungal types. Further, seedlings of different ages sampled at the same time point were colonised by significantly different fungal populations. Our results indicate that both time and host age may influence the mycorrhizal population.

Ibekwe, A. M., A. C. Kennedy, et al. (2002). "Microbial diversity along a transect of agronomic zones." FEMS Microbiology Ecology **39**(3): 183.

http://www.sciencedirect.com/science/article/B6T2V-44XTRYR-1/2/d7ce8197e745bed85b8aa70643a978fd

The diversity of microbial communities constitutes a critical component of good soil-management practices. To characterize the effects of different management practices, molecular indicators such as phospholipid fatty acid (PLFA), denaturing gradient gel electrophoresis (DGGE) and composition of ammonia-oxidizing bacteria were used to analyze bacterial community structure and diversity from four eastern Washington State soils. Samples from four sites were collected

representing a transect of high-precipitation to low-precipitation areas that covered different agronomic zones with different management and cropping practices. Biomass amounts estimated from extractable PLFA were significantly higher in the no-till (NT) soil than in the conventional-till (CT) soil. Similarities among the different 16S rDNA DGGE band profiles were analyzed quantitatively using correspondence analysis and this confirmed that the CT soil was the most dissimilar soil. DGGE analysis of 16S rDNA ammonia-oxidizing bacteria from the four soils revealed two identical bands, indicating little effect of agronomic practices and precipitation on these species. A second set of primers, specific for amoA (ammonia monooxygenase) genes, was used to examine ammonia oxidizers in the samples. Six banding patterns (clusters) from amplified rDNA restriction analysis of 16S rDNA fragments were observed after restriction analysis with HinfI. Sequencing of these clones revealed the presence of only Nitrosospira-like sequences. Analysis of the sequences showed that ammonia oxidizers from the NT soil were more diverse compared to those from the CT and conservation reserve program soils. Our data showed that management and agronomic practices had more impact on bacterial community structure than annual precipitation.

Iwamoto, T., K. Tani, et al. (2000). "Monitoring impact of in situ biostimulation treatment on groundwater bacterial community by DGGE." FEMS Microbiology Ecology **32**(2): 129.

http://www.sciencedirect.com/science/article/B6T2V-408C8X7-6/2/025a565f2f05dc59cd0d165984aa0b83

Changes in bacterial diversity during the field experiment on biostimulation were monitored by denaturing gradient gel electrophoresis (DGGE) analysis of PCR-amplified 16S rDNA fragments. The results revealed that the bacterial community was disturbed after the start of treatment, continued to change for 45 days or 60 days and then formed a relatively stable community different from the original community structure. DGGE analysis of soluble methane monooxygenase (sMMO) hydroxylase gene fragments, mmoX, was performed to monitor the shifts in the numerically dominant sMMO-containing methanotrophs during the field experiment. Sequence analysis on the mmoX gene fragments from the DGGE bands implied that the biostimulation treatment caused a shift of potential dominant sMMO-containing methanotrophs from type I methanotrophs to type II methanotrophs.

Kato, S., S. Haruta, et al. (2004). "Effective cellulose degradation by a mixed-culture system composed of a cellulolytic Clostridium and aerobic non-cellulolytic bacteria." FEMS Microbiology Ecology **51**(1): 133.

http://www.sciencedirect.com/science/article/B6T2V-4D4T73W-3/2/86c8792fd966a7ee3ce4e7a6488a0e17

A stable cellulose-degrading microflora enriched from composting materials has been analyzed in our laboratory. Cellulose-degrading efficiency of an anaerobic cellulolytic isolate, Clostridium straminisolvens CSK1, was remarkably lower than that of the original microflora. We successfully constructed bacterial communities with effective cellulose degradation by mixing C. straminisolvens CSK1 with aerobic non-cellulolytic bacteria isolated from the original microflora. Comparison of the cellulose degradation processes of the pure culture of C. straminisolvens CSK1 and the mixed-culture indicated that non-cellulolytic bacteria essentially contribute to cellulose degradation by supplying anaerobic environment, consuming metabolites, which otherwise deteriorate the cellulolytic activity, and by neutralizing pH.

La Scola, B., L. Barrassi, et al. (2000). "Isolation of new fastidious [alpha] Proteobacteria and Afipia felis from hospital water supplies by direct plating and amoebal co-culture procedures." FEMS Microbiology Ecology **34**(2): 129.

http://www.sciencedirect.com/science/article/B6T2V-41SKB53-5/2/7901f9b82ed6a16e004c79f305b4611e

As water is a source of nosocomial infections in hospitals, the presence of fastidious Gram-negative bacteria in water samples taken in a university hospital was investigated. Water samples were inoculated onto agar plates and into amoebal microplates for co-culture. Sixty-eight [alpha] proteobacteria isolates were obtained and characterized using phenotypic methods and 16S rRNA gene sequence comparison. The latter approach divided the strains into seven clusters. Of these, one corresponded to previously recognized Afipia felis and it is likely that six were closely related new species. As these bacteria are fastidious and can not be cultivated on standard microbiological media, their possible role in hospital-acquired human infections should be investigated.

Langenheder, S., V. Kisand, et al. (2003). "Salinity as a structuring factor for the composition and performance of bacterioplankton degrading riverine DOC." FEMS Microbiology Ecology **45**(2): 189.

http://www.sciencedirect.com/science/article/B6T2V-48V7K2H-1/2/e242615ffb96b67d776f906ee90a1dca

The impact of salinity on the composition and functional performance (biomass production, growth efficiency and growth rates) of bacterial communities was investigated using batch cultures growing on dissolved organic carbon from a river draining into the Northern Baltic Sea. The cultures were adjusted to riverine or estuarine salinity levels and inoculated with bacteria from these two environments. Bacterial growth efficiencies differed in response to salinity and the origin of the inoculum. When salinity was adjusted to correspond to the salinity at the site where the inoculum was retrieved, growth efficiency was relatively high (11.5+/-2.6%). However, when bacteria were confronted with a shift in salinity, growth efficiency was lower (7.5+/-2.0%) and more of the utilized carbon was respired. In contrast, growth rates were higher when bacteria were exposed to a change in salinity. The composition of the bacterial communities developing in the batch cultures differed, as shown by 16S rDNA DGGE, depending on the origin of the inoculum and salinity. Reverse and direct DNA-DNA hybridization revealed salinity optima in the growth of specific bacterial strains as well as broader phylogenetic groups. Strains belonging to the [alpha]- and [beta]-Proteobacteria, Actinobacteria and [gamma]-Proteobacteria other than the genus Pseudomonas showed higher relative abundance under freshwater conditions, whereas strains of the genus Pseudomonas and the Cytophaga-Flavobacterium-Bacteroides group were favored by estuarine conditions. Generally, our results demonstrate functional changes associated with changes in community composition. We suggest that even moderate changes in salinity affect bacterial community composition, which subsequently leads to altered growth characteristics.

Laranjo, M., J. Machado, et al. (2004). "High diversity of chickpea Mesorhizobium species isolated in a Portuguese agricultural region." FEMS Microbiology Ecology **48**(1): 101.

http://www.sciencedirect.com/science/article/B6T2V-4BP9P53-1/2/d38b09189545d4b65e47b8a2686d70d2

Chickpea rhizobia isolated from Portuguese soils were assigned to the genus Mesorhizobium by 16S-rDNA sequencing. High species diversity was found within populations of an agricultural region in the south of Portugal. Besides the expected Mesorhizobium ciceri and M. mediterraneum, some isolates were close to M. loti or M. tianshanense and some formed a clade that may represent a new species. A new PCR-based approach, named direct amplified polymorphic DNA (DAPD) analysis, supported the 16S-based phylogeny. This suggests that this method could be used as a molecular tool to assess genetic relationships. Evaluation of genetic diversity by 16S-rDNA sequence, DAPD and protein profiles showed different levels of heterogeneity in natural populations.

Leclerc, M., C. Delbes, et al. (2001). "Single strand conformation polymorphism monitoring of 16S rDNA Archaea during start-up of an anaerobic digester." FEMS Microbiology Ecology **34**(3): 213.

http://www.sciencedirect.com/science/article/B6T2V-41Y1YF9-5/2/995a7a4acce6a7fe03afa034ad648633

A laboratory-scale continuously mixed anaerobic digester was inoculated with a mix of anaerobic sludge and fed with glucose. The start-up strategy was progressive and chemical analyses were done to evaluate digester performance from day 1 to day 107. In parallel, Archaeal community dynamics were monitored by SSCP analysis of the V3 region of 16S rDNA genes and further characterized by partial sequencing of 16S rDNA genes. At day 1 the inoculum contained at least five distinct Archaeal peaks close to known methanogenic species. The dominant peak was very close to Methanosaeta concilli, the remaining species being members of the Methanobacteriales and Methanomicrobiales. A rapid shift of the Archaeal population was observed during the experiment. At day 21 Methanobacterium formicicum, which was not detected at day 1, became the dominant methanogenic species in the bioreactor and remained so until the end of the experiment.

Lin, M. and J. R. Schwarz (2003). "Seasonal shifts in population structure of Vibrio vulnificus in an estuarine environment as revealed by partial 16S ribosomal DNA sequencing." FEMS Microbiology Ecology **45**(1): 23.

http://www.sciencedirect.com/science/article/B6T2V-48CNDJS-1/2/b3ebfaf79fcb020de98f46d2ac92f59d

The partial sequence (600 bp) containing the most variable region of Vibrio vulnificus 16S ribosomal DNA (rDNA) was determined for 208 randomly selected V. vulnificus strains isolated from Galveston Bay, TX, USA between June 2000 and June 2001. A comparative analysis of the determined partial 16S rDNA sequences revealed the existence of two different partial 16S rDNA sequences (type A and type B, 1.3% base substitutions) among the 208 V. vulnificus isolates. A higher proportion of 16S rDNA type A strains was isolated in June and July while a considerably higher proportion of type B strains was isolated in September. In addition, after no V. vulnificus strains were detected during the winter months (December-February), only type A strains were isolated during the following months (March-May). The results suggest that the relative abundance of type A and type B V. vulnificus strains in Galveston Bay varies with the season and that the differences between the two 16S rDNA types may affect the viability of these organisms in the natural environment.

Lindstrom, E. S. (1998). "Bacterioplankton community composition in a boreal forest lake." FEMS

Microbiology Ecology **27**(2): 163.

http://www.sciencedirect.com/science/article/B6T2V-3TW4NYJ-7/2/753aadd464d3634aba2c0bbc4556f168

The composition of the dominating populations within a bacterioplankton community was investigated in a mesotrophic, boreal forest lake. Composite samples were collected monthly throughout the lake for two years. The community composition was determined by denaturing gradient gel electrophoresis (DGGE) of a polymerase chain reaction (PCR)-amplified part of 16S rDNA, extracted from organisms smaller than 1 [mu]m. Temporal patterns of occurrence in the lake differed among populations. There was no clear seasonal pattern of variation, but there was a gradual change. The results suggest that variation in the amount of water flowing into the lake could explain some of the changes in the bacterioplankton community.

Lowe, M., E. L. Madsen, et al. (2002). "Geochemistry and microbial diversity of a trichloroethene-contaminated Superfund site undergoing intrinsic in situ reductive dechlorination." FEMS Microbiology Ecology **40**(2): 123.

http://www.sciencedirect.com/science/article/B6T2V-45M0SXK-2/2/4b65e490bdb24fe4c1e7563ac8ad1314

This study explored the geochemistry and microbial diversity of a Superfund site containing trichloroethene (TCE) and an unusual co-pollutant, tetrakis(2-ethylbutoxy)silane. Geochemical analysis of contaminated groundwater indicated subsurface anaerobiosis, reductive dechlorination of TCE to predominantly cis-1,2-dichloroethene, and (transient) accumulation of 2-ethylbutanol and 2-ethylbutyrate as a result of tetrakis(2-ethylbutoxy)silane breakdown. Comparative analysis of 106 16S rDNA and 61 16S-23S rDNA intergenic spacer region sequences - obtained from pristine and contaminated groundwater via DNA extraction, PCR amplification, cloning and sequencing - revealed that the contaminated groundwater featured (i) a distinct microbial community, (ii) reduced species diversity, (iii) various anaerobes, and (iv) bacteria closely related to the TCE-dechlorinating, dichloroethene-accumulating genus Dehalobacter, whereas (v) the TCE-dechlorinating, ethene-producing species Dehalococcoides ethenogenes was not detectable. Thus, geochemical and molecular biological results were in excellent agreement in this first ecological field study linking in situ reductive dechlorination of TCE to metabolism of tetraalkoxysilanes.

Lukow, T., P. F. Dunfield, et al. (2000). "Use of the T-RFLP technique to assess spatial and temporal changes in the bacterial community structure within an agricultural soil planted with transgenic and non-transgenic potato plants." FEMS Microbiology Ecology **32**(3): 241.

http://www.sciencedirect.com/science/article/B6T2V-40H098K-7/2/59c6019ffbe26cdab199579b2c64c926

The aim of this study was to examine whether the terminal restriction fragment length polymorphism (T-RFLP) analysis represents an appropriate technique for monitoring highly diverse soil bacterial communities, i.e. to assess spatial and/or temporal effects on bacterial community structure. The T-RFLP method, a recently described fingerprinting technique, is based on terminal restriction fragment length polymorphisms between distinct small-subunit rRNA gene sequence types. This technique permits an automated quantification of the fluorescence signal intensities of the individual terminal restriction fragments (T-RFs) in a given community fingerprint pattern. The indigenous bacterial communities of three soil plots located within an agricultural

field of 110 m2 were compared. The first site was planted with non-transgenic potato plants, while the other two were planted with transgenic GUS and Barnase/Barstar potato plants, respectively. Once prior to planting and three times after planting, seven parallel samples were taken from each of the three soil plots. The T-RFLP analysis resulted in very complex but highly reproducible community fingerprint patterns. The percentage abundance values of defined T-RFs were calculated for the seven parallel samples of the respective soil plot. A multivariate analysis of variance was used to test T-RFLP data sets for significant differences. The statistical treatments clearly revealed spatial and temporal effects, as well as space x time interaction effects, on the structural composition of the bacterial communities. T-RFs which showed the highest correlations to the discriminant factors were not those T-RFs which showed the largest single variations between the seven-sample means of individual plots. In summary, the T-RFLP technique, although a polymerase chain reaction-based method, proved to be a suitable technique for monitoring highly diverse soil microbial communities for changes over space and/or time.

Lumini, E., M. Bosco, et al. (1996). "PCR-RFLP and total DNA homology revealed three related genomic species among broad-host-range Frankia strains." FEMS Microbiology Ecology **21**(4): 303.

http://www.sciencedirect.com/science/article/B6T2V-3W2XP98-7/2/00c68742aa06e2ac8abe3fdd47da030a

Restriction fragment length polymorphism (RFLP) analysis of the PCR amplified nifD-K intergenic spacer (IGS) region was used to cluster 22 Frankia strains of the Elaeagnus host specificity group into seven genomic groups and to measure the degree of genetic similarity among them. This PCR-RFLP analysis could assign freshly isolated strains to described genomic species and revealed genomic groups not yet described among Frankia strains of the Elaeagnus specificity group. Six broad-host-range Frankia strains, infective on both Alnus and Elaeagnus, fell into three closely related PCR-RFLP clusters. DNA-DNA hybridization was then used to establish the correlations between PCR-RFLP clusters and total DNA relatedness groups. The three PCR-RFLP clusters agreed with two new and one reference genomic species, indicating that Frankia ability to nodulate with Alnus and Elaeagnus is a monophyletic trait shared by three genomic species.

Newby, D. T., D. W. Reed, et al. (2004). "Diversity of methanotroph communities in a basalt aquifer." FEMS Microbiology Ecology **48**(3): 333.

http://www.sciencedirect.com/science/article/B6T2V-4BTY90X-1/2/17aa73a9b68f1991190283149eb95509

Methanotrophic bacteria play an important role in global cycling of carbon and co-metabolism of contaminants. Methanotrophs from pristine regions of the Snake River Plain Aquifer (SRPA; Idaho, USA) were studied in order to gain insight into the native groundwater communities' genetic potential to carry out TCE co-metabolism. Wells were selected that were proximal to a TCE plume believed to be undergoing natural attenuation. Methane concentrations ranged from 1 to >1000 nM. Carbon isotope ratios and diversity data together suggest that the SRPA contains active communities of methanotrophs that oxidize microbially produced methane. Microorganisms removed from groundwater by filtration were used as inocula for enrichments or frozen immediately and DNA was subsequently extracted for molecular characterization. Primers that specifically target methanotroph 16S rRNA genes or genes that code for subunits of soluble or particulate methane monooxygenase, mmoX and pmoA, respectively, were used to characterize the indigenous methanotrophs via PCR, cloning, RFLP analysis, and sequencing. Type I methanotroph clones aligned with Methylomonas, Methylocaldum, and Methylobacter sequences and a distinct 16S rRNA phylogenetic lineage grouped near Methylobacter. The majority of clone

sequences in type II methanotroph 16S rRNA, pmoA, and mmoX gene libraries grouped closely with sequences in the Methylocystis genus. A subset of the type II methanotroph clones from the aquifer had sequences that aligned most closely to Methylosinus trichosporium OB3b and Methylocystis spp., known TCE-co-metabolizing methanotrophs.

Palla, F., C. Federico, et al. (2002). "Identification of Nocardia restricta in biodegraded sandstone monuments by PCR and nested-PCR DNA amplification." FEMS Microbiology Ecology **39**(1): 85.

http://www.sciencedirect.com/science/article/B6T2V-44PVR7P-2/2/6c50cf9ca0079941e1cd7a7445520d62

We report the presence of Actinomycetes in degraded sandstone monuments, and on examination of 173 samples we identified Nocardia restricta as particularly prevalent. In our procedure, the extracted bacterial DNA was the template in polymerase chain reaction (PCR) experiments in order to amplify specific regions of the 16S rDNA. The fidelity of amplified fragment was confirmed by nested-PCR or restriction enzyme specific cutting. To confirm the specificity of the assay, the amplified fragments were cloned in a convenient plasmid vector, the sequence analysed and compared with the expected DNA genomic portion.

Plant, L., C. Lam, et al. (2003). "Gastrointestinal microbial community shifts observed following oral administration of a Lactobacillus fermentum strain to mice." FEMS Microbiology Ecology **43**(2): 133.

http://www.sciencedirect.com/science/article/B6T2V-46SP0DB-1/2/fc3b80421992361b7a44c107b84c0bd6

The indigenous gastrointestinal microbiota acts as an integral defense against the colonisation of orally introduced microbes. Whilst this can be important in host protection, some introduced species, including lactobacilli, can have a positive impact on existing microbial communities. The interaction of a candidate probiotic strain of Lactobacillus fermentum within the gastrointestinal tract was monitored in a mouse model and its effect on the indigenous microbiota observed. L. fermentum KLD was administered via oro-gastric doses to mice with both a specific pathogen-free (SPF) and an ampicillin-depleted gut microbiota, containing no detectable lactobacilli. Its persistence was monitored by detection in faecal homogenates using culturing methods and polymerase chain reaction with L. fermentum specific primers. Microbial population shifts were observed using denaturing gradient gel electrophoresis (DGGE). L. fermentum KLD was detected within the gastrointestinal tract of SPF mice for up to 36 h, and for greater than 11 days in the ampicillin-treated mice. The administration resulted in substantial changes within the host Lactobacillus levels, determined by DGGE of 16S rDNA from faecal samples. Denaturing gradient profiles, from faecal samples collected at a range of pre- and post-dose intervals of groups of 10 SPF mice, indicated that several other constituents of the gastrointestinal community also fluctuated following dosing. These included Bifidobacterium and Eubacterium, which increased following KLD administration. The indigenous microbiota affected the persistence of L. fermentum KLD and in SPF mice the administration of this strain induced significant shifts in the indigenous microbial community.

Ramakrishnan, B., T. Lueders, et al. (2000). "Effect of soil aggregate size on methanogenesis and archaeal community structure in anoxic rice field soil." FEMS Microbiology Ecology **32**(3): 261.

http://www.sciencedirect.com/science/article/B6T2V-40H098K-9/2/9a513c178f55704eefbcc3b8767b2012

In anoxically incubated slurries of Italian rice field soil, CH4 production is initiated after a lag phase during which ferric iron and sulfate are reduced. The production of CH4 was affected by the size of soil aggregates used for the preparation of the soil slurry. Rates of CH4 production were lowest with small aggregates (4 accumulated were positively correlated to the concentrations of acetate, propionate and caproate that transiently accumulated in the slurries prepared from different aggregate sizes and also to the organic carbon content. The addition of organic debris that was collected from large-size aggregates to the aggregate size fractions 4 production to amounts that were comparable to those measured in unamended aggregates of 200-2000 [mu]m size, indicating that CH4 production in the different aggregate size fractions was limited by substrate. The distribution of archaeal small-subunit rRNA genes in the different soil aggregate fractions was analyzed by terminal restriction fragment length polymorphism which allowed seven different archaeal ribotypes to be distinguished. Ribotype-182 (consisting of members of the Methanosarcinaceae and rice cluster VI), ribotype-389 (rice cluster I and II) and ribotype-820 (undigested DNA, rice cluster IV and members of the Methanosarcinaceae) accounted for >20, >30 and >10% of the total, respectively. The other ribotypes accounted for <10% of the total. The relative quantity of the individual ribotypes changed only slightly with incubation time and was almost the same among the different soil aggregate fractions. Ribotype-389, for example, slightly decreased with time, whereas ribotype-182 slightly increased. At the end of incubation, the relative quantity of ribotype-182 seemed to be slightly higher in soil fractions with larger than with smaller aggregates, whereas it was the opposite with ribotype-80 (Methanomicrobiaceae) and ribotype-88 (Methanobacteriaceae). Ribotype-280 (Methanosaetaceae and rice cluster V), ribotype-375 (rice cluster III), ribotype-389 and ribotype-820, on the other hand, were not much different among the different soil aggregate size fractions. However, the differences were not significant relative to the errors encountered during the extraction of polymerase chain reaction (PCR)-amplifiable DNA from soil. In conclusion, soil aggregate size and incubation time showed a strong effect on the function but only a small effect on the structure of the methanogenic microbial community.

Redfield, E., S. M. Barns, et al. (2002). "Comparative diversity and composition of cyanobacteria in three predominant soil crusts of the Colorado Plateau." FEMS Microbiology Ecology **40**(1): 55.

http://www.sciencedirect.com/science/article/B6T2V-45936K2-1/2/fa7fe153c8c51016dcc0b9f0d0f1d532

Terminal restriction fragment length polymorphism (TRF or T-RFLP) analysis and 16S rDNA sequence analysis from clone libraries were used to examine cyanobacterial diversity in three types of predominant soil crusts in an arid grassland. Total DNA was extracted from cyanobacteria-, lichen-, or moss-dominated crusts that represent different successional stages in crust development, and which contribute different amounts of carbon and nitrogen into the ecosystem. Cyanobacterial 16S rRNA genes were amplified by PCR using cyanobacteria-specific 16S rDNA primers. Both TRF and clone sequence analyses indicated that the cyanobacterial crust type is dominated by strains of Microcoleus vaginatus, but also contains other cyanobacterial genera. In the moss crust, M. vaginatus-related sequences were also the most abundant types, together with sequences from moss chloroplasts. In contrast, sequences obtained from the lichen crust were surprisingly diverse, representing numerous genera, but including only two from M. vaginatus relatives. By obtaining clone sequence information, we were able to infer the composition of many peaks observed in TRF profiles, and all peaks predicted for clone sequences were observed in TRF analysis. This study provides the first TRF analysis of biological soil crusts and the first DNA-based comparison of cyanobacterial diversity between lichen-, cyano- and moss-dominated crusts. Results indicate that for this phylogenetic group, TRF analysis, in conjunction with limited sequence analysis, can provide accurate information about

the composition and relative abundance of cyanobacterial types in soil crust communities.

Rosado, A. S., L. Seldin, et al. (1996). "Quantitative 16S rDNA-targeted polymerase chain reaction and oligonucleotide hybridization for the detection of Paenibacillus azotofixans in soil and the wheat rhizosphere." FEMS Microbiology Ecology **19**(3): 153.

http://www.sciencedirect.com/science/article/B6T2V-3VXNKDM-2/2/eb5dd2406e4e7ceeb9a6ddee0e586705

A molecular method for the detection of Paenibacillus azotofixans in soil and the wheat rhizosphere was developed. The system consisted of polymerase chain reaction (PCR) amplification of part of the variable V1 to V4 regions of the 16S ribosomal RNA gene, followed by hybridization with a specific oligonucleotide probe homologous to part of the intervening region. In vitro specificity tests showed that the detection system worked specifically for P. azotofixans strains, and did not detect other Paenibacillus species or species of other bacterial genera. Vegetative cells of a rifampicin resistant P. azotofixans derivative were trackable in Flevo silt loam (FSL) soil in 24 h experiments using both selective plating and most probable number (MPN)-PCR combined with probing, and plate counts parallelled MPN-PCR estimations of numbers of specific targets. MPN-PCR allowed for the detection of down to 102 introduced cells per g of dry soil. Introduced P. azotofixans spores did not form colonies on selective plates, but were detectable via PCR. The P. azotofixans populations introduced into the silt loam soil suffered a slow decline of the detectable plate count over a period of 14 days. MPN-PCR revealed a similar decline of the number of specific DNA targets. Greater numbers of targets were found in wheat rhizosphere from Flevo silt loam soil, and these numbers persisted throughout the experiment. Soil drying resulted in enhanced persistence of the target sequences, whereas in a constantly moist soil the numbers of target sequences declined. Rewetting of dried soil resulted in declining target sequence numbers. The MPN-PCR detection method is adequate to assess the impact of stress conditions affecting P. azotofixans in FSL and probably other soils, since it abolishes the need for culturing or specific markers and is direct and unambiguous due to its high specificity.

Scheid, D. and S. Stubner (2001). "Structure and diversity of Gram-negative sulfate-reducing bacteria on rice roots." FEMS Microbiology Ecology **36**(2-3): 175.

http://www.sciencedirect.com/science/article/B6T2V-43F78DM-C/2/14b5cc4e94d869dc41c647b0cbdc522f

Specific PCR assays were used to amplify the 16S rRNA genes of the Desulfobacteriaceae and the Desulfovibrionaceae from extracted environmental DNA from rice roots. 16S rDNA-based community patterns of the Desulfobacteriaceae were generated via terminal restriction fragment length polymorphism analysis from rice roots and compared with bulk soil. The molecular fingerprints showed no significant difference between rice roots and bulk soil, but changes during the vegetation period. 16S rDNA clone libraries and sequencing showed that the predominant terminal restriction fragments represented distinct phylogenetic groups. The 16S rDNA clone sequences of the Desulfobacteriaceae fell in the phylogenetic radiation of Desulfonema and Desulfosarcina or grouped within the Desulforhabdus-Syntrophobacter assemblage. Three of the latter sequences were closely affiliated with the MPN isolate EZ-2C2 from rice roots. All Desulfovibrionaceae 16S rDNA clone sequences, with one exception, were affiliated with the MPN isolate F1-7b from rice roots. The clustering of the clone sequences and the close phylogenetic affiliation with isolates from MPN enrichments from the same habitat in two cases indicated that these sequence clusters may represent predominant Gram-negative sulfate reducers on rice roots. Quantification of the bacterial abundances was accomplished by rRNA dot blot hybridization. In total the Gram-negative sulfate reducers accounted for approximately 2-3%

of the total rRNA content. The relative rRNA abundance of the Desulfobacteriaceae was, at 1.4%, higher than that of the Desulfovibrionaceae (0.5%).

Schlotelburg, C., C. von Wintzingerode, et al. (2002). "Microbial structure of an anaerobic bioreactor population that continuously dechlorinates 1,2-dichloropropane." FEMS Microbiology Ecology **39**(3): 229.

http://www.sciencedirect.com/science/article/B6T2V-45519BH-1/2/b28462f6bd63310989515784dd6aadfd

The bacterial diversity of an anaerobic 1,2-dichloropropane (DCP) dechlorinating bioreactor consortium derived from river sediment has been investigated by a combined molecular approach. By using rDNA clone libraries, denaturing gradient gel electrophoresis and quantitative real-time PCR, both Dehalococcoides ethenogenes- and Dehalobacter restrictus-like 16S rDNA sequences were found within the community. Both species are known for reductive dechlorination of tetrachloroethene. Furthermore, numerous yet-uncultured members of the Green non-sulfur bacteria occurred within the consortium. The community analyses over a period of 14 months revealed a clear population shift. D. restrictus 16S rDNA was enriched significantly and became the most abundant rDNA sequence type, suggesting that Dehalobacter spp. play a key role within the reductive dechlorination of DCP in this consortium. We propose the use of this species as an indicator to monitor the transformation process within the bioreactor.

Schmidt, L. M., J. F. Preston, et al. (2004). "Detection of Pasteuria penetrans infection in Meloidogyne arenaria race 1 in planta by polymerase chain reaction." FEMS Microbiology Ecology **48**(3): 457.

http://www.sciencedirect.com/science/article/B6T2V-4C4X1P7-2/2/9275428681d753d7001ca4f1341abd2d

We report on the development of a PCR-based assay to detect Pasteuria penetrans infection of Meloidogyne arenaria in planta using specific primers for recently sequenced sigE, spoIIAB and atpF genes of P. penetrans biotype P20. Amplification of these genes in crude DNA extracts of ground tomato root galls using real-time kinetic PCR distinguished infected from uninfected M. arenaria race 1 by analysis of consensus thresholds for single copy genes. Fluorescent in situ hybridization (FISH) using the sigE primer sequence as a probe shows hybridization to P. penetrans cells in various stages of vegetative (pre-endospore) development. Ratios of gene copies for sigE and 16S rDNA were obtained for P. penetrans and compared to Bacillus subtilis as a genomic paradigm of endospore-forming bacteria. Phylogenetic analysis of the sigE gene from Gram-positive, endospore-forming bacteria finds P. penetrans most closely related Paenbacillus polymyxa. The sporulation genes (spo genes), particularly sigE, have sequence diversity that recommends them for species and biotype differentiation of the numerous Pasteuria isolates that infect a large number of plant-parasitic nematodes.

Schonfeld, J., A. Gelsomino, et al. (2003). "Effects of compost addition and simulated solarisation on the fate of Ralstonia solanacearum biovar 2 and indigenous bacteria in soil." FEMS Microbiology Ecology **43**(1): 63.

http://www.sciencedirect.com/science/article/B6T2V-475B5NT-1/2/9189c45ce616b0f2daa6b1983bf9b403

The effects of compost addition and simulated solarisation of soil on the survival of Ralstonia solanacearum biovar 2 strain 1609, as well as on the structure of indigenous soil bacterial communities, were analysed. In addition, effects on the invasion of susceptible test plants by strain 1609 were assessed. In untreated soil in microcosms and the field, strain 1609 showed slow progressive declines, from 106-107 to roughly 104-105 CFU per g dry soil in around 60 days. When these soils were used in suppressiveness tests, a majority of plants developed symptoms of wilting and revealed the presence of the pathogen in their lower stem parts, as evidenced by immunofluorescence colony staining (IFC) and polymerase chain reaction (PCR). Solarisation of unamended soil did not drastically affect R. solanacearum survival or plant invasiveness. However, the addition of household compost resulted in enhanced R. solanacearum population decline rates, as well as reduced numbers of diseased plants in suppressiveness tests. Combined solarisation and compost addition yielded differential results between microcosms and the field. Some healthy-looking plants, primarily from soils treated with compost, revealed the latent presence of strain 1609 in the lower stem parts. The eubacterial and [beta]-subgroup proteobacterial communities in the differentially treated soil microcosms were rather stable, as evidenced by analysis of PCR-denaturing gradient gel electrophoresis (DGGE) generated molecular profiles. However, compost amendment clearly induced changes in these communities, which were detectable until the end of the experiment; two major bands, affiliated with Variovorax paradoxus and Aquaspirillum psychrophylum, were associated with the compost amendment. The decrease in abundance of R. solanacearum in the compost-amended soils was confirmed by the DGGE profiles.

Snell-Castro, R., J.-J. Godon, et al. (2005). "Characterisation of the microbial diversity in a pig manure storage pit using small subunit rDNA sequence analysis." FEMS Microbiology Ecology **52**(2): 229.

http://www.sciencedirect.com/science/article/B6T2V-4F0G7VW-3/2/6270996aeeecae7cfd75f05dae6b0f26

The microbial community structure of pig manure slurry (PMS) was determined with comparative analysis of 202 bacterial, 44 archaeal and 33 eukaryotic small subunit (SSU) rDNA partial sequences. Based on a criterion of 97% of sequence similarity, the phylogenetic analyses revealed a total of 108, eight and five phylotypes for the Bacteria, Archaea and Eukarya lineages, respectively. Only 36% of the bacterial phylotypes were closely related ([greater-or-equal, slanted]97% similarity) to any previously known sequence in databases. The bacterial groups most often represented in terms of phylotype and clone abundance were the Eubacterium (22% of total sequences), the Clostridium (15% of sequences), the Bacillus-Lactobacillus-Streptococcus subdivision (20% of sequences), theMycoplasma and relatives (10% of sequences) and the Flexibacter-Cytophaga-Bacteroides (20% of sequences). The global microbial community structure and phylotype diversity show a close relationship to the pig gastrointestinal tract ecosystem whereas phylotypes from the Acholeplasma-Anaeroplasma and the Clostridium purinolyticum groups appear to be better represented in manure. Archaeal diversity was dominated by three phylotypes clustering with a group of uncultured microorganisms of unknown activity and only distantly related to the Thermoplasmales and relatives. Other Archaea were methanogenic H2/CO2 utilisers. No known acetoclastic Archaea methanogen was found. Eukaryotic diversity was represented by a pluricellular nematode, two Alveolata, a Blastocystis and an Entamoebidae. Manure slurry physico-chemical characteristics were analysed. Possible inhibitory effects of acetate, sulphide and ammonia concentrations on the microbial anaerobic ecosystem are discussed.

Treude, N., D. Rosencrantz, et al. (2003). "Strain FAc12, a dissimilatory iron-reducing member of the Anaeromyxobacter subgroup of Myxococcales." FEMS Microbiology Ecology **44**(2): 261.

http://www.sciencedirect.com/science/article/B6T2V-47XPM4P-3/2/6559cdee637c28a0b0d37e2b1ffe5fe6

Dissimilatory iron reduction is of quantitative importance during anaerobic degradation of organic matter in flooded rice field soils. To isolate dissimilatory Fe(III)-reducing microorganisms from rice soil, enrichments were carried out with acetate and ferrihydrite. One of these resulted in the isolation of strain FAc12. This organism grew anaerobically in defined mineral medium with acetate as electron donor and with ferric citrate, ferrihydrite, or nitrate as electron acceptor. Strain FAc12 also grew well aerobically in defined mineral medium with acetate, citrate, glucose, or with complex medium. Comparative sequence analysis of its 16S rRNA gene revealed that strain FAc12 is most closely related to the very recently described Anaeromyxobacter dehalogenans within the order Myxococcales. The overall similarity value between the 16S rRNA gene sequences of strain FAc12 and the type strain of A. dehalogenans (2CP-1) is 99.5%. A. dehalogenans has been reported to be the first facultative anaerobic myxobacterium, while all other members of the Myxococcales were known to be strict aerobes. A. dehalogenans is able to grow by chlororespiration and to utilize nitrate as terminal electron acceptor for growth. Cultivation-independent retrieval of 16S rRNA gene sequences revealed that rice roots are also colonized by various members of this novel subgroup. This information and the metabolic capacity of strain FAc12 allows the assumption that these organisms are physiologically adapted to environments characterized by spatial and temporal fluctuations between oxic and anoxic conditions, as is typically the case for flooded rice soil.

Vettori, C., D. Paffetti, et al. (1996). "Amplification of bacterial DNA bound on clay minerals by the random amplified polymorphic DNA (RAPD) technique." FEMS Microbiology Ecology **20**(4): 251.

http://www.sciencedirect.com/science/article/B6T2V-3W2YFYP-5/2/312fe040557e77dac67467d277df64e6

Chromosomal DNA from Bacillus subtilis, bound on the clay minerals, montmorillonite (Wyoming (W) and Apache County (Ap)) and kaolinite (K), was subjected to the random amplified polymorphic DNA (RAPD) technique. DNA bound on the clays was not amplified with 0.625, 1.875, 6.25, and 12.5 U of Taq DNA polymerase, but amplification occurred when the clay-DNA complexes were diluted 10- and 20-fold or when 21 U of Taq DNA polymerase was added. DNA desorbed from the Ap-DNA and K-DNA equilibrium complexes was amplified with 0.625 U of Taq DNA polymerase, whereas amplification of DNA desorbed from the W-DNA complex occurred only after a 10-fold dilution or when 1.875 U of Taq DNA polymerase was used. These observations indicate that clay minerals differentially affect the amplification process, probably by inhibiting the activity of Taq DNA polymerase.

Watanabe, K., N. Takihana, et al. (2005). "Symbiotic association in Chlorella culture." FEMS Microbiology Ecology **51**(2): 187.

http://www.sciencedirect.com/science/article/B6T2V-4D9D99P-1/2/42d4fc0b307f657d7485feeaaf4959ed

Chlorella sorokiniana IAM C-212 has long been maintained in slant culture as a mixed strain, representing an associated natural microbial consortium. In this study, the consortium was separated and five nonalgal constituents, a fungal strain (CSSF-1), and four bacterial strains (CSSB-1, CSSB-2, CSSB-3, and CSSB-4) were isolated and identified. 16S rDNA sequence analysis revealed that strains CSSB-1, CSSB-2, CSSB-3, and CSSB-4 were close to Ralstonia pickettii (99.8% identity), Sphingomonas sp. DD38 (99.4% identity), Microbacterium

trichotecenolyticum (98.6% identity), and Micrococcus luteus (98.6% identity) respectively. 18S rDNA sequence analysis revealed that strain CSSF-1 resembled Acremonium-like hyphomycete KR21-2 (98.8%). The fungal strain CSSF-1 and one of the bacterial strains, CSSB-3, were found to promote the growth of Chlorella while the presence of bacterial strains CSSB-1 and CSSB-2 had no effect. Strain CSSB-4 could not be subcultured so its role was not elucidated. These results show that the interaction between Chlorella and its symbionts under photoautotrophic conditions involved both mutualism and commensalisms. The chlorophyll content of mixed strain was stable in long-term cultivation (7 months) while the chlorophyll content of a pure culture showed a marked decline. Electron microscopic analysis showed the two bacterial strains CSSB-2 and CSSB-3 were harbored on the sheath excreted by Chlorella, while the fungal strain CSSF-1 and the bacterial strain CSSB-1 directly adhered to the Chlorella cell surface.This report is the first observation of a symbiotic relationship among fungus, bacteria, and Chlorella, and the first observation of direct adhesion of fungus and bacteria to Chlorella in a consortium.

Wobus, A., C. Bleul, et al. (2003). "Microbial diversity and functional characterization of sediments from reservoirs of different trophic state." FEMS Microbiology Ecology **46**(3): 331.

http://www.sciencedirect.com/science/article/B6T2V-49W63K4-3/2/5569d8e64d6699455e120370e00233ea

Sediment samples from four reservoirs of different trophic state were compared with regard to chemical gradients in the pore water, composition of microbial communities and extracellular enzyme activities. The trophic state was clearly reflected by steep vertical concentration gradients of ammonium and alkalinity in the pore water. A high concentration of these parameters indicated a high microbial in situ activity in the more eutrophic reservoirs. However, the total number of bacteria in sediments seemed hardly to be influenced by the trophic conditions in the water column. Differences in the microbial composition of the sediments became evident by comparative 16S rDNA analysis of extracted DNA and by fluorescence in situ hybridization. Although a high proportion of the cells detectable with the EUB probe could not be identified at the subdomain level, members of the [beta]-Proteobacteria constituted an important fraction in the sediments of the more eutrophic reservoirs, whereas [gamma]-subgroup Proteobacteria were most frequently detected in sediment samples from the dystrophic Muldenberg reservoir. The assessment of extracellular enzyme activities (esterases, phosphatases, glucosidases and aminopeptidases, respectively) in sediment samples of the four reservoirs revealed specific patterns of metabolic potentials in accordance with the trophic state and characteristics of the catchment.

Zwart, G., R. Huismans, et al. (1998). "Divergent members of the bacterial division Verrucomicrobiales in a temperate freshwater lake." FEMS Microbiology Ecology **25**(2): 159.

http://www.sciencedirect.com/science/article/B6T2V-3S1PYDW-8/2/5face2211eccb4a97b03e7c383efd1b7

Bacterial diversity in the water column of a freshwater lake in the Netherlands was investigated by analysis of 16S rRNA gene sequences recovered through PCR amplification from total community DNA. Among 23 unique cloned sequences, two appeared to belong to the recently described bacterial division Verrucomicrobiales. One of the two sequences was most similar to a group of environmental clones that form a distinct lineage within the division. The other sequence was divergent (less than 85% similarity) from all 16S rRNA gene sequences, both from cultivated species and from environmental clones, known in this division to date. Analysis by denaturing gradient gel electrophoresis (DGGE) and sequencing of DNA recovered through excision from the DGGE gel showed that the two sequence types were present in the lake throughout the year.

FEMS Microbiology Letters (128)

Agarwal, N. and A. K. Tyagi (2003). "Role of 5'-TGN-3' motif in the interaction of mycobacterial RNA polymerase with a promoter of 'extended -10' class." FEMS Microbiology Letters **225**(1): 75.

http://www.sciencedirect.com/science/article/B6T2W-490H90F-3/2/d2ad55ad4cf3c0cba93593363618dfa7

In a systematic approach to understand the transcriptional machinery of mycobacteria, we had previously isolated and characterized mycobacterial promoter regions. In this study, we have investigated molecular interactions between mycobacterial RNA polymerase holoenzyme, reconstituted with different sigma subunits and the promoter element of the Mycobacterium tuberculosis gene pknH (Rv1266c), a representative of promoters belonging to the 'extended -10' class. In vitro transcription assays using the pknH promoter and reconstituted RNA polymerase holoenzyme demonstrated that transcription from the pknH promoter is specifically initiated by [sigma]A, the principal sigma factor of mycobacteria. DNase I protection assay and deletion studies with the pknH promoter revealed that the minimal region required for optimal transcription carries the sequence from position -37 to position +6. Moreover, mutation in the TGN motif of the pknH promoter resulted in the loss of >75% of its activity. Binding of RNA polymerase with wild-type promoter as well as its TG- mutant revealed that the TGN motif is required for the transition from a close complex into an open complex. Further, it was observed that the presence of the TGN motif reduces the thermal energy required for the conversion of a close complex into an open complex, necessary for initiation of transcription.

Agin, T. S., J. R. Cantey, et al. (1996). "Characterization of the eaeA gene from rabbit enteropathogenic Escherichia coli strain RDEC-1 and comparison to other eaeA genes from bacteria that cause attaching-effacing lesions." FEMS Microbiology Letters **144**(2-3): 249.

http://www.sciencedirect.com/science/article/B6T2W-3W263BF-S/2/395b4013b08c615b2dd210fad02aaf85

A number of enteric pathogens, including enteropathogenic (EPEC) and enterohemorrhagic (EHEC) Escherichia coli, Hafnia alvei, a strain of Citrobacter freundii, and rabbit EPEC strain RDEC-1 cause attaching-effacing (AE) lesions in the gut mucosa. These bacteria have a pathogenicity cassette (locus of enterocyte effacement or LEE) containing the eaeA gene. This gene encodes intimin, an outer membrane protein required for production of AE lesions. RDEC-1, a non-invasive enteropathogen in young rabbits, produces AE lesions morphologically indistinguishable from lesions caused by human AE bacterial strains. The RDEC-1 example of E. coli diarrhea in rabbits is an important model for studying the pathogenesis of AE bacteria in a natural infection and for analyzing specific roles of the components of LEE. In order to better understand the role of intimin in the development of AE lesions, a portion of DNA within RDEC-1 LEE, containing the eaeA gene and an upstream open reading frame (ORF), was sequenced. The RDEC-1 eaeA gene shared 87%, 92%, and 93% DNA sequence identity and > 80% amino acid sequence identity with the eaeA genes of C. freundii biotype 4280, EHEC O157:H7, and EPEC O127:H6, respectively. The carboxy-terminal 280 amino acid residues of intimin has 80%, 56%, and 54% identity with C. freundii, EHEC O157:H7, and EPEC O127:H6 intimins, respectively. The predicted protein encoded by the upstream ORF (156 amino acids) shares

95%, 97%, and 99% amino acid identity with predicted proteins from C. freundii, EHEC O157:H7, and EPEC O127:H6, respectively. The high degree of sequence homology of the ORF and the eaeA gene of RDEC-1 with those of other AE bacteria suggests an evolutionary relationship of LEE and supports and facilitates the use of the RDEC-1 model for studying the role of LEE in pathogenesis.

Ahmed, A. M. and T. Shimamoto (2004). "A plasmid-encoded class 1 integron carrying sat, a putative phosphoserine phosphatase gene and aadA2 from enterotoxigenic Escherichia coli O159 isolated in Japan." FEMS Microbiology Letters **235**(2): 243.

http://www.sciencedirect.com/science/article/B6T2W-4CBVFSJ-1/2/3f4dd7182bdb0dad641faaafbff9ecec

A class 1 integron was detected in a single multidrug-resistant strain of enterotoxigenice Escherichia coli (ETEC) O159 after examination of 23 clinical E. coli isolates. This isolate was resistant to streptomycin, kanamycin, gentamicin, chloramphenicol and ampicillin. Sequencing of the class 1 integron identified three-gene cassettes. The first is the streptothricin acetyltransferase gene, sat, which confers resistance to streptothricin. The second is an ORF whose product is a putative phosphoserine phosphatase (PSP), and the last is an aminoglycoside adenyltransferase gene, aadA2, which confers resistance to streptomycin and spectinomycin. The putative PSP gene product was found to be 39%, 38%, 28%, and 27% identical to PSP gene products of Vibrio vulnificus CMCP6, V. vulnificus YJ016, Pseudomonas syringae, and P. aeruginosa, respectively. Southern-blot hybridization showed that this integron is located on a 90 kb plasmid. This is the first report identifying a putative PSP gene in an integron.

Ahmed, A. M., S. Shinoda, et al. (2005). "A variant type of Vibrio cholerae SXT element in a multidrug-resistant strain of Vibrio fluvialis." FEMS Microbiology Letters **242**(2): 241.

http://www.sciencedirect.com/science/article/B6T2W-4DTKD6P-2/2/3df7549ee07da234acc7cdce3f668c55

Vibrio fluvialis strain H-08942 was isolated from an infant aged 6 months who was suffering from cholera-like diarrhea in India. This strain showed the typical multidrug-resistance phenotype of an SXT element. It was resistant to sulfamethoxazole (Su), trimethoprim (Tm), chloramphenicol (Cm) and streptomycin (Sm), in addition to other antibiotics such as ampicillin (Am), furazolidone (Fz), nalidixic acid (Na), and gentamicin (Gm). The SXT element is a Vibrio cholerae-derived integrative and conjugative element (ICE) that has also been referred to as a conjugative transposon. Our goal was to find a relationship between these resistant phenotypes and the presence of the SXT element in this unique strain. By using PCR, we detected the antibiotic resistance genes, the integrase gene and the attP attachment site of SXT element. Cloning and DNA sequencing results showed that both the SXT integrase gene and its attP site of V. fluvialis were similar but not identical to those of V. cholerae. The SXT integrase gene of V. fluvialis has a 99% identity to that of V. cholerae, and the attP site of SXT of V. fluvialis is variant and shorter (641 bp) than that of V. cholerae (785 bp). It was possible for the SXT of V. fluvialis to be transferred by conjugation to a laboratory strain of Escherichia coli. Here, we report the detection of a variant SXT element in species other than V. cholerae, with molecular characterization and analysis of its integrase gene and its attP site.

Andreu, N., C. Y. Soto, et al. (2004). "Mycobacterium smegmatis displays the Mycobacterium

tuberculosis virulence-related neutral red character when expressing the Rv0577 gene." FEMS Microbiology Letters **231**(2): 283.

http://www.sciencedirect.com/science/article/B6T2W-4BJK5P9-4/2/b2e27a4685680542f550a3a1644d5198

Neutral red staining is a cytochemical reaction that has been found to be related to Mycobacterium tuberculosis virulence and, therefore, the component involved in it is thought to be a virulence factor. To study the molecular basis of this reaction we constructed an M. tuberculosis cosmid library in Mycobacterium smegmatis and selected recombinant neutral red positive clones. Heterologous complementation identified Rv0577 as the gene responsible for this trait and we have also shown that it is expressed as a single polycistronic unit together with Rv0576 which could also be involved in the neutral red staining.

Beard, S. J., P. K. Hayes, et al. (2002). "The sequence of the major gas vesicle protein, GvpA, influences the width and strength of halobacterial gas vesicles." FEMS Microbiology Letters **213**(2): 149.

http://www.sciencedirect.com/science/article/B6T2W-4625S83-1/2/700f945ffce78c2a743bd3aafaa3f672

Transformation experiments with Haloferax volcanii show that the amino acid sequence of the gas vesicle protein GvpA influences the morphology and strength of gas vesicles produced by halophilic archaea. A modified expression vector containing p-gvpA was used to complement a Vac- strain of Hfx. volcanii that harboured the entire p-vac region (from Halobacterium salinarum PHH1) except for p-gvpA. Replacement of p-gvpA with mc-gvpA (from Haloferax mediterranei) led to the synthesis of gas vesicles that were narrower and stronger. Other gene replacements (using c-gvpA from Hbt. salinarum or mutated p-gvpA sequences) led to a significant but smaller increase in gas vesicle strength, and less marked effects on gas vesicle morphology.

Best, E. L., E. J. Powell, et al. (2003). "Applicability of a rapid duplex real-time PCR assay for speciation of Campylobacter jejuni and Campylobacter coli directly from culture plates." FEMS Microbiology Letters **229**(2): 237.

http://www.sciencedirect.com/science/article/B6T2W-4B3JNY6-1/2/4df497a3ce068d09e5da42f6bee9e6b5

A rapid duplex real-time polymerase chain reaction (PCR) assay for speciation of Campylobacter jejuni and Campylobacter coli using the ABI Prism 7700 sequence detection system (Applied Biosystems) was developed based on two of the genes used in a conventional multiplex PCR. A rapid turnaround time of 3 h was achieved with the use of boiled cell lysates. Applicability of the assay was tested with 6015 random campylobacter strains referred to the Campylobacter Reference Unit, with 97.6% being identified as either C. jejuni or C. coli by this technique. Rapidity, combined with specificity and sensitivity, makes this method for routine campylobacter speciation attractive to any laboratory with a Taqman system.

Bin, Y., Z. Jiti, et al. (2004). "Expression and characteristics of the gene encoding azoreductase from Rhodobacter sphaeroides AS1.1737." FEMS Microbiology Letters **236**(1): 129.

http://www.sciencedirect.com/science/article/B6T2W-4CHRMM5-

2/2/146af927804dc065edef575e146a20e2

A gene that encodes a protein with azoreductase activity was obtained by PCR amplification from Rhodobacter sphaeroidesAS1.1737. The enzyme, with a molecular weight of 18.7 kD, was heterologously expressed in Escherichia coli and its azoreductase activity was characterized. Furthermore, the reduction mechanism of azo dyes catalyzed by the azoreductase was studied in detail. The presence of a hydrazo-intermediate was identified, which provided a convincing evidence for the assumption that azo dyes were degraded via an incomplete reduction stage.

Caccio, S., W. Homan, et al. (1999). "Genetic polymorphism at the [beta]-tubulin locus among human and animal isolates of Cryptosporidium parvum." FEMS Microbiology Letters **170**(1): 173.

http://www.sciencedirect.com/science/article/B6T2W-3VGTHJK-V/2/9528ebdb97f0472b3c6c5ed2fa2b82ef

Sequence analysis of a fragment of the [beta]-tubulin gene was performed on 13 isolates of the parasite Cryptosporidium parvum, eight from humans and five from animals. A total of 12 synonymous substitutions and a deletion of two bases within the intron sequence were found. This genetic variation defined two alleles at the [beta]-tubulin locus, which can be identified by a simple polymerase chain reaction-restriction fragment length polymorphism assay. A total of 20 isolates were also tested using four available molecular markers. These analyses showed congruently that the C. parvum isolates segregate into two groups, one found exclusively in humans and the other found in both humans and animals. Since no recombinant genotypes were observed, the results are consistent with the hypothesis of a substantially clonal reproduction in this parasite.

Chavagnat, F., M. Haueter, et al. (2002). "Comparison of partial tuf gene sequences for the identification of lactobacilli." FEMS Microbiology Letters **217**(2): 177.

http://www.sciencedirect.com/science/article/B6T2W-475B5P6-4/2/21b2329fc4fe580634364e05299e5c0e

Comparative analysis of partial tuf sequences was evaluated for the identification and differentiation of lactobacilli. Comparison of the amino acid sequences allowed differentiation between species and also between the subspecies of Lactobacillus delbrueckii. The nucleotide sequence comparison allowed differentiation between other subspecies and between some strains. Lactobacilli from several collections and isolates from dairy samples were clearly identified by comparison of short tuf sequences with those of the type strains. In evaluating the taxonomy of the Lactobacillus casei-related taxa, different tuf amino acid signatures are in favour of a classification into three distinct species. The type strain designation for the L. casei species is discussed.

Chieda, Y., K. Iiyama, et al. (2005). "Pathogenicity of gacA mutant of Pseudomonas aeruginosa PA01 in the silkworm, Bombyx mori." FEMS Microbiology Letters **244**(1): 181.

http://www.sciencedirect.com/science/article/B6T2W-4FCRC72-2/2/4e37f2279782af2f363cb6441c6b9a94

To investigate the pathogenicity of Pseudomonas aeruginosa in insects, a gacA mutant of P.

aeruginosa PA01 was constructed by site-directed mutagenesis. The mutant was designated as C1. C1 was less virulent to Bombyx mori than the parent strain. To complement the gacA gene, P. aeruginosa C1 was transformed with the broad host range plasmid pJB3Km1 carrying a 3.9-kbp gacA fragment. The expression of the gacA mRNA in C1 (pgacA) was detected. In addition, the complemented mutant restored the level and timing of pyocyanin production, indicating that functional GacA is produced in the complemented strain. However, no significant difference was observed between C1 and C1 (pgacA) with respect to the killing of B. mori larvae.

Chohnan, S., Y. Kurusu, et al. (1999). "Cloning and characterization of mdc genes encoding malonate decarboxylase from Pseudomonas putida." FEMS Microbiology Letters **174**(2): 311.

http://www.sciencedirect.com/science/article/B6T2W-3WF801X-H/2/0fa9d3b754d8a4260e8cdce8bcc11675

The DNA fragment encoding malonate decarboxylase, involved in malonate assimilation, was cloned from Pseudomonas putida. The 11-kb DNA fragment contained nine open reading frames, which were designated mdcABCDEGHLM in the given order. N-terminal protein sequencing established that the mdcA, mdcC, mdcD, mdcE and mdcH genes encoded subunits [alpha], [delta], [beta], [gamma] and [epsi] of the malonate decarboxylase, respectively. Malonate decarboxylase was functionally expressed in Escherichia coli from plasmid harboring the entire gene cluster or the mdc genes lacking the mdcL and mdcM genes. The mdcL and mdcM genes encode membrane proteins and disruption of the genes of P. putida by the insertion of a kanamycin resistance cassette reduced the malonate uptake activity of the organism. Thus, we conclude that MdcLM is a malonate transporter.

Chohnan, S., J. Nonaka, et al. (2002). "Lysobacter strain with high lysyl endopeptidase production." FEMS Microbiology Letters **213**(1): 13.

http://www.sciencedirect.com/science/article/B6T2W-463Y57T-2/2/1b390996980a69f9c82be65048a83b09

A new lysyl endopeptidase producing strain, Lysobacter sp. IB-9374, was isolated from soil. This strain secreted the endopeptidase to culture medium at 6-12-fold higher levels relative to Achromobacter lyticus and Lysobacter enzymogenes. The mature Lysobacter sp. enzyme was enzymatically identical to Achromobacter lysyl endopeptidase bearing lysyl bond specificity, a high peptidase activity, a wide pH optimum, and stability against denaturants. Nucleotide sequence analysis of the Lysobacter sp. lysyl endopeptidase gene revealed that the enzyme is synthesized as a precursor protein consisting of signal peptide (20 amino acids (aa)), pro-peptide (185 aa), mature enzyme (268 aa), and C-terminal extension peptide (198 aa). The deduced amino acid sequence of the mature enzyme was totally identical to that of the Achromobacter enzyme. The Lysobacter sp. precursor protein has an 18-aa longer peptide chain following nine consecutive amino acid residues distinct from the Achromobacter counterpart at the C-terminus. Total precursor protein is 671 aa of which only 268 aa are in the finally processed exoenzyme.

Creelan, J. L., A. J. Bjourson, et al. (1999). "Characterisation of strain-specific sequences from an abortifacient strain of ovine Chlamydia psittaci using subtraction hybridisation." FEMS Microbiology Letters **171**(1): 17.

http://www.sciencedirect.com/science/article/B6T2W-3VNR780-

3/2/04bb95efe1ebf9706da3346736f3f4e0

Enzootic abortion in ewes (EAE) is caused by strains of Chlamydia psittaci which have the ability to invade and colonise the placenta of sheep. In an attempt to improve diagnostic methods for the detection of EAE, subtraction hybridisation was used to isolate unique fragments of the genome of an abortifacient strain (S26/3) of C. psittaci. One S26/3 strain-specific sequence identified was shown to encode a putative helicase which is repeated throughout the EAE genome. The labelled strain-specific helicase gene fragment was used in a dot-blot hybridisation test for the detection of EAE DNA in ovine placental samples. We report the identification of C. psittaci S26/3 strain-specific sequences with potential as diagnostic probes for the detection of EAE.

Curley, P. and D. van Sinderen (2000). "Identification and characterisation of a gene encoding aminoacylase activity from Lactococcus lactis MG1363." FEMS Microbiology Letters **183**(1): 177.

http://www.sciencedirect.com/science/article/B6T2W-3YF3Y1H-12/2/9f2d4832aa86b2ffbe803ce762be808d

Analysis of the sequence of a randomly cloned chromosomal DNA fragment (3.2 kb) from Lactococcus lactis revealed the presence of part of an open reading frame, designated amd1, which specifies a protein displaying significant similarity to aminoacylases from various bacteria. The presence of an immobilised copy of an IS982 element immediately upstream of the coding region of amd1 has probably resulted in the displacement of amd1's native promoter. This genetic organisation was shown to be retained in seven other dairy strains, one of which was only slightly different. The amd1 gene was overexpressed in L. lactis NZ9800 under the control of the inducible nisA promoter and the deacetylating capacity of its gene product was measured on a number of substrates.

Daniel, C., S. Haentjens, et al. (1999). "Characterization of the Acinetobacter baumannii Fur regulator: cloning and sequencing of the fur homolog gene." FEMS Microbiology Letters **170**(1): 199.

http://www.sciencedirect.com/science/article/B6T2W-3VGTHJK-10/2/3056788a83eb3676d075646004e6599f

Growth kinetics, siderophore activity and iron-regulated bacterial proteins of Acinetobacter baumannii BM2580 were studied in iron-restricted and iron-supplemented chemically defined media. Iron-regulated outer membrane proteins of 75 kDa and 80 kDa were expressed under iron-restricted conditions. Cloning and sequencing of the complete iron-uptake regulatory (fur) gene from A. baumannii BM2580 is reported for the first time. This gene is preceded by a single autoregulated promoter whose -10 region overlaps the Fur binding site. The open reading frame identified encodes a polypeptide consisting of 145 amino acids. The fur gene is followed by a divergent open reading frame coding for the C-terminus of a putative PilU protein. Sequence analysis indicates that the Fur protein of A. baumannii was 63% identical to the Escherichia coli Fur protein.

de las Rivas, B., A. Marcobal, et al. (2005). "Improved multiplex-PCR method for the simultaneous detection of food bacteria producing biogenic amines." FEMS Microbiology Letters **244**(2): 367.

http://www.sciencedirect.com/science/article/B6T2W-4FHJF0J-4/2/6ebc8d4ac339272d279e3c0049f8baa0

This study describes a simple and rapid multiplex-PCR method to determine the ability to produce histamine, tyramine and putrescine by bacteria. The assay is an improved method based on an assay designed for lactic acid bacteria. This improved method includes a pair of primers based on sequences from histidine decarboxylases from Gram-negative bacteria. Under the optimised conditions, the assay yielded a 367-bp DNA fragment from histidine decarboxylases of Gram-positive bacteria, 534-bp fragment from histidine decarboxylases of Gram-negative bacteria, 924-bp from bacterial tyrosine decarboxylases, and 1446-bp fragment from bacterial ornithine decarboxylases. The method was successfully applied to several biogenic amine-producing bacterial strains, even when DNAs of several target organisms were included in the same reaction. This simple method could be easily incorporated in food control laboratories to detect potentially biogenic amine-producing bacteria in foods.

Devalckenaere, A., M. Odaert, et al. (1999). "Characterization of IS1541-like elements in Yersinia enterocolitica and Yersinia pseudotuberculosis." FEMS Microbiology Letters **176**(1): 229.

http://www.sciencedirect.com/science/article/B6T2W-3WSMFR5-15/2/0f9433a630c4938cd6dd2dc897c7b1e7

We characterized Yersinia enterocolitica and Yersinia pseudotuberculosis insertion sequences related to insertion sequence 1541, recently identified in Yersinia pestis. For each of the two species, two insertion sequence copies were cloned and sequenced. Genetic elements from Y. pseudotuberculosis were almost identical to insertion sequence 1541, whereas these from Y. enterocolitica were less related. Phylogenetic analysis of the putative transposases encoded by insertion sequences from the three pathogenic members of the genus Yersinia showed that they clustered with those encoded by Escherichia coli and Salmonella enterica elements belonging to the insertion sequence 200/insertion sequence 605 group. Insertion sequences originating from Y. pestis and Y. pseudotuberculosis constitute a monophyletic lineage distinct from that of Y. enterocolitica.

Dietrich, G., U. E. Schaible, et al. (2000). "Isolation of RNA from mycobacteria grown under in vitro and in vivo conditions." FEMS Microbiology Letters **186**(2): 177.

http://www.sciencedirect.com/science/article/B6T2W-405SX5F-6/2/106162f7510af8428cdcb7a5b9582090

Isolation of RNA from mycobacteria is very difficult to perform, and the yields are generally very low. We describe an approach to isolate RNA from mycobacterial species which combines the disruption of mycobacterial cells by a silica/ceramic matrix in a reciprocal shaker with the ease and efficiency of subsequent RNA purification on spin columns with silica gel-based membranes. This method is rapid, easy to perform and yields high amounts of pure, intact total RNA. Due to its safety, this method is applicable even to group 3 biological hazard organisms like Mycobacterium tuberculosis. By combining a method for the isolation of phagosomal bacteria from infected primary macrophages with the novel RNA isolation technique, we are able to monitor gene expression during infection even in bacteria which are rather resistant to genetic manipulation, like Mycobacterium bovis.

Du, C., J. Zhou, et al. (2003). "Construction of a genetically engineered microorganism for CO2 fixation using a Rhodopseudomonas/Escherichia coli shuttle vector." FEMS Microbiology Letters **225**(1): 69.

http://www.sciencedirect.com/science/article/B6T2W-491BJCV-1/2/713d1cd3dedf4c547abee52c5cc54e8d

The CO2 fixation ability of Rhodopseudomonas palustris DH was enhanced by introducing the recombinant plasmid pMG-CBBM containing the form II ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO) gene (cbbM) isolated from Rps. palustris NO. 7. Sequencing of a 3.0-kb PstI fragment containing the cbbM gene revealed an open reading frame encoding 461 amino acids, homologous to known cbbM genes, with a ribosome binding site upstream of cbbM and a terminator downstream of cbbM, without promoter. pMG-CBBM, a Rhodopseudomonas/Escherichia coli shuttle expression plasmid, was derived from the Rhodopseudomonas/E. coli shuttle cloning vector pMG105, by inserting the promoter of the pckA gene and the cbbM gene into its multiple cloning site. Plasmid pMG-CBBM was transformed into Rps. palustris DH by electroporation, and was stably maintained when transformants were grown either photoheterotrophically or photolithoautotrophically in the absence of antibiotics. This is the first report of an expression plasmid containing a Rps. palustris-specific promoter that allows stable expression of a foreign gene in the absence of antibiotic selection.

Eaton, T. J. and M. J. Gasson (2002). "A variant enterococcal surface protein Espfm in Enterococcus faecium; distribution among food, commensal, medical, and environmental isolates." FEMS Microbiology Letters **216**(2): 269.

http://www.sciencedirect.com/science/article/B6T2W-4712DXX-1/2/93f7686690efe93c7c5072b32da5566f

Enterococci are increasingly important causes of nosocomial disease. Also, they are associated with food and have a history of use as dairy starter and probiotic cultures. An enterococcal surface protein Espfs is involved the virulence and biofilm-forming capacity of Enterococcus faecalis and recently we demonstrated the presence of a homologue Espfm in E. faecium. Here we describe the complete structure of Espfm and demonstrate that its distribution in E. faecium correlates with disease associated strains from a range of pathological sites.

Fernandez-Gonzalez, M., J. F. Ubeda, et al. (2004). "Evaluation of polygalacturonase activity in Saccharomyces cerevisiae wine strains." FEMS Microbiology Letters **237**(2): 261.

http://www.sciencedirect.com/science/article/B6T2W-4CSXM5D-4/2/ef11f2286c95cac5cd13a961411efd01

A total of 61 S. cerevisiae strains, 60 of them isolated from wine ecosystems, were evaluated for the presence of the gene encoding endopolygalacturonase (PGU1) and for polygalacturonase (PG) activity. Nine strains lack the gene PGU1 and did not exhibit PG activity on plate assays. Of the 52 strains showing an amplified band corresponding to the size of PGU1 gene, only 36 degraded polygalacturonic acid (PGA) and 17 did not degrade it at any of the pH values used. The coding region of the PGU1 gene (ORF YJR153w) was not present in some PG activity negative strains. The S. cerevisiae UCLMS-39 strain was selected for its specific activity at different pHs, temperatures and oenological parameters. The temperature and pH optima were 50 [deg]C and 3.5-5.5 respectively and it was only affected by ethanol. The PGU1 gene was cloned and sequenced. The production of a biologically functional endoPG in S. cerevisiae UCLMS-39 brings us a step closer to improving the qualities of outstanding enological yeasts naturally lacking PG activity.

Folli, C., I. Ramazzina, et al. (2003). "Purification of bacteriocin AS-48 from an Enterococcus faecium strain and analysis of the gene cluster involved in its production." FEMS Microbiology Letters **221**(1): 143.

http://www.sciencedirect.com/science/article/B6T2W-48622TK-4/2/1799b3e21a941347ee8ed12c143c4607

The cyclic bacteriocin AS-48 has previously been shown to be produced by Enterococcus faecalis strains. A bacteriocin has been purified from an E. faecium strain (E. faecium 7C5), and it has been found to possess molecular mass, cyclization and amino acid sequence typical of bacteriocin AS-48. In addition to the structural gene as-48A, the sequence analysis of the AS-48 gene cluster present in E. faecium 7C5 has revealed the presence of several putative coding regions presumably involved in bacteriocin production and immunity. The results of DNA hybridization assays have indicated that the AS-48 gene cluster and the gene pd78 are present on the same plasmid, possibly the pPD1 plasmid, in E. faecium 7C5.

Fontaine, M. and E. Guillot (2002). "Development of a TaqMan quantitative PCR assay specific for Cryptosporidium parvum." FEMS Microbiology Letters **214**(1): 13.

http://www.sciencedirect.com/science/article/B6T2W-46C0C9V-1/2/b6b5b550e7691cfa8261babfaa7ec5f8

A rapid detection method that is both quantitative and specific for the water-borne human parasite Cryptosporidium parvum is reported. Real-time polymerase chain reaction (PCR) combined with fluorescent TaqMan technology was used to develop this sensitive and accurate assay. The selected primer-probe set identified a 138-bp section specific to a C. parvum genomic DNA sequence. The method was optimized on a cloned section of the target DNA sequence, then evaluated on C. parvum oocyst dilutions. Quantification was accomplished by comparing the fluorescence signals obtained from test samples of C. parvum oocysts with those obtained from standard dilutions of C. parvum oocysts. This real-time PCR assay allowed reliable quantification of C. parvum oocysts over six orders of magnitude with a baseline sensitivity of six oocysts in 2 h.

Fontaine, M. and E. Guillot (2003). "Study of 18S rRNA and rDNA stability by real-time RT-PCR in heat-inactivated Cryptosporidium parvum oocysts." FEMS Microbiology Letters **226**(2): 237.

http://www.sciencedirect.com/science/article/B6T2W-49D1M4Y-1/2/b43fc5a511dd94c9fbc4d4ce31aad504

The public health problem posed by Cryptosporidium parvum has led the water supply industry to develop analytical tools for detecting viable oocysts in water. In this study, we report on a TaqMan real-time reverse transcription-polymerase chain reaction (RT-PCR) method that targets and quantifies C. parvum 18S rRNA. To study the suitability of 18S rRNA as an indicator of Cryptosporidium oocyst viability, the stability of 18S rRNA and rDNA was monitored by real-time RT-PCR following various Cryptosporidium heat treatments. Decay of 18S rRNA was first observed after a 20-min treatment of C. parvum oocysts at 95[deg]C and was still detectable after 4 h. In contrast, rDNA was more heat resistant. The stability of 18S rRNA and rDNA was also studied after oocyst lysis by thermal shocks in the presence and absence of Chelex-100. In the former case, both rRNA and rDNA were degraded whereas in the presence of Chelex-100 both molecules were protected from heat degradation and were still detected after 4 h at 95[deg]C following thermal shocks. Our results indicate that 18S rRNA detection may not be directly associated with viability following heat inactivation of Cryptosporidium oocysts even if in all the

experiments 18S rRNA was less stable than rDNA.

Fujiwara, T., I. Nakagawa, et al. (1994). "Inconsistency between the fimbrilin gene and the antigenicity of lipopolysaccharides in selected strains of Porphyromonas gingivalis." FEMS Microbiology Letters **124**(3): 333.

http://www.sciencedirect.com/science/article/B6T2W-476YGT6-15/2/4728622a4fd8bee493a04086e4f1dde7

Immunochemical specificity of lipopolysaccharide an the molecular property of the gene encoding the fimbrilin (fimA) of Porphyromonas gingivalis strains were examined using 'fimbriated' strains 381 and HG564 and 'non-fimbriated' strains 381FL and W50. Lipopolysaccharide from strains 381, 381FL and HG564 reacted with monoclonal antibody raised to lipopolysaccharide from strain 381 to give a fused precipitin band by the immunodiffusion test. However, silver staining and Western blotting of lipopolysaccharide clearly revealed a difference in profile of bands between strains 381 and 381FL. On the other hand, lipopolysaccharide from W50 formed another precipitin band and reacted with the antibody, but only at higher concentrations of lipopolysaccharide. The fimA genes in these strains were amplified by polymerase chain reaction and cloned. Sequencing of the fimA gene revealed thatthe fimA(W50) was almost identical to fimA(HG564), but a notable difference was observed at the start codon of the open reading frame, while the fimA(381FL) was considerably different from fimA of other strains and its open reading frame was found to be missing. These results indicate that the molecular structure of the fimA genes of these strains is not homologous, indicating that moe molecular modifications in the fimA gene should occur during in vitro passages and maintenance of strains of P. gingivalis in laboratories.

Gevers, D., G. Huys, et al. (2003). "In vitro conjugal transfer of tetracycline resistance from Lactobacillus isolates to other Gram-positive bacteria." FEMS Microbiology Letters **225**(1): 125.

http://www.sciencedirect.com/science/article/B6T2W-491H9GW-2/2/a6be59ffc670c79a6e00d4d776fd79c0

The ability of 14 Lactobacillus strains, isolated from fermented dry sausages, to transfer tetracycline resistance encoded by tet(M) through conjugation was examined using filter mating experiments. Seven out of 14 tetracycline-resistant Lactobacillus isolates were able to transfer in vitro this resistance to Enterococcus faecalis at frequencies ranging from 10-4 to 10-6 transconjugants per recipient. Two of these strains could also transfer their resistance to Lactococcus lactis subsp. lactis, whereas no conjugal transfer to a Staphylococcus aureus recipient was found. These data suggest that meat lactobacilli might be reservoir organisms for acquired resistance genes that can be spread to other lactic acid bacteria. In order to assess the risk of this potential hazard, the magnitude of transfer along the food chain merits further research.

Gihring, T. M. and J. F. Banfield (2001). "Arsenite oxidation and arsenate respiration by a new Thermus isolate." FEMS Microbiology Letters **204**(2): 335.

http://www.sciencedirect.com/science/article/B6T2W-447DF70-4/2/4de66e1dd897a1bd34c308aafb473d14

A new microbial strain was isolated from an arsenic-rich terrestrial geothermal environment. The isolate, designated HR13, was identified as a Thermus species based on 16S rDNA phylogenetic relationships and close sequence similarity within the Thermus genus. Under aerobic conditions, Thermus HR13 was capable of rapidly oxidizing inorganic As(III) to As(V). As(III) was oxidized at a rate approximately 100-fold greater than abiotic rates. Metabolic energy was not gained from the oxidation reaction. In the absence of oxygen, Thermus HR13 grew by As(V) respiration coupled with lactate oxidation. The ability to oxidize and reduce arsenic has not been previously described within the Thermus genus.

Gil, M. T., I. Perez-Arellano, et al. (2001). "Secretion of the rotavirus VP8* protein in Lactococcus lactis." FEMS Microbiology Letters **203**(2): 269.

http://www.sciencedirect.com/science/article/B6T2W-43YRFMX-1/2/6c8c3c518f3a3aaa7ca71872e3aa9dd9

Secretion of the VP8* subunit of the VP4 capsid protein of rotavirus by Lactococcus lactis has been achieved. For this purpose, a secretion vector has been constructed with the lactococcal signal sequence AL9 and the VP8*-encoding gene fragment. The amount of VP8* secreted by L. lactis in the culture supernatant was quantified and visualised by Western blot. Furthermore, it was shown to retain its hemagglutination capability, indicating that the conformation of the secreted peptide may be retaining its biological activity.

Giraffa, G. and L. Rossetti (2004). "Monitoring of the bacterial composition of dairy starter cultures by RAPD-PCR." FEMS Microbiology Letters **237**(1): 133.

http://www.sciencedirect.com/science/article/B6T2W-4CPK7GG-5/2/fb3f1c79362d37919e97bf5971b1c32c

Randomly amplified polymorphic DNA (RAPD)-PCR was used to verify the species composition of commercial dairy starters and to detect possible shifts in strain composition of these cultures. After RAPD-PCR analysis, not all the strains isolated in the years 2001 and 2002 fell within the same dendrogram cluster of the strains isolated in the year 2000 and used as reference strains. Changes in composition of the microbial population and/or voluntary immission of new biotypes with respect to the original strain formulation had occurred in the starters. The microbial composition of modern dairy starters represents a key point because the complex relationships among microorganisms can easily be altered. Little variations in the microbial composition could have unexpected effects on cheese quality.

Gonzalez-y-Merchand, J. A., I. Estrada-Garcia, et al. (1996). "A novel method for the isolation of mycobacterial DNA." FEMS Microbiology Letters **135**(1): 71.

http://www.sciencedirect.com/science/article/B6T2W-3W0P6D6-B/2/633abdae2fed99918b67aa880f80117e

DNA was isolated from mycobacteria by a simplified procedure. Cells were suspended in 6 M guanidinium chloride, the suspension was cooled to -70 [deg]C, then incubated at 65 [deg]C for 10 min, cooled in ice, deproteinized by chloroform and DNA was recovered from the supernatant. The procedure was used to obtain DNA from several mycobacteria (1 x 109) or more cells) including Mycobacterium neoaurum M. fortuitum M. phlei and M. smegmatis. Each of the species

was shown to have two ribosomal RNA operons per genome, and preliminary evidence was obtained which suggests that one of these operons is homologous with one of the operons of M. smegmatis.

Grant, K. A., J. H. Dickinson, et al. (1992). "Rapid identification of Aerococcus viridans using the polymerase chain reaction and an oligonucleotide probe." FEMS Microbiology Letters **95**(1): 63.

http://www.sciencedirect.com/science/article/B6T2W-4754C3H-2M/2/e5a86bdd7f4011b657fcecc283cd85a8

A polymerase chain reaction/oligonucleotide probe method was developed for the specific identification of the Gram-positive bacterium Aerococcus viridans. Primers for the enzymatic amplification reaction were designed from specific sequences within the 16S rRNA. The method was also highly sensitive and 10 cfu of A. viridans could be detected in 5 h although the reliability of detection was poor in mixed cultures with Escherichia coli.

Green, S. J., J. F. C. Michel, et al. (2004). "Similarity of bacterial communities in sawdust- and straw-amended cow manure composts." FEMS Microbiology Letters **233**(1): 115.

http://www.sciencedirect.com/science/article/B6T2W-4BSW4D7-3/2/e968791c057f5cb05063383f13ee4e2e

We analyzed bacterial communities in two cow manure composts derived from the same feed manure and composted in the same location, but composted with different carbon amendments, and in peat-based potting mixes amended with these composts. Bacterial communities were characterized by PCR-denaturing gradient gel electrophoresis (DGGE) analysis of extracted DNAs, and population fingerprints generated for each sample were compared. Sequence analyses of dominant DGGE bands revealed that members of the phylum Bacteroidetes were the most dominant bacteria detected in this study (19 of 31 clones). These analyses demonstrate that bacterial community profiles of individual composts were highly similar, as were profiles of compost-amended potting mixes. However, potting mix profiles differed substantially from the original compost profiles and from that of the peat base. These data indicate that highly similar bacterial populations were active in the two composts, and suggest that the effects of the initial carbon amendment on the mature compost bacterial communities were minor, while factors such as the feed manure and composting location may have been more influential.

Hallier-Soulier, S. and E. Guillot (1999). "An immunomagnetic separation polymerase chain reaction assay for rapid and ultra-sensitive detection of Cryptosporidium parvum in drinking water." FEMS Microbiology Letters **176**(2): 285.

http://www.sciencedirect.com/science/article/B6T2W-3WWV9W4-3/2/febaffc565e3078670e24f3e0255c8fe

A sensitive and rapid method was developed to detect Cryptosporidium parvum oocysts in drinking water. This molecular assay combined immunomagnetic separation with polymerase chain reaction amplification to detect very low levels of C. parvum oocysts. Magnetic beads coated with anti-cryptosporidium were used to capture oocysts directly from drinking water membrane filter concentrates, at the same time removing polymerase chain reaction inhibitory substances. The DNA was then extracted by the freeze-boil Chelex-100 treatment, followed by

polymerase chain reaction. The immunomagnetic separation-polymerase chain reaction product was identified by non-radioactive hybridization using an internal oligonucleotide probe labelled with digoxigenin. This immunomagnetic separation-polymerase chain reaction assay can detect the presence of a single seeded oocyst in 5-100-l samples of drinking water, thereby assuring the absence of C. parvum contamination in the sample under analysis.

Handal, T., I. Olsen, et al. (2005). "Detection and characterization of [beta]-lactamase genes in subgingival bacteria from patients with refractory periodontitis." FEMS Microbiology Letters **242**(2): 319.

http://www.sciencedirect.com/science/article/B6T2W-4DW3K00-1/2/ec1a7e48ce8127796cea7611c24f9c11

Fifty-three [beta]-lactamase-producing strains of oral bacteria isolated from patients with refractory periodontitis in Norway and USA were screened for the presence of the blaTEM, blaSHV, blaOXA, blaampC, blacfxA, and blacepA/cblA genes by the polymerase chain reaction (PCR). The PCR products were characterized by direct sequencing of the amplified DNA. Thirty-four of the 53 enzyme-producing strains (64%) were positive in one of the PCR assays. All [beta]-lactamase-producing Prevotella and Capnocytophaga spp. were CfxA positive. TEM-type [beta]-lactamases were identified in one strain each of Escherichia coli and Neisseria sp., and one strain of Citrobacter freundii possessed an AmpC-type [beta]-lactamase. Screening for gene cassettes and genes known to be associated with integrons did not reveal the presence of integrons in these oral bacteria. Sequence analyses showed that most CfxA positive Prevotella and Capnocytophaga isolates from patients with refractory periodontitis harboured variants of the CfxA2 and CfxA3 enzyme. The present study also showed that many different genetic determinants of [beta]-lactamase production are found in bacteria isolated from refractory periodontitis, many of which remain to be characterized.

Helgason, T., I. J. Watson, et al. (2003). "Phylogeny of the Glomerales and Diversisporales (Fungi: Glomeromycota) from actin and elongation factor 1-alpha sequences." FEMS Microbiology Letters **229**(1): 127.

http://www.sciencedirect.com/science/article/B6T2W-4B0WC17-1/2/a8e1b0217644fd8702674deb3c10c452

The arbuscular mycorrhizal (AM) fungi have been elevated to the phylum Glomeromycota based on a ribosomal gene phylogeny. In order to test this phylogeny, we amplified and sequenced small subunit ribosomal RNA (SSUrRNA), actin and elongation factor 1 (EF1)-alpha gene fragments from single spores of Acaulospora laevis, Glomus caledonium, Gigaspora margarita, and Scutellospora dipurpurescens. Sequence variation within and among spores of an isolate was low except for SSUrRNA in S. dipurpurescens, and the actin amino acid sequence was more conserved than that of EF1-alpha. The AM fungal sequences were more similar to one another than to any other fungal group. Joint phylogenetic analysis of the actin and EF1-alpha sequences suggested that the sister group to the AM fungi was a Zygomycete order, the Mortierellales.

Heuner, K., I. Bergmann, et al. (2001). "Proteolytic activity among various oral Treponema species and cloning of a prtP-like gene of Treponema socranskii subsp. socranskii." FEMS Microbiology Letters **201**(2): 169.

http://www.sciencedirect.com/science/article/B6T2W-43J81P6-9/2/a9b459f0171b774c64c5fd3d67da1698

The proteolytic activity of 11 treponemal strains representing different phylogenetic groups was investigated by SDS-polyacrylamide gel electrophoresis with copolymerised casein, gelatin and fibrinogen as substrates. The activity was specified to be trypsin- and chymotrypsin-like by the cleavage of synthetic substrates BAPNA and SAAPFNA, respectively. Nine strains degrade casein and the synthetic substrate BAPNA. Chymotrypsin-like activity specifically inhibited by phenylmethylsulfonyl fluoride was found in four treponemes. Southern blot analysis using a Treponema socranskii subsp. socranskii-specific prtP probe confirmed the presence of prtP homologous genes in these four strains. The internal fragments of the chymotrypsin-like protease genes were cloned and sequenced after PCR amplification. Here we report the cloning of the complete prtP-like gene of T. socranskii subsp. socranskii, an organism shown to possess epidemiologic relevance in periodontitis.

Heuzenroeder, M. W., C. Y. F. Wong, et al. (1999). "Distribution of two hemolytic toxin genes in clinical and environmental isolates of Aeromonas spp.: correlation with virulence in a suckling mouse model." FEMS Microbiology Letters **174**(1): 131.

http://www.sciencedirect.com/science/article/B6T2W-3W9CYSG-M/2/08f2a648bfe4a60d33c61b22dbc71c6b

Previous studies have shown that two hemolytic toxins, HlyA and AerA, contribute to the virulence of Aeromonas hydrophila. A survey was performed to gauge the distribution of hlyA and aerA genes in clinical and environmental Aeromonas isolates. For A. hydrophila, A. veronii biotype sobria and A caviae, 96%, 12% and 35% of strains, respectively, were hlyA positive, whereas, 78%, 97%, 41%, respectively, were aerA positive. All virulent A. hydrophila isolates were hlyA+ aerA+. This genotype was most common in A. hydrophila (75.4%) followed by A. caviae (29.4%) and A. veronii biotype sobria (9.6%). For A. hydrophila, a two-hemolytic toxin model of virulence provides the best prediction of virulence in an animal model.

Hookey, J. V. (1992). "Detection of Leptospiraceae by amplification of 16S ribosomal DNA." FEMS Microbiology Letters **90**(3): 267.

http://www.sciencedirect.com/science/article/B6T2W-4754BY4-11/2/4d7701a06378663efa641e2c4f9eabca

The polymerase chain reaction (PCR) was developed to detect Leptospiraceae. Primers were used to amplify a 631 base-pair (bp) 5'-region of 16S rDNA. Representative strains from the species, Leptospira interrogans sensu stricto, L. borgpetersenii, L. noguchii, L. santarosai, L. weilii, L. inadai, L. meyeri and the single member strain of Leptonema were amplified. In contrast, strains representing the saprophytic species, L. biflexa, L. wolbachii and L. parva were not amplified. There was no PCR product from 23 phylogenetically unrelated species of bacteria. As little as 10-1 pg of purified DNA and as few as 10-1 leptospires could be detected using this PCR analysis. Isolates of leptospires from clinical sources gave a positive PCR band, but those from surface waters did not.

Hu, Y. and A. R. M. Coates (2005). "Transposon mutagenesis identifies genes which control antimicrobial drug tolerance in stationary-phase Escherichia coli." FEMS Microbiology Letters **243**(1): 117.

http://www.sciencedirect.com/science/article/B6T2W-4F01KGD-3/2/8e8941d6180eefa315698d9e590c4708

Tolerance to antimicrobial agents is a universal phenomenon in bacteria which are no longer multiplying or whose growth rate slows. Since slowly multiplying bacteria occur in clinical infections, extended periods of antimicrobial chemotherapy are needed to eradicate these organisms and to achieve cure. In this study, the molecular basis of antibiotic tolerance was investigated using transposon mutagenesis. We screened 5000 Escherichia coli Tn10Cam mutants for reduction of kanamycin tolerance in late stationary phase and found that 4935 mutants were able to grow to late stationary phase. Reduced tolerance was observed in nine mutants which became sensitive to killing by kanamycin. The mutant KS639 was the most sensitive one to kanamycin, and its genome was disrupted in an intergenic region which lies between aldB and yiaW open reading frames. This mutant showed increased sensitivity not only to kanamycin but also to gentamicin, ciprofloxacin and rifampicin. Reduced tolerance of KS639 to kanamycin was also observed in a murine thigh infection model. P1 transduction to the wild type strains confirmed that the intergenic region was responsible for the tolerance of the bacterium to antibiotics. Using PCR-directed one-step gene replacement, we inactivated the genes aldB, yiaW and yiaV. We also deleted the intergenic region. There was no difference in kanamycin tolerance between each mutant ([Delta]aldB, [Delta]yiaW and [Delta]yiaV) and the parental strain. But the mutant lacking the intergenic region showed reduced tolerance to kanamycin. These data suggest that the intergenic region between aldB and yiaW genes may be involved in tolerance to antimicrobial agents in E. coli. Furthermore, they show that it is important in murine infection during antibiotic treatment and lead to a faster kill of the mutant bacteria.

Hu, Y., S. Kendall, et al. (2004). "The Mycobacterium tuberculosis sigJ gene controls sensitivity of the bacterium to hydrogen peroxide." FEMS Microbiology Letters **237**(2): 415.

http://www.sciencedirect.com/science/article/B6T2W-4CVV393-4/2/252a2bba6fbfdb8ee191f0b50464a626

Sigma factors are important global regulators which control bacterial gene expression during growth and in response to stress. Previous work showed that mRNA of the sigJ gene was up-regulated in late stationary-phase and after rifampicin treatment. In order to verify the function of SigJ, we constructed a Mycobacterium tuberculosis mutant lacking the sigJ gene. In a microaerophilic stationary-phase model, the sigJ mutant showed the same growth pattern as the wild-type strain. In an immune stasis murine model in which the bacterial number plateaued between the second and the 15th week, the mutant showed a similar growth curve to the wild-type strain. However, the sigJ mutant was more susceptible to killing by H2O2 than its parental strain. The parental level of sensitivity to H2O2 was recovered in the sigJ complemented strain. These data suggest that the SigJ protein is not essential for survival in long-term stationary phase or in bacterial stasis in mice. However, the sigJ gene may control an alternative H2O2 resistance pathway.

Huys, G. and J. Swings (1999). "Evaluation of a fluorescent amplified fragment length polymorphism (FAFLP) methodology for the genotypic discrimination of Aeromonas taxa." FEMS Microbiology Letters **177**(1): 83.

http://www.sciencedirect.com/science/article/B6T2W-3X10VC2-F/2/aa563b36e96fbb77f55b43882cb78d61

A fluorescent amplified fragment length polymorphism (FAFLP) fingerprinting assay is evaluated

for its ability to differentiate DNA hybridization groups in the genus Aeromonas. After empirical determination of optimal assay conditions using a limited set of strains, 98 well-characterized type and reference strains encompassing all known Aeromonas taxa were subjected to FAFLP fingerprinting using the standardized protocol. The present study clearly indicates that the use of fluorescent dye-labeled primers does not significantly affect the high capacity of this technique to differentiate among genotypically closely related Aeromonas taxa. Compared to the original AFLP protocol involving the application of radio-isotopes, the new FAFLP technology offers a better performance when considering speed of analysis and user safety. On the other hand, FAFLP fingerprints exhibited a significant reduction in the relative number of bands compared to the corresponding autoradiographic patterns. In our hands, the omission of the preselective amplification step and the use of a size standard mix enhanced the cost effectiveness and the reproducibility of the technique. Cluster analysis of FAFLP band patterns generated from Aeromonas type and reference strains demonstrated once more the high correlation of AFLP-generated data with DNA-DNA homology data.

Inoue, T., T. Tsuji, et al. (1993). "Amino acid sequence of heat-labile enterotoxin from chicken enterotoxigenic Escherichia coli is identical to that of human strain H 10407." FEMS Microbiology Letters **108**(2): 157.

http://www.sciencedirect.com/science/article/B6T2W-47DKW3N-23/2/1cb68e6ac40ba3c0d7e96ef7b76e1b8b

The DNA sequence of heat-labile enterotoxin from the chicken enterotoxigenic Escherichia coli 21d strain was determined by direct dideoxy sequencing of polymerase chain reaction (PCR)-amplified DNA and was compared with those of heat-labile enterotoxins from porcine and human enterotoxigenic E. coli strains EWD 299 and H 10407. The structural genes of the A and B subunits of chicken heat-labile enterotoxin were identical to those of human heat-labile enterotoxin from the human H 10407 strain. Moreover, 67 base pairs of the upstream and 60 base pairs of the downstream region of the chicken heat-labile enterotoxin gene were also identical to those of the human heat-labile enterotoxin from strain H 10407. However, the patterns of plasmids from the 21d and H 10407 strains were different. The 21d strain had no band corresponding to the 42-MDa plasmid of the H10407 strain encoding the heat-labile enterotoxin gene but it had a smaller plasmid. These data suggest that although the DNA sequence of chicken heat-labile enterotoxin is identical to that of human heat-labile enterotoxin, the plasmid encoding the chicken heat-labile enterotoxin gene in the chicken might be different from that encoding the human heat-labile enterotoxin gene in the H10407 strain.

Jain, S., K. Akiyama, et al. (2005). "Signaling via the G protein [alpha] subunit FGA2 is necessary for pathogenesis in Fusarium oxysporum." FEMS Microbiology Letters **243**(1): 165.

http://www.sciencedirect.com/science/article/B6T2W-4F29DFV-2/2/a70383cebffa3cded350ce43acc5f2a2

Cloning and disruption of fga1, the gene encoding the G protein [alpha] subunit FGA1 in phytopathogenic fungus Fusarium oxysporum, has been reported previously, and the fga1 disruptants showed altered colony morphology, increased heat resistance, reduced conidiation and pathogenicity. To further evaluate the role of G protein signaling in this fungus, cloning of fga2, which encodes the second G[alpha] protein FGA2, was performed by PCR methods. The deduced primary structure of FGA2 (355 amino acid residues) showed high identity with other G[alpha] proteins, which belong to class III of fungal G[alpha] proteins. Disruption of fga2 led to higher heat resistance, similar to the fga1 disruptants, but pathogenicity was completely lost, unlike the fga1 disruptants. Alteration of colony morphology and conidiation, which was observed

in the fga1 disruptants, was not observed in the fga2 disruptants. The fga1/fga2 double disruptants showed phenotypic alterations similar to the fga1 or fga2 single disruptants, but increase of heat resistance was much more pronounced than in each single disruptant.

Jean, J., D. D'Souza, et al. (2003). "Transcriptional enhancement of RT-PCR for rapid and sensitive detection of Noroviruses." FEMS Microbiology Letters **226**(2): 339.

http://www.sciencedirect.com/science/article/B6T2W-49D6T0W-3/2/d293862393c68069abc29fdbb23e2b0e

Previously reported nucleic acid sequence-based amplification (NASBA) primers specific for the GII Noroviruses were adapted for reverse transcriptase-polymerase chain reaction (RT-PCR), and detection sensitivity was then enhanced by a subsequent in vitro transcription of the RT-PCR amplicons. The NASBA-derived primers performed comparably to other broadly reactive GII Norovirus primers with respect to detection limits (i.e. 1 RT-PCR amplifiable unit (RT-PCRU) per reaction). Detection limits improved by approximately 1 log10 to 0.3 RT-PCRU per reaction when transcriptional enhancement and electrochemiluminescence (ECL) hybridization followed RT-PCR. The method shows promise for improved detection sensitivity in instances where very low levels of virus contamination might be anticipated.

Jensen, L. B. (1998). "Internal size variations in Tn1546-like elements due to the presence of IS1216V." FEMS Microbiology Letters **169**(2): 349.

http://www.sciencedirect.com/science/article/B6T2W-3VB4Y57-P/2/986d34451e60aacce683d79ae8612c1f

In this study, internal size variations in the VanA gene cluster Tn1546, encoding resistance to glycopeptides, is described. Studies of previously uncharacterized size variations of an internal region, encoding the vanX and vanY genes of Tn1546, revealed that these variations were due to the presence of the IS sequence, IS1216V. This IS sequence has previously been found integrated in Tn1546. Integration of the IS1216V element created both deletions and a duplication in a non-essential region of Tn1546. In several isolates, the entire vanY gene was deleted, proving that this gene is non-essential for vancomycin resistance.

Jensen, L. B., N. Frimodt-Moller, et al. (1999). "Presence of erm gene classes in Gram-positive bacteria of animal and human origin in Denmark." FEMS Microbiology Letters **170**(1): 151.

http://www.sciencedirect.com/science/article/B6T2W-3VGTHJK-R/2/2f19b0d224a41704a2bcbb2e99ce0c6b

A classification of the different erm gene classes based on published sequences was performed, and specific primers to detect some of these classes designed. The presence of ermA (Tn554), ermB (class IV) and ermC (class VI) was determined by PCR in a total of 113 enterococcal, 77 streptococcal and 68 staphylococcal erythromycin resistant isolates of animal and human origin. At least one of these genes was detected in 88% of the isolates. Four isolates contained more than one erm gene. ermB dominated among the enterococci (88%) and streptococci (90%) and ermC among staphylococci (75%) with ermA (Tn554) present in some isolates (16%). Variations in the presence of the different genes when comparing staphylococcal isolates of human and animal origin were observed.

Ji, X., F. Frati, et al. (2002). "Evolution of functional polymorphism in the gene coding for the Helicobacter pylori cytotoxin." FEMS Microbiology Letters **206**(2): 253.

http://www.sciencedirect.com/science/article/B6T2W-44PVR8K-2/2/b5da2e758acff26c4346bb4136d5f065

There are two functionally different alleles of the Helicobacter pylori vacA gene, which code for proteins with different in target cell specificity. The alleles (m1 and m2) differ by approximately 50% in amino acid sequence in a 300 amino acid region, the m-region, which determines specificity. An analysis of partial likelihood anomalies in a set of eight Chinese and six Western vacA genes revealed highly significant phylogenetic deviation of a region of the gene including the m-region. Phylogenetic analysis of the conserved regions of these genes failed to reveal any distinction between m1 alleles and m2 alleles, however clear cut geographic variation was observed. In the m-region, the m1 alleles also show separate clustering of Chinese and Western isolates, however the m-region of the m2 alleles has a phylogenetic structure markedly different from the rest of the gene. The data indicate that the m2 m-region was acquired and spread through the population by horizontal transfer of DNA.

Johanson, A., H. C. Turner, et al. (1998). "A PCR-based method to distinguish fungi of the rice sheath-blight complex, Rhizoctonia solani, R. oryzae and R. oryzae-sativae." FEMS Microbiology Letters **162**(2): 289.

http://www.sciencedirect.com/science/article/B6T2W-3SV3P41-F/2/6b02ce9e6e98b28f7eba17f4ad3a20e9

Identification of Rhizoctonia solani, R. oryzae and R. oryzae-sativae, components of the rice sheath disease complex, is extremely difficult and often inaccurate and as a result may hinder the success of extensive breeding programmes throughout Asia. In this study, primers designed from unique regions within the rDNA internal transcribed spacers have been used to develop a rapid PCR-based diagnostic test to provide an accurate identification of the species on rice. Tests on the specificity of the primers concerned showed that they provide the means for accurate identification of the Rhizoctonia species responsible for sheath diseases in rice.

Kaji, M., Y. Taniguchi, et al. (1999). "The hydA gene encoding the H2-evolving hydrogenase of Clostridium perfringens: molecular characterization and expression of the gene." FEMS Microbiology Letters **181**(2): 329.

http://www.sciencedirect.com/science/article/B6T2W-3Y0J7W3-P/2/4e8d30ab636791402e43dfccd09862d6

A putative hydrogenase (hydA) gene of Clostridium perfringens encodes a protein with strong identity to Clostridium pasteurianum hydrogenase I. Disruption of the hydA gene abolished H2 productivity, confirming its function. A putative butyrate kinase gene (buk) is adjacent to the hydA gene. When cultures were grown in medium with glucose, 1.8-kb hydA and 2.1-kb buk transcripts and a 3.9-kb transcript hybridized with both hydA and buk-probe were detectable in all the exponential growth phases. In medium without glucose, these transcripts were decreased rapidly after the mid-exponential phase. These results suggest that the transcription of these two genes is probably regulated by a similar mechanism in response to glucose availability.

Kataoka, M., K. Ueda, et al. (1997). "Application of the variable region in 16S rDNA to create an index for rapid species identification in the genus Streptomyces." FEMS Microbiology Letters **151**(2): 249.

http://www.sciencedirect.com/science/article/B6T2W-3S6D1CW-2C/2/fb5dff209eeb2eccea25e527c4c75e9c

Partial nucleotide sequences (120 bp) of the 16S rRNA gene (rDNA) containing a variable [alpha] region were compared in 89 strains of the genus Streptomyces belonging to eight major clusters of category I in Bergey's Manual of Systematic Bacteriology. Fifty-seven kinds of partial 16S rDNA sequences were observed among the 89 strains. Forty-three of the strains were grouped into 11 'identity groups', based on the fact that the strains in each group shared an identical sequence in the 120-bp region. The results of a phylogenetic analysis based on the 16S rDNA 120-bp sequences revealed that 60 of the 89 strains could be categorized into seven clusters, each consisting of four or more strains. Based on these observations it was concluded that short nucleotide sequences bearing the variable [alpha] region are useful for Streptomyces species identification.

Kato, N., C.-X. Liu, et al. (2000). "A new subtype of the metalloprotease toxin gene and the incidence of the three bft subtypes among Bacteroides fragilis isolates in Japan." FEMS Microbiology Letters **182**(1): 171.

http://www.sciencedirect.com/science/article/B6T2W-3Y6GV34-11/2/d07c7f78f20072057b8d51f4530a936c

The bft gene encoding Bacteroides fragilis toxin (BFT) has been devided into two subtypes, bft-1 and bft-2. We found a novel subtype by sequencing a segment of the bft gene from 64 enterotoxigenic B. fragilis (ETBF) strains isolated in Japan. The 1548-bp nucleotide sequences of the new bft, the bft-1, and bft-2 genes were determined for five, four, and four ETBF strains, respectively; the nucleotide sequence was identical among each bft subtype and the degree of identity between each subtype was between 89 and 94%. Most of the variations between the three subtypes were detected in the region encoding mature toxin. A multiplex PCR was developed with a four-primer mix to subtype the bft sequences. The subtyping of 143 ETBF isolates from extraintestinal and stool specimens of humans and cows showed that the bft-1 was the most prevalent subtype, followed by bft-2 and a new bft subtype. No other subtype was found among the strains tested.

Kawabata, S., Y. Terao, et al. (1997). "Nucleotide sequence and molecular characterization of a gene encoding GTP-binding protein from Streptococcus gordonii." FEMS Microbiology Letters **156**(2): 211.

http://www.sciencedirect.com/science/article/B6T2W-3RD1SSV-1K/2/0886ea185a8f59ba7c1dd283af6befcf

A 1286-bp fragment of chromosomal DNA from Streptococcus gordonii strain Challis was cloned and sequenced. The gene sgg consisted of 897-bp nucleotides encoding a 299-amino acid polypeptide (33200 Da). The deduced amino acid sequence exhibited significant similarity to Era, G protein of Escherichia coli. The nucleotide binding assay demonstrated that recombinant Sgg bound [32P]GTP but not [32P]ATP, [32P]CTP, or [32P]UTP. These findings indicate that Sgg is a member of the G protein superfamily in the genus Streptococcus.

Kawaguchi, R., J. G. Burgess, et al. (1995). "Phylogenetic analysis of a novel sulfate-reducing magnetic bacterium, RS-1, demonstrates its membership of the [delta]-Proteobacteria." FEMS Microbiology Letters **126**(3): 277.

http://www.sciencedirect.com/science/article/B6T2W-3XX6V2G-C/2/687aa640277e1a9f9e719e176dd5732a

Most of the 16S ribosomal RNA gene of a sulfate-reducing magnetic bacterium, RS-1, was sequenced, and phylogenetic analysis was carried out. The results suggest that RS-1 is a member of the [delta]-Proteobacteria, and it appears to represent a new genus. RS-1 is the first bacterium reported outside the [alpha]-Proteobacteria that contains magnetite inclusions. RS-1 therefore disrupts the correlation between the [alpha]-Proteobacteria and possession of magnetite inclusions, and that between the [delta]-Proteobacteria and possession of greigite inclusions. The existence of RS-1 also suggests that intracellular magnetite biomineralization is of multiple evolutionary origins.

Keon, J. and J. Hargreaves (1998). "Isolation and heterologous expression of a gene encoding 4-hydroxyphenylpyruvate dioxygenase from the wheat leaf-spot pathogen, Mycosphaerella graminicola." FEMS Microbiology Letters **161**(2): 337.

http://www.sciencedirect.com/science/article/B6T2W-3SF0Y74-J/2/a82bf5dc1970a31c1f623e22de4dde3b

We describe the isolation and sequence of a gene encoding 4-hydroxyphenylpyruvate dioxygenase (HPPD (EC 1.13.11.27)) from the wheat leaf-spot fungal pathogen Mycosphaerella graminicola (Septoria tritici), that directs the synthesis of 2,5-dihydroxyphenylacetate (homogentisic acid, HGA). The sequence of the deduced peptide showed homology to HPPDs from other organisms; the greatest identity was to a T-cell reactive protein, also identified as HPPD, from the human fungal pathogen Coccidioides immitis. As observed for HPPD from other sources, expression of the M. graminicola HPPD gene in Escherichia coli cells could be detected by the gradual development of a brown pigment in cultures as a result of the spontaneous oxidation and polymerisation of HGA. Pigment development in these cultures was prevented by the HPPD inhibitor sulcotrione.

Kitamoto, N., T. Kimura, et al. (1993). "Structural features of a polygalacturonase gene cloned from Aspergillus oryzae KBN616." FEMS Microbiology Letters **111**(1): 37.

http://www.sciencedirect.com/science/article/B6T2W-476W53R-11N/2/806d38db38e62130a76291cabfb5e2a9

A genomic gene encoding a polygalacturonase from Aspergillus oryzae, used in soy sauce production, was cloned and sequenced. The structural gene comprises 1227 bp coding for 363 amino acids with a putative prepropeptide of 28 amino acids and the open reading frame is disrupted by two short introns of 57 bp and 81 bp. The deduced amino acid sequence of the mature protein showed 63, 63, 63 and 64% homology with those of Aspergillus niger polygalacturonase I, Aspergillus niger polygalacturonase II, Aspergillus tubingensis polygalacturonase II and Cochliobolus carbonum polygalacturonase, respectively. There is, however, little homology among fungal, plant and bacterial polygalacturonases.

Kitazawa, H., Y. Tomioka, et al. (1994). "Expression of mRNA encoding IFN[alpha] in macrophages stimulated with Lactobacillus gasseri." FEMS Microbiology Letters **120**(3): 315.

http://www.sciencedirect.com/science/article/B6T2W-476HPGD-G9/2/bd324e0c2f401f9b1d10ee821f960319

The ability of Lactobacillus gasseri, a dairy lactic acid bacterium, to induce interferon (IFN) was investigated in murine macrophage cultures. IFN[alpha] was substantially induced by some strais of L. gasseri and the titers were the highest at a concentration of 100 [mu]g ml-1 of L. gasseri DSM20243T. The expression of mRNA encoding IFN[alpha] was detected in spleen-macrophages (SP-M[theta]) and Peyer's patch-adherent cells stimulated with L. gasseri DSM20243T. Actinomycin D and cycloheximide added to SP-M[theta] cultures showed that the mRNA was synthesized by 0.5 h, and that IFN[alpha] was produced within 3 to 6 h after the stimulation with L. gasseri DSM20243T. The results support the notion that dairy products containing L. gasseri can be 'physiologically functional foods'.

Kobayashi, Y., R. J. Forster, et al. (2000). "Development of a competitive polymerase chain reaction assay for the ruminal bacterium Butyrivibrio fibrisolvens OB156 and its use for tracking an OB156-derived recombinant." FEMS Microbiology Letters **188**(2): 185.

http://www.sciencedirect.com/science/article/B6T2W-40NG503-F/2/d9a6f8145bcdac5635543253db18e9c1

A competitive polymerase chain reaction assay targeting the 16S rDNA was developed for quantitating the rumen bacterium Butyrivibrio fibrisolvens OB156. A competitor DNA, serving as an internal control in the competitive polymerase chain reaction reaction, was constructed by polymerase chain reaction using a looped oligo longer than the normal primer. Coamplification of the target DNA with known amounts of the competitor DNA allowed quantitation of the target DNA in both pure culture and mixed culture systems, where minimum quantifiable level of OB156 was 1.7 x 102 and 5.6 x 104 cells, respectively. When an erythromycin-resistant recombinant derived from OB156 was inoculated into a rumen fluid culture, its numbers decreased with time. The rate of decrease measured by the competitive polymerase chain reaction assay was much slower than the rate determined by culture enumeration using erythromycin selection. The competitive polymerase chain reaction assay also showed 48 h persistence of the recombinant at 104 ml-1 even after disappearance of culturable recombinant, suggesting maintenance of the target DNA from uncultivable cells. In an in vivo tracking trial, the recombinant became undetectable within 72 h with either assay, indicating rapid hydrolysis and/or outflow of the cells from the rumen.

Koike, S. and Y. Kobayashi (2001). "Development and use of competitive PCR assays for the rumen cellulolytic bacteria: Fibrobacter succinogenes, Ruminococcus albus and Ruminococcus flavefaciens." FEMS Microbiology Letters **204**(2): 361.

http://www.sciencedirect.com/science/article/B6T2W-44B23S6-2/2/9d44a4e0051f572fb1500661184e719b

Competitive PCR assays were developed for the enumeration of the rumen cellulolytic bacterial species: Fibrobacter succinogenes, Ruminococcus albus and Ruminococcus flavefaciens. The assays, targeting species-specific regions of 16S rDNA, were evaluated using DNA from pure culture and rumen digesta spiked with the relevant cellulolytic species. Minimum detection levels

for F. succinogenes, R. albus and R. flavefaciens were 1-10 cells in pure culture and 103-4 cells per ml in mixed culture. The assays were reproducible and 11-13% inter- and intra-assay variations were observed. Enumeration of the cellulolytic species in the rumen and alimentary tract of sheep found F. succinogenes dominant (107 per ml of rumen digesta) compared to the Ruminococcus spp. (104-6 per ml). The population size of the three species did not change after the proportion of dietary alfalfa hay was increased. All three species were detected in the rumen, omasum, caecum, colon and rectum. Numbers of the cellulolytic species at these sites varied within and between animals.

Kulik, E. M., H. Sandmeier, et al. (2001). "Identification of archaeal rDNA from subgingival dental plaque by PCR amplification and sequence analysis." FEMS Microbiology Letters **196**(2): 129.

http://www.sciencedirect.com/science/article/B6T2W-42M1FGD-8/2/8c6eb81939cc6c1cc0ba14feb7b38345

A PCR assay for the amplification of small subunit ribosomal DNA (SSU rDNA) of Euryarchaea was developed and used to detect archaeal rDNA in 37 (77%) out of 48 pooled subgingival plaque samples from 48 patients suffering from periodontal disease. One major group of cloned periodontal sequences was identical to Methanobrevibacter oralis and a second minor group to Methanobrevibacter smithii. These two groups and a third novel group were found to be more than 98% similar to each other over an 0.65-kb segment of the 16S rRNA gene sequenced. M. oralis was found to be the predominant archaeon in the subgingival dental plaque. Phylogenetic analysis of partial SSU rDNA sequences revealed evidence for a distinct cluster for human and animal Methanobrevibacter sp. within the Methanobacteriaceae family.

Lee, C. W., R. Y. C. Lo, et al. (1994). "The detection of the sialoglycoprotease gene and assay for sialoglycoprotease activity among isolates of Pasteurella haemolytica A1 strains, serotypes A13, A14, T15 and A16." FEMS Microbiology Letters **121**(2): 199.

http://www.sciencedirect.com/science/article/B6T2W-476W4HH-PG/2/cc8e3d959b7b2a89ed43d9caf8121b8d

Polymerase chain reaction (PCR) using specific primers to the sialoglycoprotease gene (gcp) of Pasteurella haemolytica biotype A, serotype 1 amplified a 1-kb fragment from each of P. haemolytica serotypes A7, A13, A14 and A16, but not T15; which was confirmed by Southern blot hybridization analysis. Using a sialoglycoprotease (Gcp) activity assay, Gcp activity was found in serotypes A13, A14 and A16. Inclusion of these three serotypes confirms that all recognized A biotypes are positive for both gcp gene and activity, with the exception of serotype A11 (which has a different genetic organization and exhibits no Gcp activity). Furthermore, all recognized T biotypes are negative for both the gene and Gcp activity.

Lee, I. M., Y. Zhao, et al. (2005). "Novel insertion sequence-like elements in phytoplasma strains of the aster yellows group are putative new members of the IS3 family." FEMS Microbiology Letters **242**(2): 353.

http://www.sciencedirect.com/science/article/B6T2W-4DXJVD1-4/2/338134742a904aa313e888b2c9870dcd

Novel insertion sequence (IS)-like elements were isolated and characterized from phytoplasma

strains in the aster yellows (AY) group (16SrI). The IS-like elements were cloned from phytoplasma strains AY1 and NJAY or PCR-amplified from 15 additional strains representing nine subgroups in the AY group using primers based on sequences of the putative transposases (Tpases). All IS-like elements contained sequences encoding similar Tpases of 321 amino acids (320 for strain CPh). Substantial amino acid sequence variability suggested multiple species of Tpases or IS-like elements exist in the AY phytoplasma group. These Tpases have an identical DDE motif that is most similar to the DDE consensus of Tpases in the IS3 family.

Lepercq, P., P. Gerard, et al. (2004). "Epimerization of chenodeoxycholic acid to ursodeoxycholic acid by Clostridium baratii isolated from human feces." FEMS Microbiology Letters **235**(1): 65.

http://www.sciencedirect.com/science/article/B6T2W-4C6KKKH-1/2/834f2fbe9f826bca28bae82b9ce30914

Ursodeoxycholic acid-producing bacteria are of clinical and industrial interest due to the multiple beneficial effects of this bile acid on human health. This work reports the first isolation of 7-epimerizing bacteria from feces of a healthy volunteer, on the basis of their capacity to epimerize the primary bile acid, chenodeoxycholic acid, to ursodeoxycholic acid. Five isolates were found to be active starting from unconjugated chenodeoxycholic acid and its tauro-conjugated homologue, but none of these strains could epimerize the glyco-conjugated form. Biochemical testing and 16S ribosomal DNA sequencing converged to show that all five isolates were closely related to Clostridium baratii (99% sequence similarity), suggesting that this bacterial species could be responsible at least partially, for this bioconversion in the human gut.

Leung, D., J. van der Oost, et al. (1994). "Mutagenesis of a gene encoding a cytochrome o-like terminal oxidase of Azotobacter vinelandii: A cytochrome o mutant is aero-tolerant during nitrogen fixation." FEMS Microbiology Letters **119**(3): 351.

http://www.sciencedirect.com/science/article/B6T2W-47DKWH7-CT/2/3f03f936df479e8b90684ddeb0765371

The amino acid sequence obtained by translating the nucleotide sequence of a 0.55 kb fragment, amplified from Azotobacter vinelandii chromosomal DNA by PCR, was 57% identical to part of the Escherichia coli cyoB gene, encoding subunit I of the cytochrome bo-type quinol oxidase. This fragment was mutated in vitro by insertion of a kanamycin-resistance cassette and introduced into the chromosome of A. vinelandii by homologous recombination. The mutant contained no spectrally detectable cytochrome o. However, in the stationary phase of growth, the level of the alternative oxidase (cytochrome bd) was 11-fold higher than in the wild-type strain. Respiration of the mutant was insensitive to chlorpromazine, an inhibitor thought to act specifically on cytochrome o. Cytochrome o-deficient mutants fixed nitrogen in air, clearly distinguishing the role of this oxidase from that of cytochrome bd, which is required for respiratory protection of oxygen-labile nitrogenase.

Lo, R. Y. C., M.-A. Watt, et al. (1994). "Preparation of recombinant glycoprotease of Pasteurella haemolytica A1 utilizing the Escherichia coli [alpha]-hemolysin secretion system." FEMS Microbiology Letters **116**(2): 225.

http://www.sciencedirect.com/science/article/B6T2W-476W31N-7N/2/9e5f88c1c78f2736d829cc1239905e32

Three murine monoclonal antibodies were prepared against the recombinant glycoprotease of Pasteurella haemolytica A1 expressed in Escherichia coli. These monoclonal antibodies were able to recognize the authentic glycoprotease from P. haemolytica A1 culture supernatant. A recombinant plasmid which contained most of the glycoprotease gene of P. haemolytica A1 fused with the secretion signal sequence from hlyA of the E. coli [alpha]-hemolysin determinant was constructed. This recombinant plasmid expressed a fusion protein (Gcp-F) which was secreted into the culture supernantant by E. coli cells when the [alpha]-hemolysin secretion functions HlyB and HlyD are supplied in trans. Gcp-F could be readily recovered from the supernatant free from other cellular materials and is suitable for use in vaccine trials and challenge experiments in animals.

Lodge, J. M., M. Robey, et al. (1999). "Iterative selection from a Salmonella typhimurium cosmid library can lead to the isolation of an atypically small plasmid." FEMS Microbiology Letters **170**(2): 355.

http://www.sciencedirect.com/science/article/B6T2W-3VM6VBM-B/2/33f0c0ee915cd68061a1ff3f9f5e32ce

We have constructed a DNA library from a virulent Salmonella typhimurium strain, in an avirulent strain. The process of selecting the components of interest from the library involved iterative growth in liquid culture. This resulted, after four cycles, in the culture becoming homogeneous for a single plasmid, which was much smaller than the average size for the library. We have identified the larger precursor of this plasmid which has two regions of sufficient homology to allow recombination resulting in the formation of the small plasmid. S. typhimurium carrying the small plasmid have a smaller genetic burden than other members of the library and survive better in spent culture medium, facilitating selection on repeated subculture. Such rapid adventitious selection has important implications for isolation of clones of interest from genomic libraries.

Loffler, J., S. L. Kelly, et al. (1997). "Molecular analysis of cyp51 from fluconazole-resistant Candida albicans strains." FEMS Microbiology Letters **151**(2): 263.

http://www.sciencedirect.com/science/article/B6T2W-3S6D1CW-2F/2/b067320724afc141501786ac475ebd2c

The target enzyme for fluconazole is sterol 14[alpha]-demethylase, a cytochrome P450 encoded by cyp51. One mechanism of fluconazole resistance likely to occur in Candida albicans is through an altered target site. To test this hypothesis DNA sequencing of the cyp51 coding sequence from 19 fluconazole-resistant and 19 fluconazole-sensitive C. albicans was undertaken. A number of point mutations were identified in the resistant isolates which were not present in the sensitive ones: F105L (five), E266D (five), K287R (one), G448G (one), G450E (one), G464S (three) and V488I (one). These alterations are discussed in the light of a molecular model of the enzyme regarding potential roles in resistance. It was also demonstrated that sequence-specific primers can be employed to identify polymorphisms which may be associated with resistance; diagnostic tests for resistant strains will prove of value in combating this serious clinical problem.

Lozano-Leon, A., J. Torres, et al. (2003). "Identification of tdh-positive Vibrio parahaemolyticus from an outbreak associated with raw oyster consumption in Spain." FEMS Microbiology Letters **226**(2): 281.

http://www.sciencedirect.com/science/article/B6T2W-49CTCM9-

3/2/fa34ca3e8e17406c4db90bbc14e8b63d

Between August and September 1999, a total of 64 cases of illness were identified in three episodes of acute gastroenteritis associated with the consumption of live oysters from a typical outdoor street market in Galicia (northwest Spain). Nine case patients were hospitalized and analysis of their stool samples revealed the presence of Vibrio parahaemolyticus. The strains isolated from two stool samples were studied for antibiotic susceptibility, biochemical characteristics and presence of virulence factors. Both isolates were Kanagawa phenomenon positive and produced thermostable direct hemolysin, which is related to pathogenicity in humans. These results show the presence of pathogenic V. parahaemolyticus in mollusks harvested in Europe and reveal the risk of illness associated with their consumption, suggesting the revision of V. parahaemolyticus risk assessment associated with consumption of raw live shellfish.

Luft, B. J., S. Pawagi, et al. (1992). "Analysis and expression of the Borrelia burgdorferi P/Gau fla gene: Identification of heterogeneity with the B31 strain." FEMS Microbiology Letters **93**(1): 63.

http://www.sciencedirect.com/science/article/B6T2W-47DDC8H-32/2/c0a3485f8060e5d102307c85fa73c9a2

The flagellin gene from the P/Gau strain of Borrelia burgdorferi was cloned and sequenced. The translated P/Gau flagellin protein differed from the flagellin of the B31 strain at 13 of 336 amino acids. This includes seven differences between amino acids 190-234, an immunodominant and specific region for B. burgdorferi. The entire flagellin molecule, as well as peptides of the internal portion of the protein which is more specific for B. burgdorferi, has been expressed in Escherichia coli using a pET7HIs.2 expression system. These peptides may be of great value for the development of sensitive and specific recombinant-based serological assays.

Lunde, M., J. M. Blatny, et al. (2000). "The life cycles of the temperate lactococcal bacteriophage [phi]LC3 monitored by a quantitative PCR method." FEMS Microbiology Letters **192**(1): 119.

http://www.sciencedirect.com/science/article/B6T2W-41F5Y3B-P/2/3e9bf099227d81a20a4b2bfbacfa7382

We present here a new and general approach for monitoring the life cycles of temperate bacteriophages which establish lysogeny by inserting their genomes site-specifically into the bacterial host chromosome. The method is based on quantitative amplification of specific DNA sites involved in various cut-and-join events during the life cycles of the phages (i.e. the cos, attP, attB, attL and attR sites) with the use of sequence-specific primers. By comparing the amounts of these specific DNA sites at different intervals, we were able to follow the development of the lytic and lysogenic life cycles of the temperate lactococcal bacteriophage [phi]LC3 after infection of its bacterial host Lactococcus lactis ssp. cremoris IMN-C18.

Marcobal, A., B. de las Rivas, et al. (2004). "Identification of the ornithine decarboxylase gene in the putrescine-producer Oenococcus oeni BIFI-83." FEMS Microbiology Letters **239**(2): 213.

http://www.sciencedirect.com/science/article/B6T2W-4D98D7G-1/2/2f0da1bd87515118998bcfcb2170fd6d

We report here the identification of an ornithine decarboxylase (ODC) gene in the putrescine-producer Oenococcus oeni BIFI-83 strain. The gene contains a 2,235-nucleotide open reading frame encoding a 745-amino acid residues protein with a deduced molecular mass of 81 kDa. The primary structure of the ODC deduced from the nucleotide sequence has a consensus sequence containing the pyridoxal-5-phosphate (PLP) binding domain, and the critical amino acids residues involved in enzymatic activity are also conserved. As determined by BLAST analysis, the deduced amino acid sequence of the odc gene shares a 67% identity with the ODC protein from Lactobacillus 30a. The odc gene appears to be rarely present in the genome of O. oeni, since in a screening for the presence of this gene in 42 oenococcal strains none of the strains possessed an odc gene copy.

Matsumoto, M., S. Hamada, et al. (2003). "Molecular analysis of the inhibitory effects of oolong tea polyphenols on glucan-binding domain of recombinant glucosyltransferases from Streptococcus mutans MT8148." FEMS Microbiology Letters **228**(1): 73.

http://www.sciencedirect.com/science/article/B6T2W-49PYH6M-1/2/b77f5dac73003ce5f9307e509c749394

An oolong tea polyphenol (OTF6) has been shown to possess a strong anti-glucosyltransferase (GTF) activity and inhibit experimental dental caries in rats infected with mutans streptococci. The effects of OTF6 on the functional domains of GTFs of Streptococcus mutans, an N-terminal catalytic domain (CAT), and a C-terminal glucan-binding domain (GBD), were examined. The maximum velocity of glucan synthesis by recombinant GTFB (rGTFB) and GTFD (rGTFD) became significantly slower in the presence of OTF6, however, Km values remained stable when compared in their absence. These results suggest that OTF6 reduces glucan synthesis by non-competitively inhibiting the GBD of S. mutans GTFB and GTFD. Further, the recombinant proteins of CAT (rCAT) and GBD (rGBD) were expressed using Escherichia coli, and purified by affinity column chromatography. rGBD but not rCAT was found to possess dextran-binding activity, which was shown to be inhibited by OTF6. These results indicate that OTF6, a polymeric polyphenol specific for oolong tea is able to reduce glucan synthesis by inhibiting the GBD of S. mutans GTFB.

Miles, K., L. McAuliffe, et al. (2004). "Rapid detection of Mycoplasma dispar and M. bovirhinis using allele specific polymerase chain reaction protocols." FEMS Microbiology Letters **241**(1): 103.

http://www.sciencedirect.com/science/article/B6T2W-4DKDV1G-2/2/206b6c79ee7c6f94c4e77f98684a24cb

We describe an allele specific PCR based approach for the rapid detection of two bovine Mycoplasma species associated with respiratory disease. Specific and universal oligonucleotides were used in combination to detect the presence of single nucleotide polymorphisms within the 16S ribosomal DNA sequence. Presence of Mycoplasma 16S rDNA is indicated by the production of a single control fragment, whilst positive samples generate an alternative smaller specific product over the same region. This technique provides a reliable and sensitive method which, although widely used in human genetic screening, has not been documented for diagnosis of bacterial infection.

Millner, P. D., W. W. Mulbry, et al. (2001). "Taxon-specific oligonucleotide primers for detection of two ancient endomycorrhizal fungi, Glomus occultum and Glomus brasilianum." FEMS Microbiology

Letters **196**(2): 165.

http://www.sciencedirect.com/science/article/B6T2W-42M1FGD-G/2/b8bf057bc5a3e9a011a49bc668416f1b

A unique oligonucleotide pair, GOCC56:GOCC427, was designed that correctly primed specific amplification of a ~370-bp sequence spanning the ITS and 5.8S rDNA regions of Glomus occultum and Glomus brasilianum. In addition, this primer pair successfully detected G. occultum and G. brasilianum DNA in nested PCR using a primary PCR product amplified from highly diluted extracts of colonized corn (Zea mays) roots using modified ITS1:ITS4 primers. A second primer pair, GBRAS86:GBRAS388, primed specific amplification of a ~200-bp sequence spanning the ITS and 5.8S rDNA regions present only in G. brasilianum and Glomus strain GR582. Combined use of both primer pairs provides the means to detect and differentiate two ancient endomycorrhizal species, G. occultum and G. brasilianum, undetectable by standard root staining procedures. Sequence analysis showed that the purported G. occultum strain GR582 is likely a strain of G. brasilianum.

Mkay, G. J. and L. R. Cooke (1997). "A PCR-based method to characterise and identify benzimidazole resistance in Helminthosporium solani." FEMS Microbiology Letters **152**(2): 371.

http://www.sciencedirect.com/science/article/B6T2W-3S6D1CW-3D/2/9607a21b86b5b611c7310433cdc9ca47

Control of Helminthosporium solani, the cause of silver scurf in potato tubers, has been impaired by selection of benzimidazole-resistant strains as a result of repeated use of the fungicide thiabendazole. Identification of thiabendazole-resistant strains of H. solani by conventional techniques takes several weeks. Primers designed from conserved regions of the fungal [beta]-tubulin gene were used to PCR amplify and sequence a portion of the gene. A point mutation was detected at codon 198 in thiabendazole-resistant isolates causing a change in the amino acid sequence from glutamic acid to alanine or glutamine. Species-specific PCR primers designed to amplify this region were used in conjunction with a restriction endonuclease to cause cleavage in sensitive isolates only and thus provide a rapid diagnostic test to differentiate field isolates.

Moreira, D. and R. Amils (1996). "PCR-mediated detection of the chemolithotrophic bacterium Thiobacillus cuprinus using 23S rDNA- and 16S/23S intergenic spacer region-targeted oligonucleotide primers." FEMS Microbiology Letters **142**(2-3): 289.

http://www.sciencedirect.com/science/article/B6T2W-3W2YG0D-X/2/35cb87c3b6833b206f69f5aba5f2798d

Bioleaching is carried out by chemolithotrophic microorganisms, most of them belonging to the genera Thiobacillus and Leptospirillum. The role of the mixotrophic species T. cuprinus in this process is controversial, since its ecological study applying classical detection techniques to natural or industrial environments is very difficult. For this reason, we have developed an alternative method based on PCR-mediated detection using specific oligonucleotide primers that target variable regions of the 23S rRNA coding gene and of the 16S/23S intergenic spacer region. Specificity and sensitivity of PCR amplifications performed with both kinds of primers were studied.

Mori, S., A. Castoreno, et al. (2002). "Transcript levels of rbcR1, ntcA, and rbcL/S genes in cyanobacterium Anabaena sp. PCC 7120 are downregulated in response to cold and osmotic stress." FEMS Microbiology Letters **213**(2): 167.

http://www.sciencedirect.com/science/article/B6T2W-468CCFT-2/2/68ae5acfe2c530f6b5714ed86242e357

Using differential display, we identified the Anabaena sp. PCC 7120 ribulose 1,5-bisphosphate carboxylase transcriptional regulator (rbcR1) gene, a member of the LysR family of positive transcription factors. The rbcR1 transcript and its putative target gene ribulose 1,5-bisphosphate carboxylase/oxygenase (rbcL/S) were repressed by cold (20[deg]C) and osmotic (sucrose and salt) stress. Cold stress also induced a transient downregulation of the Anabaena 7120 ntcA transcriptional regulator. Expression of the ntcA gene, however, returned to normal levels 2 h after initiation of cold stress and increased significantly above normal levels 24 h after growth at 20[deg]C. The early decline in the expression of the ntcA, rbcR1, and rbcL/S transcripts appears to be part of the Anabaena 7120 global adaptation response to stress. The substantial increase in the ntcA gene expression 24 h following cold stress suggests that Anabaena 7120 experiences substantial nitrogen limitation under these conditions. These data suggest that in response to stress, Anabaena 7120 decreases its metabolic activity through regulation of the CO2 fixation machinery while enhancing its nitrogen assimilation by inducing the expression of the nitrogen global transcriptional regulator, NtcA.

Mouyna, I., C. Henry, et al. (2004). "Gene silencing with RNA interference in the human pathogenic fungus Aspergillus fumigatus." FEMS Microbiology Letters **237**(2): 317.

http://www.sciencedirect.com/science/article/B6T2W-4CTJ43J-1/2/65f4618005bfbbdc1480538e875233f5

Aspergillus fumigatus is an opportunistic pathogenic fungus which causes fatal invasive aspergillosis among immunocompromised patients. To obtain a better understanding of the key elements involved in A. fumigatus virulence and to identify possible drug targets, it is necessary to be able to generate gene-deletion strains. Unfortunately, the molecular techniques available do not include a rapid method to disrupt and identify essential genes. RNA interference, a process in which the presence of double-stranded RNA homologous to a gene of interest results in specific degradation of the corresponding message, has been successfully tested on A. fumigatus. We have shown that expression of double stranded RNA corresponding to portions of the ALB1/PKSP and FKS1 genes results in reduced mRNA levels for those genes, with phenotypic consequences similar to that of gene disruption. The two genes could also be subjected to simultaneous interference through expression of chimeric double-stranded RNA. Use of RNA interference in Aspergillus will allow easier examination of the phenotypic consequences of reducing expression of a gene of interest, especially for essential genes.

Muniesa, M. and J. Jofre (2000). "Occurrence of phages infecting Escherichia coli O157:H7 carrying the Stx 2 gene in sewage from different countries." FEMS Microbiology Letters **183**(1): 197.

http://www.sciencedirect.com/science/article/B6T2W-3YF3Y1H-15/2/0149add700ebd11bf895107f323183d6

Shiga toxin-converting bacteriophages are involved in the pathogenicity of some enteric bacteria, such as Escherichia coli O157:H7. Recent studies have demonstrated a relatively high presence of Shiga toxin 2 phages in sewage from Spain, but no data on sewage from other areas were

available. In order to evaluate the presence of such phages in sewage from diverse geographical origins, 33 sewage samples, including samples from eight different European countries as well as from New Zealand and South Africa were analysed. Using an experimental approach based on the detection of Stx 2 gene by a phage enrichment culture followed by PCR, bacteriophages infecting E. coli O157:H7 carrying the Shiga toxin 2 gene were detected in 15 of the samples studied. Results presented here show that the presence of phages carrying the Stx 2 gene is common in sewage from developed countries.

Mygind, T., S. Birkelund, et al. (2000). "Characterization of the variability of a 75-kDa membrane protein in Mycoplasma hominis." FEMS Microbiology Letters **190**(1): 167.

http://www.sciencedirect.com/science/article/B6T2W-410MFHH-11/2/de4196c81d831ae3f0e02cf88c658ae6

The gene p75 encoding a 75-kDa surface-exposed membrane protein P75 was cloned and sequenced from Mycoplasma hominis type strain PG21T. To investigate the intraspecies variability, sequences were obtained from an additional two isolates 7488 and 183, and the three sequences were compared. The nucleotide and amino acid differences were not confined to specific regions of the gene/protein, but when comparing the three sequences, differences were present as single site substitutions or small insertions or deletions of nucleotides/amino acids. The intraspecies variability was further investigated by restriction enzyme analysis with two restriction enzymes (AluI and MboII) of PCR products amplified from p75 from 28 M. hominis isolates. On the basis of band patterns produced by the two restriction enzymes, the isolates could be divided into five and six groups. These groups neither matched categories of the M. hominis vaa gene nor the M. hominis p120 gene classes, indicating that the three genes vary by different mechanisms and possibly indicating horizontal gene transfer.

Mygind, T., I. Zeuthen Sogaard, et al. (2000). "Cloning, sequencing and variability analysis of the gap gene from Mycoplasma hominis." FEMS Microbiology Letters **183**(1): 15.

http://www.sciencedirect.com/science/article/B6T2W-3YF3Y1H-3/2/5f89a611a7746d20d742aaf75a7b746a

The gap gene encodes the glycolytic enzyme glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The gene was cloned and sequenced from the Mycoplasma hominis type strain PG21T. The intraspecies variability was investigated by inspection of restriction fragment length polymorphism (RFLP) patterns after polymerase chain reaction (PCR) amplification of the gap gene from 15 strains and furthermore by sequencing of part of the gene in eight strains. The M. hominis gap gene was found to vary more than the Escherichia coli counterpart, but the variation at nucleotide level gave rise to only a few amino acid substitutions. To verify that the gene was expressed in M. hominis, a polyclonal antibody was produced and tested against whole cell protein from 15 strains. The enzyme was expressed in all strains investigated as a 36-kDa protein. All strains except type strain PG21T showed reaction to a 104-kDa band in addition to the expected 36-kDa band. The protein reacting at 104 kDa is a M. hominis protein with either an epitope similar to one on GAPDH, or it is an immunoglobulin binding protein.

Nakamura, Y., M. Aki, et al. (2004). "Differences in gene expression of the ciliate Paramecium caudatum caused by endonuclear symbiosis with Holospora obtusa, revealed using differential display reverse transcribed PCR." FEMS Microbiology Letters **240**(2): 209.

http://www.sciencedirect.com/science/article/B6T2W-4DH262J-6/2/69f17ff107a386916140589d62cf5e0c

We identified six genes of Paramecium caudatum, which differentially expressed in Holospora obtusa-bearing and H. obtusa-free cells using differential display reverse transcribed PCR (DDRT-PCR). Northern blot analyses revealed that two of the genes, CA10-3 and CA20-2, were expressed extensively in the H. obtusa-free cell, while the other four, AS16-1, CS14, CS21 and CA17-1, were expressed more in the H. obtusa-bearing cell. Putative amino acid sequences of CA10-3, AS16-1 and CA17-1 showed high homologies with known genes related to intracellular signaling, transcription and aerobic metabolism. CS14 and CS21 also showed homologies with some genes whose products are still functionally unknown, but CA20-2 encoded a novel protein. We show in this study that H. obtusa alters multiple gene expression of the host after establishing endosymbiosis.

Namy, O., M. Mock, et al. (1999). "Co-existence of clpB and clpC in the Bacillaceae." FEMS Microbiology Letters **173**(2): 297.

http://www.sciencedirect.com/science/article/B6T2W-3W4XH5W-4/2/7e50ad9b81f0d17d2b5d70c38e20777a

The gene encoding ClpC in Bacillus anthracis was amplified from the chromosome by polymerase chain reaction using degenerate oligonucleotide primers. These primers also amplified a second DNA fragment identified as a clpB homolog. Both genes were suggested to be functional. Contrary to Bacillus subtilis which possesses clpC but not clpB, many Bacillus species were found to harbor both clpC and clpB. We also found that Clostridium strains could possess clpB, clpC, or both. All the Gram-negative strains tested had clpB only.

Nubel, U., P. M. Schmidt, et al. (2004). "Oligonucleotide microarray for identification of Bacillus anthracis based on intergenic transcribed spacers in ribosomal DNA." FEMS Microbiology Letters **240**(2): 215.

http://www.sciencedirect.com/science/article/B6T2W-4DHWSY6-1/2/2d1caa9c6ba97d8f211e993e25b6c1b6

We developed a DNA microarray for identification of Bacillus anthracis and other phylogenetic groupings within the "Bacillus cereus group". Nucleotide sequences of 16S-23S ribosomal DNA internal transcribed spacers containing genes for tRNAIle from 52 B. anthracis strains were found to be identical to sequences from seven strains published previously and different from all other bacteria. When 42 oligonucleotide probes targeting polymorphic sites were immobilized on glass slides and hybridized to fluorescently labeled PCR amplification products, one or more mismatches could be discriminated in all but one cases. Hence, hybridization events were highly specific and identification of B. anthracis was straightforward.

Obregon, V., P. Garcia, et al. (2003). "Molecular and biochemical analysis of the system regulating the lytic/lysogenic cycle in the pneumococcal temperate phage MM1." FEMS Microbiology Letters **222**(2): 193.

http://www.sciencedirect.com/science/article/B6T2W-48CX32Y-4/2/a97a678a7d37f7cc93656a66882eaae4

The temperate phage MM1 forms stable lysogens in Streptococcus pneumoniae. We report here the first characterization of the lysogenic control region in Pneumococcus which contains two functional divergent promoters (PR and PL). MM1 encodes a 14-kDa cI protein (CI) that appears to be responsible for maintaining the lysogenic state in Pneumococcus since it prevents elongation of the transcripts controlled by PR and PL.

Olivier, C. and R. Loria (1998). "Detection of Helminthosporium solani from soil and plant tissue with species-specific PCR primers." FEMS Microbiology Letters **168**(2): 235.

http://www.sciencedirect.com/science/article/B6T2W-3V5V0PK-D/2/8d0b48c1e67a8ff649e579fff3677b76

Two PCR primer pairs specific for Helminthosporium solani, which causes silver scurf on potato tubers, were designed from nucleotide sequences of the nuclear ribosomal internal transcribed spacer regions of H. solani. Both primer pairs amplified a single product with DNA from 48 North American and European isolates of H. solani, but not with DNA from 42 other fungi. Primers also amplified a single product with DNA extracted from silver scurf lesions on potato tubers and other plant tissue inoculated with spores of H. solani. Detection of the fungus in infested soil was only possible with nested PCR and after processing soil with a bead beater. Specific amplification of H. solani DNA can be used to study the saprophytic and pathogenic activity of this fungus in soil and plant tissue.

Palmer, H. M., C. B. Gilroy, et al. (1991). "Development and evaluation of the polymerase chain reaction to detect Mycoplasma genitalium." FEMS Microbiology Letters **77**(2-3): 199.

http://www.sciencedirect.com/science/article/B6T2W-47DKVJ3-F/2/3968b6551dfe82fa2337d95d7b14b585

The polymerase chain reaction (PCR) was developed to detect Mycoplasma genitalium. Oligonucleotide primers were used to amplify a 374 bp region of the attachment protein of the mycoplasma. DNA from three strains of M. genitalium tested gave a characteristic PCR product which was not seen with DNA from any other source. As little as 10-15 g of M. genitalium DNA could be detected and it was found in the vagina of progesterone-treated BALB/c mice inoculated with M. genitalium organisms later than they could be cultured from this site, but not in mice that never became colonised vaginally.

Perez-Arellano, I. and G. Perez-Martinez (2003). "Optimization of the green fluorescent protein (GFP) expression from a lactose-inducible promoter in Lactobacillus casei." FEMS Microbiology Letters **222**(1): 123.

http://www.sciencedirect.com/science/article/B6T2W-48CNDK1-2/2/9bfe50071f6ac34c6be3bfd8b462cbf1

An expression vector for Lactobacillus casei has been constructed containing the inducible lac promoter and the gene encoding ultraviolet visible green fluorescent protein (GFPUV) as reporter. Different conditions to grow L. casei were assayed and fluorescence as well as total protein synthesized were quantified. The maintenance of neutral pH had the greatest incidence on GFPUV expression, followed by aeration and a temperature of 30[deg]C. Environmental factors favoring GFPUV accumulation did not exactly correlate with those enhancing fluorescence.

Therefore, oxygenation, by stirring the culture, had the greatest influence on the proportion of fluorescent protein, which is in accordance with the structural requirements of this protein. The highest yield obtained was 1.3 [mu]g of GFP per mg of total protein, from which 55% was fluorescent.

Perilli, M., D. Ettorre, et al. (2004). "Overexpression system and biochemical profile of CTX-M-3 extended-spectrum [beta]-lactamase expressed in Escherichia coli." FEMS Microbiology Letters **241**(2): 229.

http://www.sciencedirect.com/science/article/B6T2W-4DNR95G-1/2/30120cf4d36788675292dba48f345f38

An efficient over-expression system was developed for CTX-M-3 extended-spectrum-[beta]-lactamase. The recombinant enzyme was purified from 1 l of culture to yield 22 mg of pure enzyme. The N-terminal amino acid sequence was determined to be NH2-QTADVQ. Determination of kinetic parameters with the purified CTX-M-3 revealed efficient hydrolysis of penicillins and cephalosporins, while ceftazidime and aztreonam were very poor substrates. Clavulanic acid, sulbactam and especially tazobactam inhibited the CTX-M-3 enzyme.

Pitcher, D. and J. Hilbocus (1998). "Variability in the distribution and composition of insertion sequence-like elements in strains of Mycoplasma fermentans." FEMS Microbiology Letters **160**(1): 101.

http://www.sciencedirect.com/science/article/B6T2W-3S4P7HB-K/2/9789b5229190ae3089dac6bc7ba70896

Mycoplasma fermentans has been reported to be pathogenic for man. All fourteen strains tested contain an insertion sequence-like element (ISLE) which may be present in multiple copies. To determine whether ISLE copies are similarly distributed in different strains of M. fermentans, restriction enzyme digest fragments of genomic DNA from 14 isolates, from a variety of sources, were separated by electrophoresis, blotted and hybridized to a biotin labelled polymerase chain reaction (PCR) amplified fragment of ISLE. A range of patterns was observed suggesting that the element has a tendency to undergo rearrangement within the genome. Analysis of ISLE sequences revealed inter- and intra-strain polymorphisms.

Plante, I., D. Centron, et al. (2003). "Direct sequencing and PCR mapping of integrons reveals multiple class 1 integrons in the multiresistant strain Enterobacter cloacae SCH88040794." FEMS Microbiology Letters **221**(1): 59.

http://www.sciencedirect.com/science/article/B6T2W-484KM89-4/2/33956f1859f2bf1143f39649a21f2818

We have characterized the variable region of three integrons found in the multiresistant strain Enterobacter cloacae SCH88040794, using polymerase chain reaction and direct sequencing of amplicons. The first integron has the gene cassette arrangement dfrAI-aadA1 in its variable region and the second aadB-oxa9. The third integron is In40 (aacA4-qacF-cmlB-oxa9), which has recently been described. The multiresistance expressed by this strain to amikacin, gentamicin, trimethoprim, sulfonamides, oxacillin, chloramphenicol and quaternary ammonium compounds is due, at least in part, to these three integrons.

Prod'hom, G., B. Lagier, et al. (1998). "A reliable amplification technique for the characterization of genomic DNA sequences flanking insertion sequences." FEMS Microbiology Letters **158**(1): 75.

http://www.sciencedirect.com/science/article/B6T2W-3S0DMMP-D/2/141b333a3c15c32adfa66fc36f3a1f4f

A simple and efficient ligation-mediated PCR (LMPCR) is described for amplifying DNA adjacent to known sequences. The method uses one primer specific for the known sequence and a second specific for a synthetic linker ligated to restricted genomic DNA. Perkin-Elmer AmpliTaq Gold polymerase is used to minimize non-specific primer annealing and amplification. This LMPCR method was successfully applied to isolate DNA sequences flanking mobile elements present in mycobacterial mutants generated by transposon mutagenesis.

Ramseier, T. M., J. Reizer, et al. (1995). "In vitro binding of the CcpA protein of Bacillus megaterium to cis-acting catabolite responsive elements (CREs) of Gram-positive bacteria." FEMS Microbiology Letters **129**(2-3): 207.

http://www.sciencedirect.com/science/article/B6T2W-41M3YXC-H/2/f7d46ba4c9a5be228ebc532ce3f418b4

Using DNA band migration retardation assays, specific binding of the CcpA protein of Bacillus megaterium to the ds-acting catabolite responsive element (CRE) of the xyl operon of B. subtilis has been demonstrated. Binding of CcpA was specifically inhibited by addition of unlabeled DNA fragments containing CREs of other operons but not by DNA fragments lacking a CRE. Binding was stimulated by high concentrations of phosphate, pyrophosphate, and organic phosphate esters and specifically inhibited by serine phosphorylated HPr and its conformational analogue, S46D HPr. This report therefore documents the specific binding of CcpA to a target CRE and defines its regulation by HPr(ser-P) and phosphorylated metabolites.

Rich, C., S. Favre-Bonte, et al. (1999). "Characterization of enteroaggregative Escherichia coli isolates." FEMS Microbiology Letters **173**(1): 55.

http://www.sciencedirect.com/science/article/B6T2W-3W37YDV-8/2/54acab227781ab786e0102b61e2a2ea1

Forty enteraggregative Escherichia coli (EAggEC) previously characterized by their ability to adhere to HEp-2 cells or/and their hybridization with the 1-kb EAggEC DNA probe were investigated for the presence of adherence factors and heat-stable enterotoxin (EAST1)-encoding genes. Only 45% of the isolates harbored the EAST1-encoding genes as detected by polymerase chain reaction. None of them hybridized with an AAF/II-encoding gene specific DNA probe and 35% (14/40) were positive in a PCR assay using primers specific for aggC, an accessory gene of the AAF/I-encoding operon. Cloning and sequence analysis of the aggA variant from one isolate, EAggEC 457, revealed 68.9% identity between its deduced amino acid sequence and those of the aggA product from the AAF/I-producing reference strain, E. coli 17.2. No major protein subunit was detected at the surface of EAggEC 457 compared to the bacterial surface extract of E. coli 17.2.

Robinson, P. N., B. Heidrich, et al. (1996). "Species-specific detection of Legionella using polymerase chain reaction and reverse dot-blotting." FEMS Microbiology Letters **140**(2-3): 111.

http://www.sciencedirect.com/science/article/B6T2W-3W0FD5J-1G/2/313f3d43f064dca5e9bc8222317f805c

Legionella pneumophila and some other Legionella species are capable of causing Legionnaire's disease, a potentially fatal pneumonia. The identification of legionellae by standard laboratory techniques such as culture is difficult and time-consuming. In the present work, the DNA sequence of the 23S-5S spacer region was determined for 43 Legionella isolates, and the sequence information was used to develop a species-specific detection system using PCR and reverse dot-blotting which employs just one PCR amplicon to perform genus- and species-specific detection. L. pneumophila serogroups 1-16 as well as 21 non-pneumophila isolates could be identified and differentiated at the species level using this system.

Rodriguez-Lazaro, D., M. Hernandez, et al. (2004). "Simultaneous quantitative detection of Listeria spp. and Listeria monocytogenes using a duplex real-time PCR-based assay." FEMS Microbiology Letters **233**(2): 257.

http://www.sciencedirect.com/science/article/B6T2W-4BVRJXG-2/2/52c2de6a6ab77e9245cb4a1c0c520e59

We report a duplex real-time PCR-based assay for the simultaneous quantitative detection of Listeria spp. and the food-borne pathogen Listeria monocytogenes. The targets of this single tube reaction were the 23S rDNA and hly genes of Listeria spp. and L. monocytogenes, respectively. Our assay was efficient, 100% selective (i.e., it allowed accurate simultaneous identification of 52 L. monocytogenes and 120 Listeria spp. strains through the FAM-labelled hly and the VIC-labelled 23S rDNA probes, respectively); and had a detection limit of one target molecule in 100% (23S rDNA) and 56% (hly) of the reactions. Simultaneous quantification was possible along a 5-log dynamic range, with an upper limit of 30 target molecules and R2 values >0.995 in both cases. Our results indicate that this assay based on the amplification of the 23S rDNA gene can accurately quantify any mixture of Listeria species and simultaneously unambiguously quantify L. monocytogenes.

Ryu, k. H. and W. M. Park (1995). "Rapid detection and identification of odontoglossum ringspot virus by polymerase chain reaction amplification." FEMS Microbiology Letters **133**(3): 265.

http://www.sciencedirect.com/science/article/B6T2W-3Y45GPM-25/2/30705d94daa91e4068a975aff9627634

The odontoglossum ringspot Tobamovims (ORSV) movement and coat proteins genes were selected for the design of oligonucleotide primers for amplification of a 1,085 bp fragment. A combined assay of reverse transcription and the polymerase chain reaction (RT-PCR) was performed with 20-mer ORSV-specific primers and crude nucleic acid extracts from virus-infected orchids for rapid detection of the virus. The lowest concentration of template viral RNA required for detection was 10 fg. The RT-PCR is a 103 times more sensitive, reproducible and time-saving method than the enzyme-linked immunosorbent assay. No PCR product was observed when cymbidium mosaic potexvirus or a crude extract of healthy Cymbidium sp. were used as a template in RT-PCR with the same primers. The specificity of the primers was verified using other tobamoviruses RNAs.

Saito, K., Y. Oda, et al. (2003). "Molecular cloning of the gene for 2,6-[beta]--fructan 6-levanbiohydrolase from Streptomyces exfoliatus F3-2." FEMS Microbiology Letters **218**(2): 265.

http://www.sciencedirect.com/science/article/B6T2W-47C4CF2-G/2/d0524eebe274eb16efb456918ab591a7

The gene encoding a 2,6-[beta]--fructan 6-levanbiohydrolase (LF2ase) (EC 3.2.1.64) that converts levan into levanbiose was cloned from the genomic DNA of Streptomyces exfoliatus F3-2. The gene encoded a signal peptide of 37 amino acids and a mature protein of 482 amino acids with a total length of 1560 bp and was successfully expressed in Escherichia coli. The similarities of primary structure were observed with levanases from Clostridium acetobutylicum, Bacillus subtilis, B. stearothermophilus (51.0-54.3%) and with LF2ase from Microbacterium levaniformans (53.9%). The enzyme from S. exfoliatus F3-2 shared the conserved six domains and the completely conserved five amino acid residues with family 32 glycosyl hydrolases, which include levanase, inulinase, and invertase. These observations led to the conclusion that the enzyme belongs to family 32 glycosyl hydrolases.

Sandvang, D., F. M. Aarestrup, et al. (1997). "Characterisation of integrons and antibiotic resistance genes in Danish multiresistant Salmonella enterica Typhimurium DT104." FEMS Microbiology Letters **157**(1): 177.

http://www.sciencedirect.com/science/article/B6T2W-3RFPJW3-Y/2/a0a1477e99a62b195a5f67d28153a9b0

The presence and genetic content of integrons was investigated in eight Salmonella enterica Typhimurium DT104 isolates from different pig herds in Denmark. Two different integrons were identified using PCR and sequencing. Each of the integrons carried a single resistance cassette in addition to the sul1 and qacE[Delta]1 genes characteristic of integrons. The first integron encoded the ant (3'')-Ia gene that specified resistance to spectinomycin and streptomycin. The second contained the pse-1 [beta]-lactamase gene. All the multiresistant strains contained both integrons. The presence of these two integrons did not account for the total phenotypic resistance of all the isolates and does not exclude the presence of other mobile DNA elements.

Sandvang, D., F. M. Aarestrup, et al. (1998). "Characterisation of integrons and antibiotic resistance genes in Danish multiresistant Salmonella enterica Typhimurium DT104." FEMS Microbiology Letters **160**(1): 37.

http://www.sciencedirect.com/science/article/B6T2W-3S4P7HB-6/2/77c9a9b1fc130f1c94238dc95cd8e06d

The presence and genetic content of integrons was investigated in eight Salmonella enteritica Typhimurium DT104 isolates from different pig herds in Denmark. Two different integrons were identified using PCR and sequencing. Each of the integrons carried a single resistance cassette in addition to the sul1 and qacE[Delta]1 genes characteristic of integrons. The first integron encoded the ant (3'')-Ia gene that specified resistance to spectinomycin and streptomycin. The second contained the pse-1 [beta]-lactamase gene. All the multiresistant strains contained both integrons. The presence of these two integrons did not account for the total phenotypic resistance of all the isolates and does not exclude the presence of other mobile DNA elements.

Saruta, K., T. Matsunaga, et al. (1997). "Rapid identification and typing of Staphylococcus aureus by nested PCR amplified ribosomal DNA spacer region." FEMS Microbiology Letters **146**(2): 271.

http://www.sciencedirect.com/science/article/B6T2W-3S7XFSM-K/2/fc42ac1aa1681d2ab5a646032df3073f

We designed a polymerase chain reaction (PCR) assay for rapid detection of prokaryotic 16S-23S spacer regions. This PCR assay consisted of nested DNA amplifications. The first-step PCR was able to detect the general presence of eubacteriales with a unified set of universal primers. The universal primers were selected from highly conserved regions in 16S and 23S ribosomal RNA (rRNA) genes and amplified DNAs from all 62 different species of bacteria tested. In the second-step PCR, the identification primers could detect four important bacterial species through amplification of the rRNA spacer regions between the 16S-23S rRNA genes. For Staphylococcus aureus, intraspecies variation in spacer amplification products was observed with S. aureus specific primers. We suggest that the nested PCR assay could be used as a novel method for the identification and typing in epidemiological studies of S. aureus.

Schmidt, H., B. Henkel, et al. (1997). "A gene cluster closely related to type II secretion pathway operons of Gram-negative bacteria is located on the large plasmid of enterohemorrhagic Escherichia coli O157 strains." FEMS Microbiology Letters **148**(2): 265.

http://www.sciencedirect.com/science/article/B6T2W-3S6D1CW-V/2/c6268ca3c3e71533aeffbdfc3d4acef0

Analysis of 14.162 kb of DNA derived from plasmid pO157 of enterohemorrhagic Escherichia coli (EHEC) O157:H7 strain EDL933, extending in the 5' direction of the recently described EHEC-hly operon, revealed 13 open reading frames (ORF) which showed great similarities to genes of members of the type II pathway secretion systems of Gram-negative bacteria. We named the ORFs etpC to etpO for HEC ype II secretion athway. In addition, an IS911-like insertion element was found to separate the etp genes from the EHEC-hlyC gene. Hybridization experiments with a specific etp probe and various categories of enteric E. coli pathotypes revealed that the etp gene cluster occurred in all 30 EHEC strains of serogroup O157 (100%) tested and is distributed sporadically among other EHEC serogroups (60%). In addition, the etp genes were rarely detected in STEC isolated from bovine feces (10%). Moreover, it was found not to occur in enteropathogenic E. coli, enteroaggregative E. coli, enterotoxigenic E. coli and enteroinvasive E. coli. The results obtained with the etp probe were confirmed by a PCR approach to specifically detect an internal fragment of the etpD gene.

Schmidt, H., J. Scheef, et al. (1997). "An ileX tRNA gene is located close to the Shiga toxin II operon in enterohemorrhagic Escherichia coli O157 and non-O157 strains." FEMS Microbiology Letters **149**(1): 39.

http://www.sciencedirect.com/science/article/B6T2W-3PH8MTT-21/2/c7bc1daac6c3d97d07bf29a4f0d3d8b0

A cosmid library of enterohemorrhagic Escherichia coli (EHEC) O157:H7 strain EDL 933 was constructed and clones carrying the stx2 gene were identified by colony blot hybridization with a stx2B specific probe. Nucleotide sequencing upstream of the stx2A gene revealed high sequence identities of 89.5% to the ileX tRNA gene found in E. coli. The ileX gene was located 260 bp from

the translational start codon of stx2A. PCR analysis with primers specific for this analyzed region showed that in 11 Stx2-producing EHEC strains from patients with hemolytic uremic syndrome, all PCR-positive strains carried the ileX tRNA gene. However, PCR analysis of the respective region in 11 Stx1-producing EHEC strains detected no ileX genes. Although the role of ileX in Stx2-producing EHEC strains is not clear, its function in regard to the use of rare codons and as an integration site is discussed.

Scybert, S., R. Pechous, et al. (2003). "NaCl-sensitive mutant of Staphylococcus aureus has a Tn917-lacZ insertion in its ars operon." FEMS Microbiology Letters **222**(2): 171.

http://www.sciencedirect.com/science/article/B6T2W-48GNYN9-6/2/e0b5216e69e39a97a1ebcbe3c1e02238

Staphylococcus aureus is a Gram-positive bacterium that is extremely halotolerant. To investigate the molecular mechanisms by which S. aureus can cope with osmotic stress, Tn917-lacZ-induced NaCl-sensitive mutants were isolated. An NaCl-sensitive mutant showed a longer lag period, slower growth rate, and lower final culture turbidity than the parent strain in liquid medium containing 1.5 M NaCl. Electron microscopic observation of the NaCl-sensitive mutant under NaCl stress conditions revealed large, pseudo-multicellular cells. Addition of exogenous osmoprotectants, such as glycine betaine, choline, -proline, and proline betaine, did not relieve the NaCl sensitivity of the mutant. The region flanking the transposon insertion site in the NaCl-sensitive S. aureus chromosome was sequenced. The mutated gene was 99% identical to arsR, the arsenic operon regulatory protein present on the pI258 plasmid of S. aureus. The ars operon from pI258 was subcloned into the shuttle vector pLI50 and transferred into the NaCl-sensitive mutant. The ars operon in trans restored NaCl tolerance in the mutant, suggesting that NaCl sensitivity is due to the mutation in arsR.

Sekeyova, Z., V. Roux, et al. (1999). "Intraspecies diversity of Coxiella burnetii as revealed by com1 and mucZ sequence comparison." FEMS Microbiology Letters **180**(1): 61.

http://www.sciencedirect.com/science/article/B6T2W-3XP0GHW-9/2/bd8ede92edef4c1d72fde50debe6099e

Coxiella burnetii is classified within the [gamma] subgroup of the Proteobacteria. All strains tested to date have an identical 16S rRNA sequence but 20 different genotypes have been determined by pulsed field gel electrophoresis (PFGE). In this study, intraspecies genetic diversity was investigated by sequence comparison of 715 bp of the Com1 encoding gene (com1) and 774 bp of the MucZ encoding gene (mucZ) in 37 strains isolated from animals and humans with acute or chronic Q fever in Europe, North America and Africa. Five and four groups were established from sequence analysis of com1 and mucZ, respectively. Neither relation of the defined groups to geographical distribution of the isolates was noted nor relation to disease form (acute/chronic). The same isolates were grouped together regardless of the gene being investigated. Comparison of the five proposed groups to previous groups, yielded after digestion by NotI PFGE, allowed for an intermediate classification of C. burnetii isolates between those obtained by using 16S rDNA (one group) and PFGE (20 groups).

Sellstedt, A., B. Wullings, et al. (1992). "Identification of Casuarina-Frankia strains by use of polymerase chain reaction (PCR) with arbitrary primers." FEMS Microbiology Letters **93**(1): 1.

http://www.sciencedirect.com/science/article/B6T2W-47DDC8H-2P/2/ce46a6dd47c75bf51a2952daa3e2a8db

Free-living N2-fixing Frankia strains isolated from Casuarina sp. were investigated for genomic polymorphism. We used six 10-mer oligonucleotides as single arbitrary primers (AP) for the polymerase chain reaction (PCR) in order to amplify random DNA fragments in the genome of free-living Frankia strains. Agarose-gels of the amplified genomic DNA revealed that two of the six arbitrary primers showed polymorphism in the eight different Frankia genomes. Analysis of the AP-PCR products showed 9 polymorphic bands ranging from 4.1-0.60 kb. We conclude that single arbitrary primers can be used to amplify genomic DNA, and that polymorphism can be detected between the amplification products of the different Frankia genomes.

Sergeyenko, T. V. and D. A. Los (2003). "Cyanobacterial leader peptides for protein secretion." FEMS Microbiology Letters **218**(2): 351.

http://www.sciencedirect.com/science/article/B6T2W-47P8RN4-1/2/53d0eb8929aaadb119af4e875e505cea

The leader peptide of the major secreted protein PilA1 of the cyanobacterium Synechocystis sp. strain PCC 6803 and several artificial leader peptides have been used to study secretion of the reporter protein lichenase to the culture medium. The strains of Synechocystis carrying lichenase with the leader sequences of PilA and with the leader sequence of Slr2016 efficiently secreted the reporter protein. The artificial leader sequence that was characterized by the overall positive charge (as PilA1 and Slr2016 leaders) also allowed secretion. The artificial leader with negative charge, however, did not allow secretion of the reporter protein. Moreover, no secreted proteins have been isolated from this strain using conventional techniques for preparation of secreted proteins. These data suggest that the general secretion pathway in cyanobacteria, at least for pilins, recognizes the overall charge of the leader sequences, and operates in a sequence-non-specific manner.

Sharma, V. K., S. A. Carlson, et al. (2005). "Hyperadherence of an hha mutant of Escherichia coli O157:H7 is correlated with enhanced expression of LEE-encoded adherence genes." FEMS Microbiology Letters **243**(1): 189.

http://www.sciencedirect.com/science/article/B6T2W-4F1J58F-3/2/0bb7697991a9a8c57055c0c614996960

Enterohemorrhagic Escherichia coli (EHEC) O157:H7 virulence factors, specifically those conferring intimate adherence to and formation of attaching and effacing lesions (A/E) on host cells, are encoded by a horizontally acquired locus of enterocyte effacement (LEE). Expression of several LEE-encoded genes, which are organized into operons LEE1 through LEE5, is under the positive regulation of ler, the first gene in the LEE1 operon. We have recently demonstrated that EHEC O157:H7 lacking hha exhibited greater than a 10-fold increase in ler expression and that the repression of ler results from the binding of Hha to the ler promoter. In this report, we show that an hha mutant of EHEC O157:H7 exhibited increased adherence to Hep-2 cells, had increased transcriptional activities of LEE1, LEE2, LEE3, and LEE5 as determined by reverse transcriptase-polymerase chain reaction assays, and expressed LEE5:lac transcriptional fusion at levels that were several-fold higher than that expressed by the parental hha+ strain. These results demonstrate that hha is an important regulatory component of the cascade that governs the expression of LEE operons and the resulting ability of EHEC O157:H7 to intimately adhere to host cells.

Shigematsu, T., K. Yumihara, et al. (2003). "Purification and gene cloning of the oxygenase component of the terephthalate 1,2-dioxygenase system from Delftia tsuruhatensis strain T7." FEMS Microbiology Letters **220**(2): 255.

http://www.sciencedirect.com/science/article/B6T2W-482YHXM-5/2/840b0b38e370910497865ac2bc29a36e

The terephthalate 1,2-dioxygenase system (TERDOS) was found in cell extracts of Delftia tsuruhatensis strain T7 (=IFO16741) grown in terephthalate-salt medium. The cell extract was separated by anion exchange chromatography to yield two fractions (R and Z) that were necessary for oxygenation of terephthalate with NADH and Fe2+. The oxygenase component of TERDOS (TerZ) was purified from fraction Z by gel filtration chromatography to near homogeneity. An [alpha]3[beta]3 subunit structure was deduced from the molecular masses of 235, 46 and 17 kDa of the native complex and the [alpha]- and [beta]-subunits, respectively. The N-terminal amino acid sequences of the two subunits of TerZ allowed polymerase chain reaction primers to be deduced and the DNA sequence of the [alpha]-subunit was determined. The amino acid sequence of the [alpha]-subunit (TerZ[alpha]) showed significant similarities to the large subunits of multicomponent ring-hydroxylating oxygenases. Two motifs in the deduced amino acid sequence, a Rieske [2Fe-2S] center and a mononuclear Fe(II) binding site, were observed. Phylogenetic analyses indicated that TerZ[alpha] and the large oxygenase component subunits ortho-halobenzoate 1,2-dioxygenase and salicylate-5-hydroxylase form a cluster that is distant from the rest of the large oxygenase subunits of multicomponent ring-hydroxylating oxygenases.

Silva, F. J., R. C. H. J. van Ham, et al. (1998). "Structure and evolution of the leucine plasmids carried by the endosymbiont (Buchnera aphidicola) from aphids of the family Aphididae." FEMS Microbiology Letters **168**(1): 43.

http://www.sciencedirect.com/science/article/B6T2W-3V3XR4C-7/2/ce4fb1e4ebdcee17152403c6bbc9cc55

In all examined species of the family Aphididae, the bacterial endosymbiont Buchnera aphidicola carries a plasmid encoding the genes leuABCD (involved in leucine biosynthesis) along with repA1, repA2 and ORF1. The gene organisation of the leucine plasmids was conserved, except in Buchnera isolated from Pterocomma populeum, where ORF1 was located in a different position. An inverted repeat (LIR1) located between repA2 and leuA is found in all of the Buchnera leucine plasmids examined. The predicted secondary structure of the LIR1 transcript conforms to a long hairpin loop, suggesting an involvement in transcription termination or messenger stability. Phylogenetic reconstruction based on repA2 sequences suggests that horizontal transfer of Buchnera leucine plasmids has not occurred.

Smith, H. E., L. van Bruijnsvoort, et al. (1999). "Rapid PCR test for Streptococcus suis serotype 7." FEMS Microbiology Letters **178**(2): 265.

http://www.sciencedirect.com/science/article/B6T2W-3X9YTRF-9/2/c30ac0d47adb52812509e452737f4287

Recent epidemiological studies on Streptococcus suis infections in pigs indicated that, besides serotypes 1, 2 and 9, serotype 7 is also frequently associated with diseased animals. For the latter serotype, however, no rapid and sensitive diagnostic methods are available. This hampers

prevention and control programs. Here, we describe the development of a type-specific PCR test for the rapid and sensitive detection of S. suis serotype 7. The test is based on DNA sequences of capsular (cps) genes specific for serotype 7. These sequences were identified by cross-hybridization of several individual cps genes with the chromosomal DNAs of 35 different S. suis serotypes.

Solovieva, I. M., R. A. Kreneva, et al. (2005). "The riboflavin kinase encoding gene ribR of Bacillus subtilis is a part of a 10 kb operon, which is negatively regulated by the yrzC gene product." FEMS Microbiology Letters **243**(1): 51.

http://www.sciencedirect.com/science/article/B6T2W-4DXBVYX-1/2/338741088ac4362b2d748a0d5229945e

The riboflavin kinase encoding gene ribR is situated within a 12 genes locus ytmI-ytnM of the Bacillus subtilis chromosome. Here we demonstrate that ribR is transcribed as part of a 10 kb ytmI-ytnM operon. The riboflavin overproduction phenotype of B. subtilis ribC mutant strains, which is a result of the strongly reduced flavokinase activity of the riboflavin kinase/FAD synthetase RibC, was suppressed by ribR expression. Analysis of mutations with an upregulated ribR gene revealed 2 different groups of mutants. One class of mutants contained base substitutions in an 8 nucleotide sequence of the promoter region of the ytmI-ytnM operon. A second class of mutants had single point mutations within the yrzC gene or in the RBS of this gene. Dot-blot analysis of ytmI-ytnM transcription and the results of in trans complementation experiments for the yrzC mutants confirmed a role of the yrzC gene product as a negative regulator for the ytmI-ytnM operon.

Stanton, T. B., M. G. Thompson, et al. (2003). "Detection of bacteriophage VSH-1 svp38 gene in Brachyspira spirochetes." FEMS Microbiology Letters **224**(2): 225.

http://www.sciencedirect.com/science/article/B6T2W-48W8VSF-5/2/75cd2440c51bec05afedc0bcf541ae68

VSH-1 is a mitomycin C-inducible, non-lytic, phage-like agent that packages random 7.5-kb fragments of the Brachyspira hyodysenteriae genome. VSH-1 is the first recognized mechanism for gene transfer between B. hyodysenteriae cells. To analyze the distribution of VSH-1 among spirochetes, a 344-bp probe for gene svp38, encoding the VSH-1 major head protein, was amplified by polymerase chain reaction and used in Southern blot hybridizations with genomic DNA from various spirochete genera. The svp38 probe hybridized to a 40-kb SalI-SmaI fragment of the B. hyodysenteriae B78T chromosome, indicating VSH-1 DNA insertion into the chromosome at a unique site. Restriction endonuclease digested DNAs of 27 spirochete strains representing six Brachyspira species (B. hyodysenteriae, B. innocens, B. pilosicoli, B. murdochii, B. intermedia, B. alvinipulli) contained a single fragment hybridizing with the svp38 probe. DNAs from spirochete species of the genera Treponema, Spirochaeta, Borrelia, and Leptospira did not hybridize with the probe. VSH-1-like agents appear to be widely distributed among Brachyspira species and, as has been demonstrated for B. hyodysenteriae, may serve as useful gene transfer agents for those other species.

Stroup, D. and J. N. Reeve (1992). "Histone HMf from the hyperthermophilic archaeon Methanothermus fervidus binds to DNA in vitro using physiological conditions." FEMS Microbiology Letters **91**(3): 271.

http://www.sciencedirect.com/science/article/B6T2W-4754C0H-1M/2/a126c307cb07908b1c1647c5504f6228

The concentration of HMf (istone ethanothermus ervidus) in vivo has been shown to be between 1 and 2 x 104 molecules per genome. At this mass ratio the amount of HMf bound to pUC19 DNA in vitro was found to be dependent on the topology of the plasmid DNA. M. fervidus grows optimally between 80 and 85[deg]C and contains approx. 1 M K+ plus 300 mM 2'3'(cyclic) diphosphoglycerate. (Hensel, R. and Konig, H. 1988. FEMS Microbiol. Lett. 49, 75-79). HMf binding to DNA in vitro under these conditions has been demonstrated.

Studholme, D. J., R. A. Jackson, et al. (1999). "Phylogenetic analysis of transformable strains of thermophilic Bacillus species." FEMS Microbiology Letters **172**(1): 85.

http://www.sciencedirect.com/science/article/B6T2W-3VW874P-G/2/0fe5287a150813f905c81fbe908a9bb3

Few strains of thermophilic Bacillus spp are readily transformable with plasmid DNA. Given the considerable phylogenetic and phenotypic diversity amongst thermophilic bacilli, we have examined whether transformability is a trait associated with a particular phylogenetic group, by sequencing the 16S ribosomal RNA genes from transformable strains NUB3621, K1041, and NRRL1174. Although all of these strains were described in the literature as B. stearothermophilus, only NRRL1174 is closely related to the type strain of this species. Based on its 16S rDNA sequence and physiological data K1041 appeared to belong to the species B. thermodenitrificans, while NUB3621 showed a slightly closer relationship to B. thermoglucosidasius than to B. stearothermophilus. Therefore we conclude that the trait of transformability, though possibly strain-specific, is not limited to a single species of thermophilic Bacillus.

Suyama, T., H. Hosoya, et al. (1998). "Bacterial isolates degrading aliphatic polycarbonates." FEMS Microbiology Letters **161**(2): 255.

http://www.sciencedirect.com/science/article/B6T2W-3SF0Y74-6/2/ae602e6477a4745b1520a248a6c76192

Bacteria that degrade an aliphatic polycarbonate, poly(hexamethylene carbonate), were isolated from river water in Ibaraki Prefecture, Japan, after enrichment in liquid medium containing poly(hexamethylene carbonate) suspensions as carbon source, and dilution to single cells. Four of the strains, 35L, WFF52, 61A and 61B2, degraded poly(hexamethylene carbonate) on agar plate containing suspended poly(hexamethylene carbonate). Degradation of poly(hexamethylene carbonate) was confirmed by gel permeation chromatography. Besides poly(hexamethylene carbonate), the strains were found to degrade poly(tetramethylene carbonate). The strains were characterized morphologically, physiologically, and by 16S rDNA sequence analysis. Strains 35L and WFF52 were tentatively identified as Pseudomonas sp. and Variovorax sp., respectively, while strains 61A and 61B2 constitute an unidentified branch within the [beta] subclass of the Proteobacteria.

Suzuki, T. and K. Yamasato (1994). "Phylogeny of spore-forming lactic acid bacteria based on 16S rRNA gene sequences." FEMS Microbiology Letters **115**(1): 13.

http://www.sciencedirect.com/science/article/B6T2W-476HNT3-7K/2/07163524a73e4c1b14c4cd64b29ecb82

The phylogeny of spore-forming lactic acid bacteria was investigated on the basis of 16S rRNA gene sequences. Sixteen strains were separated into three lines of descent; one consisted of 14 strains assigned to Sporolactobacillus spp. and Bacillus spp., and the other two each consisted of "Sporolactobacillus dextrus" and Bacillus coagulans. Strains of all the first lineage but one composed a cluster of similarity values of 97.2% and higher, and were represented by the type of S. inulinus. The cluster was further separated into five subclusters, four catalase negative and one positive. The definition of the genus Sporolactobacillus should be amended to accomodate catalase positive strains. Spore-forming lactic acid bacteria originated at different phylogenetic positions, and would have evolved convergently in the area of Bacillus.

Te'o, V. S. J., A. E. Cziferszky, et al. (2000). "Codon optimization of xylanase gene xynB from the thermophilic bacterium Dictyoglomus thermophilum for expression in the filamentous fungus Trichoderma reesei." FEMS Microbiology Letters **190**(1): 13.

http://www.sciencedirect.com/science/article/B6T2W-410MFHH-3/2/946955a9bbaa55b83e2f331176259093

The catalytic domain of the xynB (xylanase) gene from the thermophilic bacterium Dictyoglomus thermophilum was reconstructed by PCR to match the codon preference of Trichoderma reesei. The 0.6-kb DNA fragment encoding the enzyme was first amplified by primer extension with a mixture of eight overlapping oligonucleotides, followed by PCR with outside primers containing restriction enzyme sites for directional cloning into Escherichia coli and T. reesei vectors. The synthetic gene was expressed in both organisms, producing a clearing halo around transformant colonies in plate assay utilizing an overlay of oat spelts xylan. Effective transcription of xynB in T. reesei was obtained after changing 20 codons.

Turner, S. J., D. J. Saul, et al. (2002). "A heteroduplex method for detection of targeted sub-populations of bacterial communities." FEMS Microbiology Letters **208**(1): 9.

http://www.sciencedirect.com/science/article/B6T2W-451SCY4-3/2/d52849a46f3b89123e90de52bc6637eb

We describe a simple method, based on heteroduplex mobility analysis of 16S rDNA fragments, for targeted detection of sub-populations of bacteria within diverse microbial communities. A small (ca. 200 bp) polymorphic fragment of the bacterial 16S rRNA gene was amplified from sample DNA using universal primers. Sample products were hybridised with a fluorescently labelled fragment amplified from a selected 'reporter' organism representing the target group. The resulting products were resolved and the labelled heteroduplex pairs detected on non-denaturing gels using automated DNA detection technology. A model, based on analysis of samples with known 16S rDNA sequences, demonstrates that heteroduplex mobility is inversely correlated with genetic distance and that beyond 26% genetic difference, heteroduplex products are not detected. The utility of the method was tested by field studies in which stream biofilms could be characterised by heteroduplex profiles generated with heterotrophic and autotrophic reporter organisms representing target groups.

Vandekerckhove, T. T. M., S. Watteyne, et al. (1999). "Phylogenetic analysis of the 16S rDNA of the

cytoplasmic bacterium Wolbachia from the novel host Folsomia candida (Hexapoda, Collembola) and its implications for wolbachial taxonomy." FEMS Microbiology Letters **180**(2): 279.

http://www.sciencedirect.com/science/article/B6T2W-3XT6BRP-S/2/ed5637e35fd3e1b6e0ce92e5e372b55b

Wolbachia pipientis are intracellular, transovarially inherited [alpha]-Proteobacteria in invertebrates. Four major Wolbachia groups exist: A, B (contained in divergent arthropods), C and D (harbored by Nematoda). By means of transmission electron microscopy, we observed Wolbachia-like bacteria in a primitive insect, Folsomia candida (Hexapoda, Collembola, Isotomidae). 16S rDNA analysis proved them to constitute a novel lineage, henceforth named group E, in the wolbachial phylogenetic tree. It shares 97.8% 16S rDNA homology with its nearest neighbors, groups A and B, which diverged from it more recently. We propose (i) a new taxon E for the Wolbachia strain in F. candida, (ii) that the single-described Wolbachia pipientis fall apart into at least three species: C, D and the large E-A-B complex. F. candida's group E Wolbachia rekindle the question about invasive capacities of free-living ancestral wolbachiae and horizontal transfer.

Wang, J. and J.-H. Liu (2004). "Mutations in the chloramphenicol acetyltransferase (S61G, Y105C) increase accumulated amounts and resistance in Pseudomonas aeruginosa." FEMS Microbiology Letters **236**(2): 197.

http://www.sciencedirect.com/science/article/B6T2W-4CK7MT4-1/2/3158bc1b4a15bdb888325fc7922ab22f

A chloramphenicol acetyltransferase (catB7) gene containing two point mutations, 181A/G and 314A/G, has been recently reported to be a determinant for high-level chloramphenicol resistance phenotype in a Pseudomonas aeruginosa strain PAhcr1. The mutant CATB7 was further characterized in vitro and in vivo to elucidate the molecular basis of high-level resistance. CAT assay demonstrated that the mutant and wild-type recombinant CATB7 had similar specific activities. Dot blotting revealed that the accumulated amounts of CATB7 in P. aeruginosa strains PAO1 and PAhcr1 were proportionate to the respective anti-chloramphenicol level. Site-directed mutagenesis showed that G61S and Y105C contributed synergistically to the PAhcr1 resistance phenotype. It could be proposed that the mutant CATB7 was more structurally stable than catalytically efficient as a chloramphenicol resistance determinant in PAhcr1.

Weitz, H. J., J. M. Ritchie, et al. (2001). "Construction of a modified mini-Tn5 luxCDABE transposon for the development of bacterial biosensors for ecotoxicity testing." FEMS Microbiology Letters **197**(2): 159.

http://www.sciencedirect.com/science/article/B6T2W-42TC8Y4-5/2/33be3887e08fdd4a08d9a509a4abe361

A mini-Tn5 transposon was modified to introduce a promoterless luxCDABE cassette from Vibrio fischeri into environmentally relevant bacterial strains in order to develop bioluminescence-based biosensors for toxicity testing. The mini-Tn5 luxCDABE transposon was chromosomally integrated downstream from an active promoter into two Pseudomonas strains (Pseudomonas fluorescens 8866 and Pseudomonas putida F1). Characterisation of the bioluminescent transconjugants demonstrated that the transposon integration was stable and had no effect on growth rate. Both P. fluorescens 8866 Tn5 luxCDABE and P. putida F1 Tn5 luxCDABE were used to assess the toxicity of standard solutions (Cu, Zn and 3,5-DCP) as well as Cu- and 3,5-

DCP-spiked groundwater samples. They were successfully used for bioluminescence-based bioassays and the potential value of using different bacterial biosensors for ecotoxicity testing was shown.

Widada, J., H. Nojiri, et al. (2001). "Quantification of the carbazole 1,9a-dioxygenase gene by real-time competitive PCR combined with co-extraction of internal standards." FEMS Microbiology Letters **202**(1): 51.

http://www.sciencedirect.com/science/article/B6T2W-43PGJY7-8/2/84ec87c26f9fe16a0d755b53f149126f

The fluorogenic probe assay, competitive polymerase chain reaction (PCR) and co-extraction with internal standard cells were combined to develop a rapid, sensitive, and accurate quantification method for the copy number of a target carbazole 1,9a-dioxygenase gene (carAa) and the cell number of Pseudomonas sp. strain CA10. The internal standard DNA was modified by replacement of a 20-bp long region with one for binding a specific probe in fluorogenic PCR (TaqMan). The resultant DNA fragment was similar to the corresponding region of the intact carAa gene in terms of G+C content. When used as a competitor in the PCR reaction, the internal standard DNA was distinguishable from the target carAa gene by two specific fluorogenic probes with different fluorescence labels, and was automatically detected in a single tube using the ABI7700 sequence detection system. To minimize variations in the efficiency of cell lysis and DNA extraction between the samples, the co-extraction method was combined. A mini-transposon was used to introduce competitor DNA into the genome of other pseudomonads, and the resultant construct was used as the standard cell. After the addition of a fixed amount of the internal standard cells to soil samples, total DNA was extracted (co-extraction). Using this method, the copy number of the carAa gene and the cell number of strain CA10 in soil samples could be quantified rapidly.

Yano, A., N. Kaneko, et al. (2002). "Real-time PCR for quantification of Streptococcus mutans." FEMS Microbiology Letters **217**(1): 23.

http://www.sciencedirect.com/science/article/B6T2W-47184YJ-1/2/f2b6f91726155e6fc0857708d9156b3e

A real-time polymerase chain reaction (PCR) assay was developed for the quantification of Streptococcus mutans. Primers targeting gtf genes of S. mutans were designed and tested for their specificity using 28 oral streptococcal strains, three other bacterial strains, and human DNA. The primers could amplify specifically the target DNA fragment from a mixture of oral streptococcus genomic DNA containing about 10 fg to 10 ng of S. mutans genome DNA. The real-time PCR produced a linear quantitative detection range over concentrations spanning seven exponential values, with a detection limit of a few copies of S. mutans' genomic DNA per reaction tube. The results of the real-time PCR assay corresponded well to those of conventional culture assays for S. mutans in saliva samples. A real-time PCR assay for Streptococcus sobrinus and Streptococcus downei was also established and produced results that corresponded well to those from conventional culture assays for S. sobrinus in saliva samples. These assays will be useful as a new means to assess one of the important risk factors for caries.

Yasuda, M. and M. P. Shiaris (2005). "Differentiation of bacterial strains by thermal gradient gel electrophoresis using non-GC-Clamped PCR primers for the 16S-23S rDNA intergenic spacer

region." FEMS Microbiology Letters **243**(1): 235.

http://www.sciencedirect.com/science/article/B6T2W-4F29DFV-3/2/f26b39f427b2cb39565085c58dbee709

The method for DNA fingerprinting of the 16S-23S rDNA intergenic spacer region was modified to increase resolution of bacterial strains by thermal gradient gel electrophoresis (TGGE) analysis. By utilizing the high melting temperature region of the tRNA gene located in the middle of the 16S-23S rDNA intergenic spacer region as an internal clamp for TGGE, multiple melting domain problems were solved. PCR primers lacking a stretch of GC-rich sequences (GC-clamp) amplified the intergenic spacer region more efficiently than GC-clamped primers. Therefore, PCR artifacts were avoided by using low, 17-cycle, PCR. The method was successfully applied to diverse bacterial species for strain differentiation by TGGE without requiring a special PCR primer set.

Zhang, D., J. Manos, et al. (2004). "Transcriptional analysis and operon structure of the tagA-orf2-orf3-mop-tagD region on the Vibrio pathogenicity island in epidemic V. cholerae." FEMS Microbiology Letters **235**(1): 199.

http://www.sciencedirect.com/science/article/B6T2W-4C889TD-4/2/a3acb4ee3f3b93e93accd77ed6af31f6

The Vibrio pathogenicity island (VPI) in epidemic Vibrio cholerae is an essential virulence gene cluster. The VPI can excise from the chromosome and form extrachromosomal circular excision products. The VPI is 41.2-kb in size and encodes 29 potential proteins, several of which have no known function and whose regulation is not well understood. To determine the transcriptional organization of the tagA-orf2-orf3-mop-tagD region located at the 5'-(left) end of the VPI, we used reverse-transcriptase-PCR (RT-PCR), Northern blot analysis and DNA sequencing. RT-PCR primers were designed to transcribe and amplify regions spanning two or more open reading frames so as to establish the transcriptional organization. RT-PCR and Northern blot results demonstrated that the tagA-tagD region is transcribed as a polycistronic message and organized into several potential operons including tagA-orf2, orf3-mop, orf3-mop-tagD and tagD alone. Transcriptional lacZ fusions supported the existence of a promoter upstream of orf3 that was toxT-dependent. Interestingly, our data suggests that the orf3 promoter can drive the expression of either a long transcript (orf3-mop-tagD) or a short transcript (orf3-mop) without tagD. Our data also suggests that tagD can be expressed from two different promoters and that tagD is either transcribed alone or co-expressed with orf3-mop under certain conditions. These studies provide new insight into the genetic structure, transcriptional organization and regulation of a cluster of virulence genes on the VPI of epidemic V. cholerae.

Zhang, Y. and F. I. Molina (1995). "Strain typing of Lentinula edodes by random amplified polymorphic DNA assay." FEMS Microbiology Letters **131**(1): 17.

http://www.sciencedirect.com/science/article/B6T2W-3Y45F4C-31/2/60c12f3b0745cbfdb6b5775816e2c6bb

Single 10-base primers were used to generate randomly amplified polymorphic DNA (RAPD) markers in the shiitake mushroom, Lentinula edodes. Seven primers produced polymorphisms in all 15 strains tested, producing 12-19 bands ranging from 0.34 to 2.52 kb. Thirteen of the 15 strains had unique DNA fingerprints, whereas L. edodes ATCC 28759 and ATCC 28760 exhibited identical RAPD profiles for all the primers. Molecular-genetic markers obtained with the RAPD assay can be used to differentiate strains of L. edodes and have potential applications in

mushroom breeding and strain improvement programs.

Zhu, P., M. J. Klutch, et al. (2001). "Genetic analysis of conservation and variation of lipooligosaccharide expression in two L8-immunotype strains of Neisseria meningitidis." FEMS Microbiology Letters **203**(2): 173.

http://www.sciencedirect.com/science/article/B6T2W-43XFCNV-1/2/fc92cc737dc14b318c5bf5598fd9c1d1

Neisseria meningitidis strains A1 and M978 both express the lipooligosaccharide (LOS) L8 immunotype [Gu et al., J. Clin. Microbiol. 30 (1992) 2047-2053]. Under different growth conditions, strain A1 did not change its LOS profile whereas strain M978 produced variable LOS profiles on SDS-PAGE. To understand the genetic basis of LOS conservation and variation, their lgt locus encoding glycosyltransferases responsible for the biosynthesis of the [alpha]-chain of LOS was analyzed. Strain A1 possessed only two genes, lgtA and lgtH, at the lgt locus. The lgtA gene was inactivated due to a frameshift mutation; thus strain A1 expressed only L8 LOS. In contrast, strain M978 contained five genes lgtZ, lgtC, lgtA, lgtB and lgtE at this locus, thus it had a potential to express L1, L3,7 in addition to the L8 LOS. The data showed that strain A1 is a better reference strain for the L8 immunotype because of the stability of L8 LOS expression resulting from its unique lgt locus. In addition, these two strains had two new genetic organizations, lgtAH and lgtZCABE, compared to the reported gene organization at the lgt locus in N. meningitidis.

FEMS Microbiology Reviews (1)

Holden, P. J. and R. W. Brown (1993). "Amplification of ribulose bisphosphate carboxylase/oxygenase large subunit (RuBisCO LSU) gene fragments from Thiobacillus ferrooxidans and a moderate thermophile using polymerase chain reaction." FEMS Microbiology Reviews **11**(1-3): 19.

http://www.sciencedirect.com/science/article/B6T37-47DKYBV-5/2/faf527517fbbcbccb850a11a28d7e4fc

Southern blot analysis of DNA from an iron-oxidising moderate thermophile NMW-6 and from Thiobacillus ferrooxidans strain TF1-35 demonstrated sequences homologous to the RuBisCO LSU gene of Synechococcus. DNA fragments (457 bp) encoding part of the RuBisCO LSU gene (amino acids 73-200) were amplified from the genomic DNA of Thiobacillus ferrooxidans and the moderate thermophile NMW-6 using the polymerase chain reaction (PCR) technique (Saiki et al. (1985) Science 233, 1350-1354). A comparison with the LSU sequences from T. ferrooxidans, Alcaligenes eutrophus, Chromatium vinosum, Synechococcus and Spinacea oleracea, which all have RuBisCOs with a hexadecameric structure, showed that the RuBisCO LSU gene sequence from NMW-6 appeared to be most closely related to that of the hydrogen bacterium A. eutrophus which showed 71.9% homology at the amino acid level. Despite its physiological similarity, T. ferrooxidans showed only 64.1% homology to the amino acid sequence from NMW-6 and had the lowest DNA homology (60.9%) of the hexadecameric type RuBisCOs. In the region sequenced, T. ferrooxidans and the RuBisCOs of the phototrophs C. vinosum, Synechococcus and S. oleracea, had 17 residues that were completely conserved which were substituted in both NMW-6 and A. eutrophus, 11 of these being identical substitutions. Comparison of the nucleotide and derived amino acid sequences of the RuBisCO LSU fragment from T. ferrooxidans with other

RuBisCO sequences indicated a closer relationship to the hexadecameric type LSU genes of photosynthetic origin than to that of A. eutrophus. The T. ferrooxidans amino acid sequence showed 93.8%, 78.9% and 77.3% homology, respectively, to the C. vinosum, Synechococcus and S. oleracea (spinach) sequences but only 56.2% to A. eutrophus. The DNA sequence from Rhodospirillum rubrum, which has the atypical large subunit dimer RuBisCO structure with no small subunit, showed 39.2% and 42.7% homology, respectively, with the sequences of NMW-6 and T. ferrooxidans, and 25.0% and 29.7% amino acid homology, indicating that the DNA homology was substantially random in nature. PCR fragments (126 bp) that overlaped the last 15 codons of the fragments above were also amplified and sequenced. They showed incomplete homology with the larger fragments, supporting evidence obtained from Southern hybridizations that T. ferrooxidans and the moderate thermophile NMW-6 have multiple copies of RuBisCO LSU genes.

FEMS Yeast Research (3)

Hansen, J., S. V. Bruun, et al. (2002). "The level of MXR1 gene expression in brewing yeast during beer fermentation is a major determinant for the concentration of dimethyl sulfide in beer." FEMS Yeast Research **2**(2): 137.

http://www.sciencedirect.com/science/article/B6W8C-45JY96G-1/2/02f05665945b5df3872f9c4655a7bbe6

DMS (dimethyl sulfide) is an important beer flavor compound which is derived either from the beer wort production process or via the brewing yeast metabolism. We investigated the contribution of yeast MXR1 gene activity to the final beer DMS content. The MXR1-CA gene from Saccharomyces carlsbergensis (synonym of Saccharomyces pastorianus) lager brewing yeast was isolated and sequenced, and found to be 88% identical with Saccharomyces cerevisiae MXR1. Inactive deletion alleles of both genes were substituted for their functional counterparts in S. carlsbergensis. Such yeasts fermented well and did not form DMS from dimethyl sulfoxide. Overexpression in brewing yeast of MXR1 from non-native promoters with various strengths and transcription profiles resulted in an enhanced and correlated DMS production. The promoters of MXR1 and MXR1-CA contain conserved Met31p/Met32p binding sites, and in accordance with this were found to be co-regulated with the genes of the sulfur assimilation pathway. In addition, conserved YRE-like DNA sequences are present in these promoters, indicating that Yap1p may also take part in the control of these genes.

Johannesen, P. F. and J. Hansen (2002). "Differential transcriptional regulation of sulfur assimilation gene homologues in the Saccharomyces carlsbergensis yeast species hybrid." FEMS Yeast Research **1**(4): 315.

http://www.sciencedirect.com/science/article/B6W8C-44JJF6D-1/2/52a6b88f02d2a3d31171a2a10dd7e8c7

The allopolyploid yeast Saccharomyces carlsbergensis appears to be a relatively newly formed species hybrid, and therefore constitutes a good model for studying early steps in hybrid speciation. Using reverse transcription-coupled polymerase chain reaction to monitor derepression of the S. carlsbergensis homologues of the sulfur assimilation genes MET14 and

MET2, we found that both homologues of these genes are regulated in the same pathway-specific manner, but surprisingly, with different kinetics, as the genes derived from one of the parent species (the non-Saccharomyces cerevisiae-like) are alleviated from repression much faster than the genes from the other parent (the S. cerevisiae-like). This probably reflects differing physiological adaptation of the parent species, and the finding may contribute to the general understanding of hybrid speciation.

Latouche, G. N., T. C. Sorrell, et al. (2002). "Isolation and characterisation of the phospholipase B gene of Cryptococcus neoformans var. gattii." FEMS Yeast Research **2**(4): 551.

http://www.sciencedirect.com/science/article/B6W8C-468CC2G-1/2/28e6cd4cd0a91aa37b2df9da37ea87d4

Cryptococcus neoformans var. gattii (serotypes B and C) is a human pathogen, ecologically, biochemically, clinically and genetically different from C. neoformans var. grubii (serotype A) and C. neoformans var. neoformans (serotype D). The phospholipase B (PLB1) gene from serotypes B and C was isolated and characterised. It resembled the serotype A and D genes, with an overall sequence homology of more than 85%. The respective open reading frames were 2236 bp (serotype B) and 2239 bp (serotype C) in length. Each contained six introns and encoded a 68-kDa protein destined for secretion. PLB1 was located on the second smallest chromosome in both serotypes. Gene expression, measured as mRNA, was not regulated by temperature, pH or exogenous nutrients.

Fertility and Sterility (34)

Barbieri, R. L. and X. Gao (1997). "Presence of 17[beta]-hydroxysteroid dehydrogenase type 3 messenger ribonucleic acid transcript in an ovarian Sertoli-Leydig cell tumor." Fertility and Sterility **68**(3): 534.

http://www.sciencedirect.com/science/article/B6T6K-3S3N1PM-Y/2/9c48bdf631d7aeeec6fd96cdd3d521e5

Objective: To evaluate the level of 17[beta]-hydroxysteroid dehydrogenase (17[beta]-HSD) type 1, 2, and 3 transcripts in a Sertoli-Leydig cell tumor, adjacent theca lutein ovarian tissue, and normal control ovarian stromal tissue.Setting: An academic medical center.Design: Total RNA was extracted from formalin-fixed, paraffin-embedded tissue slides and used for reverse transcription-polymerase chain reaction (RT-PCR) with primers specific for 17[beta]-HSD types 1, 2, or 3. The PCR products were subjected to Southern hybridization with 5' [32p] end-labeled internal primers for each type of the isozymes.Patient(s): A 35-year-old woman with a Sertoli-Leydig cell tumor on her right ovary. The tumor and the right ovary were surgically removed. Control ovarian stromal tissue was obtained from a woman undergoing hysterectomy for uterine leiomyomata.Result(s): In the control ovarian stromal tissue, the transcripts for the type 1 and type 2 isoforms were the predominant transcripts detected. In the Sertoli-Leydig cell tumor, the transcript for the type 3 isoform was the predominant transcript detected. Ovarian tissue from the same ovary as the Sertoli-Leydig cell tumor (diagnosed as theca lutein cysts on histologic examination) expressed the type 2 and 3 transcripts.Conclusion(s): The 17[beta]-HSD type 3 isoform efficiently converts androstenedione to T and is the predominant HSD isoform in the

testis. In hyperandrogenism caused by a Sertoli-Leydig cell tumor, both the tumor tissue and nontumor tissue from the same ovary expressed the "testicular" form of the 17[beta]-HSD.

Briton-Jones, C., I. Hung Lok, et al. (2001). "Regulation of human oviductin mRNA expression in vivo." Fertility and Sterility **75**(5): 942.

http://www.sciencedirect.com/science/article/B6T6K-42WX434-H/2/7d23c67487064d3b6b9205d49c29e71a

Objective: To examine changes in oviductin mRNA expression in oviductal mucosal tissue from fertile women throughout an ovulatory cycle.Design: Semiquantitative reverse-transcriptase-polymerase chain reaction (RT-PCR) analysis of oviductin mRNA.Setting: University-based obstetrics and gynecology department.Subject(s): Twenty women undergoing laparoscopy for tubal sterilization or hysterectomy for uterine fibroids.Intervention(s): The mucosal layer was isolated from the oviduct tissue, and semiquantitative RT-PCR was performed.Main Outcome Measure(s): The relationship between serum estradiol, luteinizing hormone, and progesterone concentrations and the expression of oviductin mRNA.Result(s): There was a significant positive correlation between serum estradiol and luteinizing hormone concentrations and oviductin mRNA expression. There was a significant inverse correlation between serum progesterone concentrations and oviductin mRNA expression.Conclusion(s): Little is known about the regulation of human oviductin. This study was the first to examine the relationship between oviductin mRNA expression and serum estradiol and luteinizing hormone and progesterone concentrations in fertile women. Estradiol and luteinizing hormone both have a stimulatory effect on oviductin mRNA in humans, however, it is difficult to determine whether the effects are independent of one another, as the luteinizing hormone surge is dependent on the estradiol increase. Progesterone shows a clear inhibitory effect on oviductin mRNA.

Briton-Jones, C., I. H. Lok, et al. (2004). "Estradiol regulation of oviductin/oviduct-specific glycoprotein messenger ribonucleic acid expression in human oviduct mucosal cells in vitro." Fertility and Sterility **81**(Supplement 1): 749.

http://www.sciencedirect.com/science/article/B6T6K-4C6JJFF-3/2/80dd92228bc668f86a8eff6bc6d26c5a

ObjectiveTo determine whether oviduct mucosal cell culture with exogenous 17[beta] E2 supports the continued production of oviductin, a putative embryotrophic protein.DesignSemiquantitative reverse-transcriptase polymerase chain reaction analysis of oviductin mRNA expression after oviduct mucosal cell culture in the presence of 17[beta] E2. Three different culture systems were studied to investigate the response to E2.SettingUniversity-based obstetrics and gynecology department.SubjectsOviduct tissue was obtained from 18 women undergoing laparoscopy for benign gynecologic conditions.Intervention(s)The mucosal layer was isolated from the oviduct tissue and exposed to three different culture systems, which contained various concentrations of 17[beta] E2, or vehicle-only control.Main outcome measure(s)The relationship between exposure to 17[beta] E2 and expression of oviductin messenger (m)RNA by cultured oviduct mucosal cells.Result(s)There was a significant increase in oviductin mRNA expression after the addition of 17[beta] E2 to the culture system in which the in vivo cell-to-cell and cell-to-basement-membrane contacts of the oviduct had been maintained.Conclusion(s)Estradiol failed to alter oviductin mRNA expression in oviduct mucosal cells cultured under conditions in which the ciliated mucosal cell phenotype plus the cell-to-cell and cell-to-basement-membrane contacts of the oviduct were lost. However, with a culture system that maintained the cell architecture, E2 initiated and significantly increased oviductin mRNA expression.

Briton-Jones, C., I. H. Lok, et al. (2003). "Human chorionic gonadotropin and 17-[beta] estradiol regulation of human oviductin/oviduct specific glycoprotein mRNA expression in vitro." Fertility and Sterility **80**(Supplement 2): 720.

http://www.sciencedirect.com/science/article/B6T6K-49J881K-6/2/2c2cd0cca6cc4467da03b361f02bcb1c

ObjectiveTo determine whether oviduct mucosal cell culture with exogenous hCG or 17-[beta] estradiol (E2) supports the continued production of oviductin, a putative embryotrophic protein.DesignSemiquantitative reverse transcriptase-polymerase chain reaction (RT-PCR) analysis of oviductin mRNA expression after oviduct mucosal cell culture in the presence of hCG or 17-[beta] E2.SettingUniversity-based Obstetrics and Gynecology Department.Subject(s)Ten women undergoing laparoscopy for tubal sterilization or hysterectomy for uterine fibroids.Intervention(s)The mucosal layer was isolated from the oviduct tissue, subjected to routine culture conditions with the addition of various concentrations of hCG or 17-[beta] E2 or the equivalent vehicle-only control and semiquantitative RT-PCR performed.Main outcome measure(s)The relationship between exposure to hCG or 17-[beta] E2 and expression of oviductin mRNA by cultured oviduct mucosal cells.Result(s)There was a significant increase in oviductin mRNA expression after the addition of hCG to the culture medium but only in samples that had maintained a baseline level of oviductin expression. Addition of 17-[beta] E2 to the culture medium had no significant effect on oviductin mRNA expression.Conclusion(s)Under standard cell culture conditions, baseline human oviductin mRNA expression is increased by the addition of hCG. This effect is likely to be a secondary or synergistic effect as exogenous hCG failed to restore oviductin mRNA expression in samples where expression was lost after culture. E2 failed to alter oviductin mRNA expression in oviduct mucosal cells cultured under these conditions.

Briton-Jones, C., I. H. Lok, et al. (2002). "Human oviductin mRNA expression is not maintained in oviduct mucosal cell culture." Fertility and Sterility **77**(3): 576.

http://www.sciencedirect.com/science/article/B6T6K-456BW2B-X/2/947f144b8ae9910d6074306ccda1a0e2

Objective: To determine whether oviduct mucosal cell culture supports the continued production of oviductin, a putative embryotrophic protein.Design: Semiquantitative reverse-transcriptase polymerase chain reaction (RT-PCR) analysis of oviductin messenger RNA (mRNA) expression after oviduct mucosal cell culture.Setting: University-based obstetrics and gynecology department.Patient(s): Ten women undergoing laparoscopy for tubal sterilization or hysterectomy for uterine fibroids.Intervention(s): The mucosal layer was isolated from the oviduct tissue and subjected to routine culture conditions; semiquantitative RT-PCR was performed.Main Outcome Measure(s): The relationship between duration of cell culture and expression of oviductin mRNA.Result(s): There was a significant reduction in oviductin mRNA expression after 3 days in culture, with a complete loss after 6 days in 70% of the samples and after 12 days in the remaining 30%.Conclusion(s): This is the first study to investigate whether oviductin mRNA continues to be expressed in cultured human oviduct mucosal cells. Our results suggest that oviduct mucosal cells lose their ability to produce oviductin after short-term culture. This method of culture does not appear to be appropriate for a coculture system reliant upon oviductal secretion of oviductin.

Chang, C.-C., Y.-Y. Hsieh, et al. (2002). "The proline form of p53 codon 72 polymorphism is associated with endometriosis." Fertility and Sterility **77**(1): 43.

http://www.sciencedirect.com/science/article/B6T6K-44T0V32-6/2/f26f9f62cc2a81dea5d8373a74282503

Objective: To evaluate the association between endometriosis and the p53 polymorphism.Design: Prospective study.Setting: Department of gynecology and genetics in a medical center.Patient(s): Women with and without endometriosis.Intervention(s): Women were categorized as having moderate or severe endometriosis (n = 118) or no endometriosis (n = 140).Main Outcome Measure(s): Polymerase chain reaction was used to detect p53 codon 72 polymorphisms (arginine homozygosity, heterozygosity, and proline homozygosity). Associations between endometriosis and p53 polymorphisms were evaluated.Result(s): The distributions of different p53 polymorphisms differed significantly between groups. The respective proportions of arginine homozygotes, heterozygotes, and proline homozygotes were 10.2%, 66.9%, and 22.9% in the group with endometriosis and 30.7%, 50%, and 19.3% in the group without endometriosis.Conclusion(s): Endometriosis is associated with p53 polymorphism. p53 arginine homozygotes have lower risk for endometriosis. Heterozygotes and proline homozygotes have higher risk for endometriosis.

Chen, C.-P., S.-R. Chen, et al. (1999). "A frame shift mutation in the DNA-binding domain of the androgen receptor gene associated with complete androgen insensitivity, persistent mullerian structures, and germ cell tumors in dysgenetic gonads." Fertility and Sterility **72**(1): 170.

http://www.sciencedirect.com/science/article/B6T6K-3WS633J-14/2/8df9859637c4597b7bd730517814f1cf

Objective: To describe the molecular, cytogenetic, immunohistochemical, and endocrinologic characteristics of a young 46,XY female with persistent mullerian structures and germ cell tumors in dysgenetic gonads.Design: Descriptive case study.Setting: Mackay Memorial Hospital and National Yang-Ming University, Taipei, Taiwan.Patient(s): A 22-year-old 46,XY female with persistent mullerian structures, a low level of serum testosterone, and no apparent adnexal masses.Intervention(s): Laparoscopic removal of the dysgenetic gonads.Main Outcome Measure(s): Detection of an androgen receptor gene mutation by a semiautomated DNA sequencer, of the chromosomal complement by cytogenetic examination, of placental alkaline phosphatase activity by immunohistochemical analysis, and of neoplasms in dysgenetic gonads by histologic studies.Result(s): A unilateral gonadoblastoma and a contralateral gonadoblastoma associated with a dysgerminoma were found in the excised gonads. The tumors had a 46,XY complement. Placental alkaline phosphatase was present in the tumor cells. A frameshift mutation in the DNA-binding domain of the androgen receptor gene was detected in the patient's blood and the tumor tissues. A five-nucleotide "AGGAA" deletion at codons 608 and 609 of the androgen receptor gene resulted in a missing arginine and lysine as well as a frameshift that introduced a stop codon 12 amino acid downstream from the mutation.Conclusion(s): Molecular genetic analysis of the androgen receptor gene aids in the rapid diagnosis of complete androgen insensitivity irrespective of atypical clinical phenotypes and endocrinologic parameters.

Chen, H.-F., J.-Y. Shew, et al. (1999). "Expression of leukemia inhibitory factor and its receptor in preimplantation embryos." Fertility and Sterility **72**(4): 713.

http://www.sciencedirect.com/science/article/B6T6K-3XK6T78-X/2/bde87da96c159376263afc89a222b3d5

Objective: To examine the expression of leukemia inhibitory factor (LIF) and its receptor (LIF-R) transcripts in human and murine preimplantation embryos.Design: Prospective study.Setting: University medical center.Patient(s): Human oocytes were obtained from patients undergoing IVF treatment. Two-cell murine embryos were obtained from ICR strain mice.Intervention(s): Second-day intracytoplasmic sperm injection procedures were performed on oocytes that failed to be fertilized by IVF. Embryos were cultured to various stages and collected for study.Main Outcome Measure(s): The transcript levels of LIF and LIF-R in these embryos were examined and semiquantitated using single-cell reverse transcription-polymerase chain reaction methodology.Result(s): Leukemia inhibitory factor and LIF-R transcripts were detectable in most human preimplantation embryos (30 of 34 and 31 of 34 embryos showed LIF and LIF-R messenger RNA, respectively). There was a trend toward decreased expression of both transcripts in embryos at the four-cell stage and in embryos in which growth had been arrested for 24-48 hours. The expression of LIF and LIF-R genes in murine embryos was inconsistent.Conclusion(s): Preimplantation human embryos express LIF and LIF-R messenger RNA. It is suggested that LIF may be able to affect embryo development through its action at stages before implantation in an autocrine or paracrine manner.

Daneshmand, S., S. R. Weitsman, et al. (2002). "Overexpression of theca-cell messenger RNA in polycystic ovary syndrome does not correlate with polymorphisms in the cholesterol side-chain cleavage and 17[alpha]-hydroxylase/C17-20 lyase promoters." Fertility and Sterility **77**(2): 274.

http://www.sciencedirect.com/science/article/B6T6K-45082BJ-D/2/fa22b9e7e84cd4c7dd0f06023d9600f7

Objective: To determine whether overexpression of CYP17 or CYP11A messenger (m)RNA in theca cells from polycystic ovaries is related to polymorphic regions in the gene promoters that may increase transcription.Design: Case-control study.Setting: Research institute.Patient(s): Fifty-one women with PCOS and 280 regularly cycling controls underwent genotyping. Thecal cells were obtained from 23 women with PCOS and 51 controls.Main Outcome Measure(s): Ovarian tissue was obtained from women with PCOS undergoing wedge resection for treatment of their infertility and from controls undergoing ovariectomy for indications unrelated to the study. Expression of mRNA in theca cells was measured by using competitive reverse transcriptase polymerase chain reaction. Genotype analysis for polymorphisms in the CYP11A and CYP17 promoters was performed by using polymerase chain reaction.Result(s): Although expression of CYP11A and CYP17 mRNA was higher in women with PCOS, no significant dose effects of CYP11A or CYP17 alleles were observed with respect to serum testosterone; follicular fluid androstenedione, estradiol, and androstenedione-to-estradiol ratio; or CYP11A or CYP17 mRNA expression.Conclusion(s): Overexpression of CYP17 and CYP11A mRNA in theca cells from polycystic ovaries is explained by polymorphic differences in the gene promoters.

Erhart, B., P. J. Chan, et al. (1998). "Ofloxacin: the next generation of antibiotic in sperm and embryo cultures for assisted reproductive technologies." Fertility and Sterility **69**(2): 246.

http://www.sciencedirect.com/science/article/B6T6K-3VKJXCV-1S/2/ebd34c781f7337c45f8d4317da588ba4

Objective: To analyze the effect of different concentrations of ofloxacin on sperm kinematic parameters and to determine the embryotoxicity of ofloxacin at physiologic and at 100 x concentrations.Design: Prospective comparative study.Setting: Clinical and academic research environment.Patient(s): Pooled cryopreserved donor sperm (n = 7).Intervention(s): Human sperm were processed through two-layer discontinuous Percoll gradients, and the resultant pellet was resuspended in either HEPES-buffered human tubal fluid medium containing different

concentrations of ofloxacin or the control medium. After measuring the kinematic parameters, the percentages of apoptosis and viability were obtained. Next, the sperm DNA was extracted and polymerase chain reaction of P-globin gene was performed followed by denaturing gradient gel electrophoresis. Mouse embryos recovered at the one-cell pronuclear or zygote stages were cultured in the presence or absence of ofloxacin up to the hatched blastocyst stage and differences in development were recorded.Main Outcome Measure(s): Sperm kinematic parameters, sperm P-globin gene, and number of embryos reaching the hatched blastocyst stage.Result(s): The number of embryos exposed to control and physiologic ofloxacin concentrations showed comparable excellent growth. However, the 100 x concentration significantly arrested development. Rates of sperm viability and apoptosis measured 48 hours after exposure to the above concentrations were not different from controls. No differences were noted in the sperm kinematic parameters of sperm exposed to ofloxacin concentrations (1 X, 10 x, and 100X) or control medium after 0, 4, and 48 hours of incubation. Denaturing gradient gel electrophoresis of [beta]-globin genes from DNA exposed to varying ofloxacin concentrations failed to show any point mutations.Conclusion(s): Ofloxacin was embryotoxic at pharmacologic concentrations (100 x). At physiologic or higher concentrations, ofloxacin appears to be safe and does not affect sperm kinematic parameters when compared with controls. This may indicate that sperm motility parameters alone cannot be relied on to evaluate the effects of drugs on fertility and that in vitro embryologic studies are essential. Ofloxacin at any concentration did not alter the rates of sperm apoptosis or viability. Ofloxacin does not appear to be mutagenic as evidenced by the [beta]-globin gene analysis.

Giltay, J. C., M. Deege, et al. (2004). "Apparent primary follicle-stimulating hormone deficiency is a rare cause of treatable male infertility." Fertility and Sterility **81**(3): 693.

http://www.sciencedirect.com/science/article/B6T6K-4BY331V-1B/2/60049cc3143f1dab9a48ce0cbb9b9050

ObjectiveTo find the underlying defect in a case of primary FSH deficiency and to estimate the beneficial effect of FSH treatment.DesignCase report.SettingUniversity hospital fertility clinic.Patient(s)Normal, healthy, 37-year-old male patient with severe oligoteratozoospermia.Intervention(s)Levels of FSH, LH, LHRH provocation test, karyotyping, genomic analysis on the Y-chromosomal AZF region and sequencing of the FSHB gene, FSH treatment.Main outcome measure(s)We compiled detailed clinical and molecular data on four pregnancies. We compare this case with a similar case published recently.Result(s)There were detectable but very low FSH levels after LHRH provocation; the LH response was not entirely normal, and no genomic abnormalities were found in the FSHB gene. The FSH treatment resulted in four pregnancies, two of which ended in abortion; the other two resulted in the birth of two healthy children. Both our case and the published case had detectable but abnormally low FSH levels on some occasions, but normal or highly normal inhibin B levels that differed from the expected low levels. Both patients had a normal male phenotype and no detectable mutation in the FSHB gene. The published case differed from our patient in that the published case was azoospermic whereas ours was extremely oligoteratozoospermic. The beneficial effect of FSH treatment was only shown in our patient.Conclusion(s)The published case and ours may have a common, as yet unidentified, underlying defect. The dramatic and immediate effect of FSH treatment on our patient's fertility was clearly demonstrated.

Hsieh, Y.-Y., C.-C. Chang, et al. (2005). "Estrogen receptor [alpha] dinucleotide repeat and cytochrome P450c17[alpha] gene polymorphisms are associated with susceptibility to endometriosis." Fertility and Sterility **83**(3): 567.

http://www.sciencedirect.com/science/article/B6T6K-4FM9D09-

9/2/3cda3b300cfc292ec5ca21baa6f9380f

ObjectiveTo investigate the association of endometriosis with estrogen receptor alpha (ER[alpha]) and cytochrome P450c17[alpha] (CYP17) gene polymorphisms in light of the fact that estrogen plays a role in the pathogenesis of endometriosis and the CYP17 enzyme is involved with estrogen biosynthesis.DesignProspective study.SettingGenetics and gynecology units.Patient(s)All patients were divided into two groups: group 1, women with endometriosis (n = 119); group 2, normal controls (n = 108).Intervention(s)A dinucleotide (thymine-adenine [TA]) repeat polymorphism lying upstream of the ER[alpha] gene and A1/A2 polymorphism of the CYP17 gene were amplified by polymerase chain reaction, enzyme restriction, and electrophoresis.Main outcome measure(s)The ER genotypes were classified into A through T (TA repeats, 10-29). The CYP17 genotypes included indigestible (A1 homozygote), heterozygote, and digestible (A2 homozygote). We compared these polymorphism distributions in both groups.Result(s)The percentage of genotypes D-G (TA, 13-16) in both groups were 10.5%, 29.4%, 13.0%, and 11.3% in group 1 and 7.9%, 16.7%, 19.9%, and 17.6% in group 2. The genotype E (14 TA repeats) is associated with a higher risk of endometriosis. Proportions of A1 homozygote/heterozygote/A2 homozygote for CYP17 were 26.1%/46.2%/27.7% for group 1 and 14.8%/44.5%/40.7% for group 2, respectively. The A1 homozygote and allele were associated with a higher susceptibility of endometriosis.Conclusion(s)ER[alpha]* 14 TA repeats and the CYP17* A1 allele are associated with an increased risk of endometriosis. Both polymorphisms are useful markers for predicting endometriosis susceptibility.

Hsieh, Y.-Y., C.-C. Chang, et al. (2005). "T homozygote and allele of epidermal growth factor receptor 2073 gene polymorphism are associated with higher susceptibility to endometriosis and leiomyomas." Fertility and Sterility **83**(3): 796.

http://www.sciencedirect.com/science/article/B6T6K-4FM9D09-1S/2/85ab2a38941778472772a0efdbf983ea

Epidermal growth factor receptor (EGFR) is a regulator of angiogenesis and mediator of sex steroid-induced cell growth and differentiation. We observed that EGFR gene 2073*T-related genotypes and allele are associated with higher susceptibilities to endometriosis and leiomyoma.

Hsieh, Y.-Y., C.-C. Chang, et al. (2004). "Tumor necrosis factor-[alpha]-308 promoter and p53 codon 72 gene polymorphisms in women with leiomyomas." Fertility and Sterility **82**(Supplement 3): 1177.

http://www.sciencedirect.com/science/article/B6T6K-4DH19R3-11/2/9b45e313228da532c4b8ba51d81ebce7

ObjectiveTumor necrosis factor-[alpha] (TNF-[alpha]), a proinflammatory cytokine, plays an important role in the process of autoimmune diseases. p53 is related to the regulation of cell growth and prevention of carcinogenesis. We propose to investigate whether gene polymorphisms for TNF-[alpha]-308 promoter and p53 could be used as markers of susceptibility in leiomyomas.DesignProspective basic study.SettingDepartments of gynecology and genetics in a medical center.Patient(s)Group 1: leiomyoma (n = 159); group 2: non-leiomyoma (n = 131).Intervention(s)Genomic DNA was obtained from peripheral leukocyte. The TNF-[alpha] and p53 gene polymorphisms were amplified by polymerase chain reaction (PCR), enzyme restriction, and electrophoresis.Main outcome measure(s)Two gene polymorphisms were identified: [1] the A (cuttable)/G (uncuttable) polymorphisms of the TNF-[alpha] gene on chromosome 6p21.3; [2] A (cuttable)/P (uncuttable) polymorphisms of the p53 gene on chromosome 17p. Genotype and allelic frequencies in both groups were compared.Result(s)Genotype distribution and allele

frequency of TNF-[alpha] gene polymorphism in both groups were significantly different. Proportions of A homozygote/heterozygote/G homozygote for TNF-[alpha] in both groups were: (group 1) 61%/34.6%/4.4% and (group 2) 81.7%/14.5%/3.8%. Proportions of allele A/G for TNF-[alpha] in both groups were: (group 1) 78.3%/21.7% and (group 2) 88.9%/11.1%. Distributions of p53 polymorphisms in both groups were not different. The proportions of A homozygotes/heterozygotes/P homozygotes for p53 were (group 1) 32.7%/42.1%/25.2% and (group 2) 28.2%/48.9%/22.9%.Conclusion(s)G homozygote and G allele for TNF-[alpha] promoter are related to a higher risk of leiomyomas. The p53 codon 72 gene polymorphism is not associated with the susceptibility of leiomyomas.

Hsieh, Y.-Y., C.-C. Chang, et al. (2003). "Estrogen receptor thymine-adenine dinucleotide repeat polymorphism is associated with susceptibility to leiomyoma." Fertility and Sterility **79**(1): 96.

http://www.sciencedirect.com/science/article/B6T6K-47MH9GK-M/2/813be840971242eaba85fd4880ef360d

ObjectiveTo evaluate the association between leiomyomas and estrogen receptor gene polymorphism.DesignProspective study.SettingDepartment of gynecology and genetics.Patient(s)Women with (n = 159) or without leiomyomas (n = 131).Main outcome measure(s)Polymerase chain reaction was used to detect dinucleotide (thymine-adenine [TA]) repeat polymorphisms upstream of the estrogen receptor gene. Genotypes were classified as A through P according to the number of the TA repeats from 12 to 27. Distributions of TA repeat for estrogen receptor in both groups were compared.Result(s)Genotypes A to E were detected in 10.7%, 18.9%, 15.7%, 16.4%, and 4.4%, respectively, of women with leiomyomas and 4.2%, 9.5%, 20.6%, 19.1%, and 10.3% of women without leiomyomas. Women with genotypes A and B (12 or 13 TA repeats) have a higher risk for leiomyomas, and those with genotype E (16 TA repeats) have a lower risk.Conclusion(s)Estrogen receptor gene polymorphism probably contributes to the pathogenesis of leiomyoma and may predict the susceptibility to leiomyoma. The 12 and 13 TA repeats are associated with a higher risk of leiomyoma.

Hsieh, Y.-Y., C.-C. Chang, et al. (2001). "Androgen receptor trinucleotide polymorphism in endometriosis." Fertility and Sterility **76**(2): 412.

http://www.sciencedirect.com/science/article/B6T6K-43JY68D-1B/2/b8c5737f5e6fa87d2d7c663a4505881e

Iwabe M.D, T., T. Harada M.D, et al. (1998). "Pathogenetic Significance of Increased Levels of Interleukin-8 in the Peritoneal Fluid of Patients with Endometriosis." Fertility and Sterility **69**(5): 924.

http://www.sciencedirect.com/science/article/B6T6K-3V5N66Y-T/2/01a768dc73c5e5ff42e5b6849e44ebc5

Objective: To investigate the role of interleukin-8 (IL-8) in peritoneal fluid of patients with endometriosis in the pathogenesis of endometriosis.Design: Peritoneal fluid was collected by laparoscopy. Endometrial and endometriotic stromal cells were obtained from normal endometrium and from chocolate cyst linings of the ovary.Setting: Department of Obstetrics and Gynecology of Tottori University Hospital, Yonago, Japan.Patient(s): Forty women who underwent either laparoscopy or laparoscopic surgery.Main Outcome Measure(s): The peritoneal

fluid concentration of IL-8 was analyzed by enzyme-linked immunosorbent assay, and the correlation between the IL-8 concentration and the extent of active endometriosis was investigated. The effect of IL-8 on cell proliferation was examined by tetrazolium bromide and thymidine incorporation. The expression of IL-8 receptor was examined in stromal cells by reverse transcription polymerase chain reaction.Result(s): The level of IL-8 in peritoneal fluid was significantly higher in patients with endometriosis than in patients without endometriosis. A significant correlation was noted with the extent of active endometriosis. Interleukin-8 significantly increased the number of cells and DNA synthesis in the endometrial and endometriotic stromal cells in a dose-dependent manner. Transcripts of IL-8 receptor type A were detected in stromal cells.Conclusion(s): The present study suggests that IL-8 found in the peritoneal fluid of patients with endometriosis contributes to the pathogenesis of endometriosis.

Kok, H. S., K. M. van Asselt, et al. (2004). "Age at natural menopause is not linked with the follicle-stimulating hormone receptor region: a sib-pair study." Fertility and Sterility **81**(3): 611.

http://www.sciencedirect.com/science/article/B6T6K-4BY331V-V/2/331b21f34f19265e63aeb06ad06bb7e0

ObjectiveStudies have shown that age at natural menopause is heritable. Mutations in the FSH-receptor have been identified in women with premature ovarian failure (POF) and the FSH-receptor gene may, therefore, be considered a candidate gene for (early) menopausal age. This study investigates whether there is linkage between genetic markers in the FSH-receptor region and (early) age at menopause using a sib-pair design.DesignSib-pair based linkage analysis.SettingSister pairs and their first-degree family members from The Netherlands.Patient(s)The inclusion criteria for a family were natural menopause in upper or lower tail of the distribution of menopausal age in at least two sisters. A total of 126 families with at least one sib-pair were included in this study. Six polymorphic markers encompassing the FSH-receptor gene were genotyped.Intervention(s)None.Main outcome measure(s)Single point and multipoint logarithm of the odds (LOD) scores.Result(s)None of the markers showed evidence in favor of linkage with overall age at natural menopause or early age at natural menopause.Conclusion(s)Possibly, age at natural menopause in the more or less normal range is not part of the spectrum of phenotypes determined by mutations in the FSH-receptor gene. Alternatively, our results might be explained by genetic heterogeneity in the left tail of the distribution of menopausal age. This can limit the chance of finding a genetic locus, especially if this factor has a modest contribution to the phenotype.

Korhonen, S., E.-L. Romppanen, et al. (2003). "Two exonic single nucleotide polymorphisms in the microsomal epoxide hydrolase gene are associated with polycystic ovary syndrome." Fertility and Sterility **79**(6): 1353.

http://www.sciencedirect.com/science/article/B6T6K-48S3GMC-G/2/59f2a5c5a1f26d4bdf4368371140a96c

ObjectiveTo determine whether genetic variability in the gene encoding microsomal epoxide hydrolase (EPHX) contributes to individual differences in susceptibility to the development of polycystic ovary syndrome (PCOS).DesignRetrospective case-control study.SettingUniversity-based clinic.Patient(s)One hundred twelve white women with PCOS and 115 healthy controls.Intervention(s)None.Main outcome measure(s)The presence of two single nucleotide polymorphisms (SNPs), T->C (Tyr113His) in exon 3 and A->G (His139Arg) in exon 4, in the EPHX gene. Single point analysis was expanded to pair of loci haplotype analysis to examine the estimated haplotype frequencies of the two SNPs, of unknown phase, in the PCOS and control groups. Estimated haplotype frequencies were assessed using the maximum-likelihood method,

using an expectation-maximization algorithm.Result(s)Single point allele and genotype distributions in exon 3 and exon 4 of the EPHX gene were not statistically different between the groups. However, according to the haplotype estimation analysis, we observed a significantly elevated frequency of haplotype C-G (His113-Arg139) in the PCOS group versus the control group. The odds ratio for PCOS associated with the low activity haplotype C-G (His113-Arg139) was 2.28 (95% confidence interval 1.1-4.8).Conclusion(s)The use of two intragenic single nucleotide polymorphisms jointly in haplotype analysis of association demonstrated that the genetically determined low activity haplotype C-G (His113-Arg139) was significantly associated with PCOS.

Kutteh, W. H., V. M. Park, et al. (1999). "Hypercoagulable state mutation analysis in white patients with early first-trimester recurrent pregnancy loss." Fertility and Sterility **71**(6): 1048.

http://www.sciencedirect.com/science/article/B6T6K-3WK3SS6-D/2/7bcccc447477ca41cd01edca54de2cff

Objective: Antiphospholipid antibodies (APA) and other coagulation abnormalities have been associated with an increased risk of venous, arterial, and placental thrombosis and recurrent pregnancy loss (RPL). Factor V Leiden (a point mutation [1691G->A] in the factor V gene), the prothrombin 20210G->A mutation, and homozygosity for a common polymorphism in the methylene tetrahydrofolate reductase (MTHFR) gene (677C->T) have been associated with arterial and venous thrombosis and arterial occlusive disease. We explored an association between these markers of thrombophilic states and RPL.Design: Prospective case-control evaluation.Setting: University-associated private practice.Patient(s): Fifty nonpregnant women with three or more pregnancy losses and 50 healthy, nonpregnant controls.Intervention(s): None.Main Outcome Measure(s): Anticardiolipin and antiphosphatidylserine antibodies were detected in serum by ELISA. Polymerase chain reaction was performed to identify the factor V Leiden (1691G->A) mutation, the thermobile MTHFR (677C->T) mutation, and the prothrombin 20210G->A mutation.Result(s): The following were identified by restriction fragment-linked polymorphism analyses: 1 (2%) factor V Leiden heterozygosity; 1 (2%) prothrombin 20210G->A heterozygosity; and 4 (8%) thermolabile MTHFR homozygosity. None of these mutation frequencies in women with RPL were statistically significantly different from controls.Conclusion(s): These data suggest that factor V Leiden, thermolabile MTHFR (677C->T), and prothrombin 20210G->A are not found at an increased frequency in women with a history of early RPL.

Lam, P. M., C. Briton-Jones, et al. (2004). "Increased messenger RNA expression of vascular endothelial growth factor and its receptors in the implantation site of the human oviduct with ectopic gestation." Fertility and Sterility **82**(3): 686.

http://www.sciencedirect.com/science/article/B6T6K-4DB52W5-13/2/86d243eaf1e9daceecbf556a5ca1f654

ObjectiveTo compare the mRNA expression of vascular endothelial growth factor (VEGF) and its receptors (KDR and flt-1) in the implantation and nonimplantation sites of the human oviduct with ectopic gestation.DesignProspective observational study.SettingUniversity-based Obstetrics and Gynecology Department.Patient(s)Ten women undergoing laparoscopic salpingectomy for tubal pregnancy.Intervention(s)The mucosal layer was isolated from the implantation and nonimplantation sites of the oviduct tissue with ectopic gestation. Semiquantitative reverse transcriptase-polymerase chain reaction was performed.Main outcome measure(s)The differences in the mRNA expression of VEGF and its receptors between the implantation and nonimplantation sites of the oviduct tissue.Result(s)The mRNA expression of VEGF and its

receptors, both KDR and flt-1, was significantly higher in the implantation site of the human oviduct with ectopic gestation compared with the nonimplantation site.Conclusion(s)The results suggest that VEGF may be the angiogenic factor responsible for the implantation and placentation of an ectopic pregnancy in the oviduct.

Lam, P. M., C. Briton-Jones, et al. (2004). "In vivo regulation of mRNA expression of vascular endothelial growth factor receptors (KDR and flt-1) in the human oviduct." Fertility and Sterility **81**(2): 416.

http://www.sciencedirect.com/science/article/B6T6K-4BN4RKT-15/2/c4ac3941524b36f4145d94732fbafa0b

ObjectiveTo examine the localization of vascular endothelial growth factor receptors (VEGF-R) and the changes in VEGF-R messenger ribonucleic acid (mRNA) expression in various regions of the oviduct from fertile women throughout the ovulatory cycle.DesignProspective observational study.SettingUniversity-based obstetrics and gynecology department.Patient(s)Twenty-two women who underwent laparoscopic tubal sterilization or hysterectomy for a benign gynecological condition.Intervention(s)The mucosal layer was isolated from the oviduct tissue. Immunohistochemistry and a semiquantitative reverse-transcriptase-polymerase chain reaction (RT-PCR) was performed.Main outcome measure(s)Immunohistochemical localization of VEGF-R proteins in oviduct tissue, and the differences of VEGF-R mRNA expression in the various regions of the oviduct and in the various stages of the ovulatory cycle.Result(s)Immunohistochemical study localized VEGF-R, both KDR and flt-1, in the oviduct luminal epithelium, smooth muscle cells as well as blood vessels within the oviduct. Messenger RNA expression of KDR, but not flt-1, was significantly higher in the ampullary and infundibular regions than in the isthmus. Messenger RNA expression of flt-1, but not KDR, varied significantly in the oviduct along the course of an ovulatory cycle, with the highest level in the periovulatory stage.Conclusion(s)These results suggest that the two VEGF receptors may have different roles in the oviduct. Our data support a role for KDR in oviduct angiogenesis whereas flt-1 appears to be important in the temporal regulation of oviductal secretion.

Licht, P., M. von Wolff, et al. (2003). "Evidence for cycle-dependent expression of full-length human chorionic gonadotropin/luteinizing hormone receptor mRNA in human endometrium and decidua." Fertility and Sterility **79**(Supplement 1): 718.

http://www.sciencedirect.com/science/article/B6T6K-48CGY15-B/2/0dba5619be9abc0507d5f070d18fd6d4

ObjectiveTo investigate the expression of full-length and truncated hCG/LH-receptor mRNA in human endometrium and decidua.DesignIn vitro experiment.SettingTertiary university center.Patient(s)Premenopausal women undergoing hysterectomy because of benign diseases or induced abortions.Intervention(s)Isolation of RNA from endometrial samples, reverse transcription, selective preamplification of full-length hCG/LH receptor mRNA and several shorter fragments of the receptor gene (exons 1-11, 1-10, and 1-5), nested polymerase chain reaction with internal primers.Main outcome measure(s)Appropriately sized cDNA product confirmed by sequencing.Result(s)All samples derived from the proliferative as well as from the early and mid-luteal phases were positive for all four amplification products, suggesting the expression of a full-length hCG/LH receptor mRNA. Only 5 of 8 samples derived from the late secretory phase and 2 of 12 samples derived from early decidua amplified the entire receptor sequence. In contrast, the shortest fragment (exons 1-5), coding for part of the extracellular receptor domain, was amplified in all samples.Conclusion(s)The data suggest cycle-dependent regulation of hCG/LH-receptor mRNA by changes in the alternative splicing pattern and down-regulation of full-length hCG/LH receptor mRNA in early decidua. The major splicing site appears to be located between introns 5

and 9. Alternative splicing may be a mechanism regulating hCG/LH-receptor down-regulation.

Matovina, M., K. Husnjak, et al. (2004). "Possible role of bacterial and viral infections in miscarriages." Fertility and Sterility **81**(3): 662.

http://www.sciencedirect.com/science/article/B6T6K-4BY331V-13/2/08bf4a1bb444aa5807aad50425289d01

ObjectiveTo determine the role of infections in miscarriages. Chorionic villi from aborted material were subjected to cytogenetic evaluation and analyzed for the presence of Chlamydia trachomatis, Ureaplasma urealyticum, Mycoplasma hominis, human cytomegalovirus (HCMV), adeno-associated virus (AAV), and human papillomaviruses (HPV).DesignRetrospective study.SettingUniversity hospital and academic research institution.Main outcome measure(s)Karyotyping and detection of bacterial and viral DNA by means of polymerase chain reaction (PCR) in placenta specimens.Result(s)In 54 (50%) of 108 samples the karyotype was normal, in 38 (35%) samples it was abnormal, and in 16 (15%) samples karyotype was undetermined. No U. urealyticum, M. hominis, HCMV, or AAV-2 DNA was detected, while C. trachomatis DNA was detected in one (1%) and HPV DNA in eight (7%) samples. No significant correlation of HPV-positive findings with karyotype status was established.Conclusion(s)Our findings do not support a role of C. trachomatis, U. urealyticum, M. hominis, HCMV, or AAV infections in miscarriages during the first trimester of pregnancy. However, further investigation should be made to determine a possible involvement of HPVs in the development of genetic abnormalities of the fetus and in miscarriages.

Nabeshima, H., T. Murakami, et al. (2003). "Analysis of the clonality of ectopic glands in peritoneal endometriosis using laser microdissection." Fertility and Sterility **80**(5): 1144.

http://www.sciencedirect.com/science/article/B6T6K-49XHS6W-G/2/2961bc77d8c01205307705b2287d1a3c

ObjectiveTo investigate the clonality of ectopic gland cells in peritoneal endometriosis.DesignProspective study.SettingUniversity hospital.Patient(s)Seventeen women with surgically diagnosed endometriosis.Intervention(s)Samples of peritoneal endometriotic lesions were obtained from patients during laparoscopic surgery.Main outcome measure(s)Clonality analysis used the laser microdissection technique, a phosphoglycerate kinase (PGK) gene polymorphism assay, and an androgen receptor (AR) gene polymorphism assay after digestion of the DNA with methylation-sensitive endonuclease.Result(s)Each ectopic gland of the peritoneal endometriotic lesion showed a monoclonal pattern in both the PGK gene and AR gene assays, but the methylation pattern of the PGK gene and/or AR gene was divergent among adjacent glands in the lesion. These data indicate that the peritoneal endometriotic lesions are multicellular in origin, although individual glands of the lesion are derived from single precursor cells.Conclusion(s)The colored peritoneal endometriotic lesion in the present study was multicellular in origin. Peritoneal endometriotic lesions may thus be initiated by transplantation of a cluster of eutopic endometrial tissues into the pelvis.

Ondrizek, R. R., P. J. Chan, et al. (1999). "An alternative medicine study of herbal effects on the penetration of zona-free hamster oocytes and the integrity of sperm deoxyribonucleic acid." Fertility and Sterility **71**(3): 517.

http://www.sciencedirect.com/science/article/B6T6K-3VW837T-P/2/0fce01eca6ff5e83c0da725b1f529472

Objective: To analyze the effects of certain herbs on sperm DNA and on the fertilization process.Design: Prospective comparative study.Setting: Clinical and academic research environment.Patient(s): Donor sperm specimens.Intervention(s): Zona-free hamster oocytes were incubated for 1 hour in saw palmetto (Serenoa repens), echinacea purpura, ginkgo biloba, St. John's wort (Hypericum perforatum), or control medium before sperm-oocyte interaction. The DNA of herb-treated sperm was analyzed with denaturing gradient gel electrophoresis.Main Outcome Measure(s): Oocyte penetration and integrity of the sperm BRCA1 exon 11 gene.Result(s): Pretreatment of oocytes with 0.6 mg/mL of St. John's wort resulted in zero penetration. A lower concentration (0.06 mg/mL) had no effect. High concentrations of echinacea and ginkgo also resulted in reduced oocyte penetration. Exposure of sperm to echinacea purpura and St. John's wort resulted in DNA denaturation. In contrast, saw palmetto and ginkgo had no effect. Sperm exposed to 0.6 mg/mL of St. John's wort showed mutation of the BRCA1 exon 11 gene.Conclusion(s): High concentrations of St. John's wort, echinacea, and ginkgo had adverse effects on oocytes. Saw palmetto had no effect. The data suggested that St. John's wort, ginkgo, and echinacea at high concentrations damage reproductive cells. St. John's wort was mutagenic to sperm cells.

Patrizio, P., S. M. Ricci, et al. (2000). "Identification of meiotic and postmeiotic gene expression in testicular tissue of patients histologically classified as Sertoli cell only." Fertility and Sterility **74**(4): 785.

http://www.sciencedirect.com/science/article/B6T6K-41GNKR4-14/2/f0eb3a4d4a273ac927da0f3cbd615ef3

Objective: To determine whether meiotic and postmeiotic germ cell gene products could be detected in biopsy specimen from patients with Sertoli cell only (SCO) and maturation arrest.Design: Prospective clinical study.Setting: University-based departments and laboratories.Patient(s): Nine patients, seven with nonobstructive azoospermia (12 biopsies) and two with obstructive azoospermia (controls) (2 biopsies).Intervention(s): Specimens were divided into three parts: IVF laboratory, histology, and molecular analysis. Germ cell-specific messenger RNAs (mRNAs) were detected by extracting total RNA for Northern blotting or reverse transcription-polymerase chain reaction.Main Outcome Measure(s): Detection of meiotic (lactate dehydrogenase C4) and postmeiotic (transition protein 1 and protamine 1 and 2) gene expression and correlation with histologic and IVF laboratory findings.Result(s): The IVF laboratory identified spermatozoa in 3 of 14 biopsies (controls and severe hypospermatogenesis). Histologically, 6 of 14 biopsies (43%) were diagnosed as SCO, 4 (29%) maturation arrest, 2 (14%) severe hypospermatogenesis, and 2 normal. Molecular analysis showed mRNA for meiotic and postmeiotic genes in 12 of 14 biopsies (86%) (P =.006), of which 4 (67%) in SCO and 3 (75%) in maturation arrest.Conclusion(s): Differentiated germ cells are present in biopsies of men histologically diagnosed as SCO. Absence of these molecular markers strengthens the histologic diagnosis and helps the physician in counseling the infertile couple.

Stefansson, H., A. Einarsdottir, et al. (2001). "Endometriosis is not associated with or linked to the GALT gene." Fertility and Sterility **76**(5): 1019.

http://www.sciencedirect.com/science/article/B6T6K-44C0HF9-X/2/52db759e0db98a44f2d6731fa11d4fab

Objective: To investigate a possible association between the carrier frequency of the N314D mutation in the galactose-1-phosphate uridyl transferase (GALT) gene and endometriosis and linkage to the short arm of chromosome 9, where the GALT gene resides.Design: Association and linkage study.Setting: Population material collected for case and family studies in endometriosis.Patient(s): Women diagnosed with endometriosis by laparotomy or laparoscopy.Intervention(s): Association with the GALT gene investigated by genotyping 85 affected women and 213 unrelated control women and a scan for linkage to chromosome 9 in 205 women from 64 families with endometriosis.Main Outcome Measure(s): Multipoint parametric lod scores and frequency of alleles.Result(s): There was no significant difference in allele frequency for the N314D polymorphism in patients compared with control subjects. No evidence for linkage was found to chromosome 9p, where the GALT gene resides.Conclusion(s): The experiments reported herein provide no evidence supporting involvement of the GALT locus in the development of endometriosis.

Suzumori, N., M. Sato, et al. (1999). "Expression of secretory leukocyte protease inhibitor in women with endometriosis." Fertility and Sterility **72**(5): 857.

http://www.sciencedirect.com/science/article/B6T6K-3XRP7P8-J/2/050c4fc7b3e874d105a1de7b18576bd9

Objective: To explore endometriosis-related molecules in patients with use of differential display analysis.Design: Prospective study.Setting: Nagoya City University Medical School, Nagoya, Japan.Patient(s): Women with endometriosis (n = 27) and without endometriosis (n = 21).Intervention(s): Surgery was scheduled in the proliferative or secretory phase of the menstrual cycle.Main Outcome Measure(s): Differentially expressed products of endometrioma samples were sequenced at nucleotides. One of the candidate genes, secretory leukocyte protease inhibitor (SLPI) gene, was analyzed with use of in situ hybridization and Northern blot analyses. Distribution of SLPI was determined by immunohistochemistry, and the amount of SLPI in the peritoneal fluid and serum was measured by ELISA.Result(s): Distinct expression of SLPI messenger RNA could be detected in the endometrial-type epithelium of extrauterine endometriotic tissues and in the eutopic endometrium of women with endometriosis. SLPI was localized in the endometrial-type epithelium of endometriomas immunohistochemically. The amount of SLPI in the peritoneal fluid was markedly elevated in the endometriosis group (91.6 +/- 6.6 ng/mL compared with 68.4 +/- 5.3 ng/mL in the controls).Conclusion(s): Secretory leukocyte protease inhibitor may be involved in the pathogenesis of endometriosis.

TuerlingsM.D, J. H. A. M., B. Mol Ph.D, et al. (1998). "Mutation Frequency of Cystic Fibrosis Transmembrane Regulator is not Increased in Oligozoospermic Male Candidates For Intracytoplasmic Sperm Injection." Fertility and Sterility **69**(5): 899.

http://www.sciencedirect.com/science/article/B6T6K-3V5N66Y-N/2/ee957afe01a6583be59c6ebd31a40c71

Objective: To examine the frequency of anomalies of the vas deferens and the frequency of mutations of the cystic fibrosis transmembrane regulator (CFTR) gene in male candidates for intracytoplasmic sperm injection (ICSI) who had severe oligoasthenoteratozoospermia.Design: The clinical data for male candidates for ICSI were studied. The three most frequent cystic fibrosis (CF)-causing CFTR mutations in the Dutch population ([Delta]F508, A455E, and G542X) and the three most frequent CFTR mutations potentially causing congenital bilateral absence of the vas deferens (CBAVD) in the Dutch population ([Delta]F508, R117H, and IVS8-5T) were analyzed. Delta I507 is also detected by the [Delta]F508 test. Samples of DNA from patients identified as CFTR mutation carriers were subjected to denaturing gradient gel electrophoresis

analysis with use of a two-dimensional electrophoretic technique.Setting: University-based center for reproductive medicine and clinical genetics.Patient(s): Male candidates for ICSI who had oligoasthenoteratozoospermia and no history of operative sterilization and refertilization. Males with a chromosomal aberration or a Y-chromosome microdeletion were excluded.Intervention(s): Semen and blood samples were collected from the patients at their first visit to the clinic.Main Outcome Measure(s): Frequency of anomalies of the vas deferens and frequency of mutations of the CFTR gene in male candidates for ICSI who had oligoasthenoteratozoospermia.Result(s): None of the patients had abnormalities of the vas deferens at physical examination. In 4 of the 150 chromosomes (75 patients), a CFTR mutation was found, yielding a CFTR mutation frequency of 2.7% (95% confidence interval, 1.0-6.7%). None of the patients had two CFTR mutations.Conclusion(s): The frequency of congenital abnormalities of the vas deferens in patients with oligoasthenoteratozoospermia is low. The frequencies of the CFTR mutations identified in this cohort did not differ significantly from the frequencies found in the normal Dutch population.

Wang, H., M. Mahadevappa, et al. (2003). "Distinctive proliferative phase differences in gene expression in human myometrium and leiomyomata." Fertility and Sterility **80**(2): 266.

http://www.sciencedirect.com/science/article/B6T6K-496N8HW-7/2/83f88bf3c112652fb55f0dd791eb10e6

ObjectiveTo gain a comprehensive view of the gene expression and regulation involved in uterine leiomyomata and matched normal myometrium using oligonucleotide microarray-based hybridization analysis.DesignRetrospective analyses of tissue obtained in a prospective randomized clinical study.SettingAcademic institution.Patient(s)Seven patients with leiomyomata scheduled for surgery during the proliferative phase.Intervention(s)Seven paired samples of leiomyomata and adjacent myometrium were obtained from patients undergoing hysterectomy.Main outcome measure(s)The total RNA extracted from leiomyomata and myometrium was used for gene expression profiling of 6,800 human genes using high-density oligonucleotide microarrays. In addition, reverse transcriptase-semiquantitative polymerase chain reaction and immunohistochemistry were used to validate tumor-specific gene expression.Result(s)A comparison of expression patterns in each paired sample revealed 68 genes significantly up- or down-regulated in each paired tissue sample, of which 23 genes showed increased expression and 45 showed decreased expression in leiomyomata compared with normal myometrium. Cluster analysis supported the relevance of these candidate genes for distinguishing between normal myometrium and leiomyomata biologic activity.Conclusion(s)Expression profiling of uterine leiomyomata using high-density oligonucleotide microarrays yields signature patterns that reflect the distinctive differences between normal human myometrium and leiomyomata during the proliferative phase. These observations suggest that a number of genes are involved in the tumorigenesis of leiomyomata.

Wolczynski, S. l., P. Laudanski, et al. (2003). "A case of complete hypogonadotropic hypogonadism with a mutation in the gonadotropin-releasing hormone receptor gene." Fertility and Sterility **79**(2): 442.

http://www.sciencedirect.com/science/article/B6T6K-47T5CDW-18/2/aa5c336ef74528cd5e6a7bd4726a46a8

ObjectiveTo screen for mutations in the GnRH receptor gene in a case of complete hypogonadotropic hypogonadism (HH) with GnRH resistance.DesignCase report.SettingA university hospital.Patient(s)A male patient with the complete form of HH without anosmia.Intervention(s)Physical examination and laboratory and genetic studies.Main outcome

measure(s)Gonadotropins at the basal state and after GnRH administration and GnRH receptor DNA sequencing.Result(s)A novel missense mutation, localized in the first amino acid of the extracellular loop found in the heterozygous state, and another mutation, Arg139His (R139H), located in the conserved aspartate-arginine-serine motif at the junction of the third transmembrane and second intracellular loop of the GnRH receptor, were identified in the homozygous state. Pedigree studies reveal that both parents were heterozygous for R139H, while the mother carried the missense mutation at codon 1(M1T).Conclusion(s)GnRH receptor mutations may account for a larger proportion of cases of HH than previously thought. The phenotypic spectrum of HH seems to vary, and this heterogeneity may be related, at least in part, to the degree of impaired biological activity of the mutated GnRH receptor caused by the allelic type of mutations.

Wu, Y., Z. Basir, et al. (2003). "Resolution of clonal origins for endometriotic lesions using laser capture microdissection and the human androgen receptor (HUMARA) assay." Fertility and Sterility **79**(Supplement 1): 710.

http://www.sciencedirect.com/science/article/B6T6K-48CGY15-9/2/9a34582f6d0f2cf6373afcfd1f37149b

ObjectiveTo determine the clonal origins of endometriotic lesions using laser capture microdissection and PCR-based HUMARA assay.DesignMolecular genetic study of human tissue.SettingMolecular genetics laboratory in an academic setting.Patient(s)Twenty patients with endometriosis. Forty specimens of endometriotic lesions from these patients and one specimen of normal endometrium were analyzed.Intervention(s)Laser capture microdissection was used to harvest epithelial cells from single and multifocal endometrial lesions from paraffin-embedded and frozen tissues, and their clonality was determined with the HUMARA assay.Main outcome measure(s)Polymerase chain reaction-based HUMARA assay of clonality.Result(s)Thirty-eight specimens were polymorphic and thus informative. Most specimens were monoclonal, as determined by the HUMARA assay. In four specimens of multifocal lesions, polyclonality was detected, but upon more refined microdissections and further analyses, we found that each focus was monoclonal individually.Conclusion(s)Previously reported polyclonality is very likely to be attributed to the pooling of multifocal lesions or contamination of normal tissues. These results suggest that endometriotic lesions were monoclonal in origin, and in the case of multifocal lesions, each focus originates monoclonally; hence, different foci have independent origins. The monoclonality of endometriotic lesions suggests that they may carry neoplastic potentials, and the apparent independent origins of multifocal lesions suggest that reconstruction of individual lesion histories may help us to understand the initiation and progression of endometriosis.

Zhong, W., T. Sun, et al. (2004). "SAPK[gamma]/JNK1 and SAPK[alpha]/JNK2 mRNA transcripts are expressed in early gestation human placenta and mouse eggs, preimplantation embryos, and trophoblast stem cells." Fertility and Sterility **82**(Supplement 3): 1140.

http://www.sciencedirect.com/science/article/B6T6K-4DH19R3-V/2/42477cd1302ccf29e0bd58c50d1cae64

ObjectiveTo test early-gestation human placenta, a human trophoblast cell line, mouse eggs, preimplantation embryos, and a mouse trophoblast cell line for the expression of mRNA transcripts for stress-activated protein kinase/c-Jun N-terminal kinase (SAPK[gamma]/JNK1, SAPK[alpha]/JNK2, and SAPK[beta]/JNK3).DesignWhole RNA was isolated from the tissue sources listed above and control tissues, and reverse transcription-polymerase chain reaction (RT-PCR) was performed to assay for the qualitative and semiquantitative presence of SAPK[gamma]/JNK1, SAPK[alpha]/JNK2, and

SAPK[beta]/JNK3.SettingNone.Patient(s)None.Intervention(s)None.Main outcome measure(s)The presence and magnitude of amplimer amounts in gels or gene hybridization on Affymetrix cDNA arrays of RT-PCR products of reactions for SAPK[gamma]/JNK1, SAPK[alpha]/JNK2, and SAPK[beta]/JNK3.Result(s)SAPK[gamma]/JNK1 and SAPK[alpha]/JNK2 mRNA transcripts are present in early-gestation human placenta, a human trophoblast cell line, mouse eggs, preimplantation embryos, and a mouse trophoblast cell line at levels similar to positive control levels. SAPK[alpha]/JNK2 is expressed at the highest level of the three transcripts in the family. SAPK[beta]/JNK3 is present at levels that are 1/100-1/1,000 those of the positive control and in some cases at the apparent level of the negative control (previously measured by the less-sensitive Northern blot analysis). Analysis with an Affymetrix cDNA array suggested that SAPK[alpha]/JNK2 and 38 kDa mitogen-activated protein kinase had the highest mRNA expression measured for each of three family members.Conclusion(s)Mitotic placental trophoblast cell lines and primary conceptus/embryo samples containing early placental trophoblasts express SAPK[alpha]/JNK2 at higher levels than SAPK[gamma]/JNK1, but not (only low background levels of) SAPK[beta]/JNK3 mRNA transcripts. This suggests that SAPK[gamma]/JNK1 and SAPK[alpha]/JNK2 may be important mediators of stress-induced responses in early implanting conceptuses that could mediate embryo loss.

Fish & Shellfish Immunology (3)

Grove, S., S. Hoie, et al. (2003). "Distribution and retention of antigens of Aeromonas salmonicida in Atlantic salmon (Salmo salar L.) vaccinated with a [Delta]aroA mutant or formalin-inactivated bacteria in oil-adjuvant." Fish & Shellfish Immunology **15**(4): 349.

http://www.sciencedirect.com/science/article/B6WFN-48B5JPD-8/2/d1cf80edb3a4f103d11c34607def1c6e

In this study we report the differences in distribution and retention of Aeromonas salmonicida antigens after vaccination with two different vaccines. Parr of Atlantic salmon (Salmo salar) were given intraperitoneal injections of either a commercial, monovalent furunculosis vaccine (Apoject) or live, attenuated A. salmonicida ([Delta]aroA). Fish were sampled at weeks 2, 4 and 12 post-vaccination and head kidney and spleen were collected. Presence of LPS and 16S rDNA in isolated leukocytes were investigated by immunocytochemistry and polymerase chain reaction (PCR).16S rDNA was detected in head kidney and spleen of all [Delta]aroA vaccinated and most Apoject-vaccinated fish at weeks 2 and 4. At week 12, 16S rDNA was detected in none of the [Delta]aroA vaccinated fish, but it was detected in head kidney of 75% of Apoject-vaccinated fish. LPS was detected in both vaccination groups at all sampling times, but most frequently in the [Delta]aroA vaccinated fish (in head kidney 75-83% vs. 50%, in spleen 58-67% vs. 17-25%).

Hirono, I., B.-H. Nam, et al. (2003). "Cloning and characterisation of a cDNA encoding Japanese flounder Paralichthys olivaceus IgD." Fish & Shellfish Immunology **15**(1): 63.

http://www.sciencedirect.com/science/article/B6WFN-48PDMG3-4/2/371baa9af1e9ee06a718311fe1a885e6

A cDNA containing the gene for Japanese flounder IgD consisted of 3240 bp encoding 998 amino acid residues. The amino acid sequence of the constant region of Japanese flounder IgD shares

38-80% identity with the sequences of previously reported teleost IgDs. The structure of the constant region of Japanese flounder IgD, which contains the [mu]1, [delta]1, [delta]2, [delta]3, [delta]4, [delta]5, [delta]6, [delta]7, and TM regions, is similar to the structures of the constant regions of the IgDs of channel catfish and Atlantic salmon. Southern blot hybridisation showed that the Japanese flounder IgD gene exists as a single locus. The Japanese flounder IgD gene was mainly detected in peripheral blood leucocytes (PBLs) and small amounts were detected in the spleen, head and trunk kidney, although IgM mRNA was detected in similar amounts in PBLs, the head kidney, and spleen. The copy number of IgM mRNA in Japanese flounder PBL was 56-fold higher than that of IgD.

Verri, T., L. Ingrosso, et al. (2003). "Assessment of DNA vaccine potential for gilthead sea bream (Sparus aurata) by intramuscular injection of a reporter gene." Fish & Shellfish Immunology **15**(4): 283.

http://www.sciencedirect.com/science/article/B6WFN-48B5JPD-2/2/f15c209698da07fa0b20f5b1d37d1974

Naked circular plasmid DNA containing the cytomegalovirus (CMV)-promoter-driven lacZ reporter gene (pCMV-LacZ) was injected in the epaxial muscle of gilthead sea bream (Sparus aurata). A mosaic pattern of expression of [beta]-galactosidase ([beta]-gal) in the myofibres at the site of injection was visualised by in situ histochemical staining using 5-bromo-4-chloro-3-indolyl-[beta]--galactopyranoside. As measured by o-nitrophenyl-[beta]--galactopyranoside assay, [beta]-gal enzymatic activity was found to steadily increase for at least 50 days post injection (p.i.) in pCMV-LacZ-injected muscle. In parallel, foreign DNA was detected by polymerase chain reaction in injected muscles (but not in other tissues) up to 60 days p.i., persisting most probably in an extrachromosomal, non-replicative, circular form. Neither [beta]-gal activity nor pCMV-LacZ-related amplification products were found 90 days p.i. Antibodies against [beta]-gal were demonstrated in pCMV-LacZ-injected fish sampled 45 days p.i. The results suggest that intramuscular delivery of foreign genes represents a realistic approach for DNA vaccine technology for the prevention of infectious diseases in gilthead sea bream.

Food and Chemical Toxicology (1)

Chattopadhyay, P. (2003). "RAPD markers system as a useful tool for rapid identification of the origin of lizard contaminants in food." Food and Chemical Toxicology **41**(12): 1719.

http://www.sciencedirect.com/science/article/B6T6P-49DN50R-1/2/2d6a1a09f88d64785fc88772cb9e81e0

In food poisoning, detection of the nature of causative agent is important for management of trauma and forensic investigation. Most of the methods in clinical toxicology are developed for detection of toxins and poisons. A RAPD-based method has been described for detection of species of animal from its morphologically unrecognizable fragments, recovered from food substances, consumption of which caused even death. Pre-mixed RAPD reaction beads and six RAPD primers were used in PCR analysis. Among six RAPD primers used, any one of them was sufficient in resolving this practical forensic situation. But to enhance the probability values for matching in the present study of fixing identity of an animal, six set of market available RAPD primers were used. This is the first report of a forensic application of RAPD DNA typing in

identification of charred skeleton remnants of Lizard sp. in food material. Furthermore unique amplicons were generated for different reptilian species, which can be used as species specific markers for species identification in forensic situation, however no variations among individuals of same species were observed.

Food Control (6)

Alary, R., A. Serin, et al. (2002). "Comparison of simplex and duplex real-time PCR for the quantification of GMO in maize and soybean." Food Control **13**(4-5): 235.

http://www.sciencedirect.com/science/article/B6T6S-452WB84-1/2/3776188bb0c8e10575b45ac8f006baf9

Birch, L., C. L. Archard, et al. (2001). "Evaluation of LabChipTM technology for GMO analysis in food." Food Control **12**(8): 535.

http://www.sciencedirect.com/science/article/B6T6S-446578R-7/2/57747a740c191be12455ab73de5d7b6f

Cardarelli, P., M. R. Branquinho, et al. "Detection of GMO in food products in Brazil: the INCQS experience." Food Control **In Press, Corrected Proof**
http://www.sciencedirect.com/science/article/B6T6S-4DDXRGD-2/2/c43f3565bab355332ff818b850d7a1c2

Regulations for the use and labeling of genetically modified organism products and derived ingredients are being implemented worldwide, what demands reliable and accurate methods to detect genetically modified organisms (GMO) in raw materials and food products. This study aimed at monitoring products derived from GMO in the Brazilian market using detection methods for the presence of Roundup Ready soybean, Bt176 and MON 810 maize. The results demonstrate for the first time the presence of GM-soy in Brazilian food products, reinforcing the need for the development of accurate quantitative methods in routine analyses.

Dooley, J. J., H. D. Sage, et al. (2005). "Improved fish species identification by use of lab-on-a-chip technology." Food Control **16**(7): 601.

http://www.sciencedirect.com/science/article/B6T6S-4CYGS6G-5/2/d7ba90f893ca06af15571240305c8111

Work reported here shows the outcome of improvements made to a published PCR-RFLP approach for fish species identification. The objective of the improved method was to replace the gel-electrophoretic steps for fragment separation, detection and analysis, by employing a chip-based capillary electrophoresis (CE) system. Fragment resolution on the system was sensitive, with detection of small fragments not observed with the published conventional gel-based

method. Experimental repeatability was less than 3%, allowing species identification without the need to run reference materials with every sample. Using DNA admixtures, the discrimination of 5% salmon DNA in trout DNA was readily achieved. Results showed that the CE system was quick and easy to use, providing post-restriction digestion results for 12 samples within 40 min. This, along with the relatively low cost of the instrument, should make this method suitable for use in a wide range of analytical laboratories involved with species identification.

Lau, L.-T., R. A. Collins, et al. (2004). "Detection and characterization of recombinant DNA in the Roundup Ready(R) soybean insert." Food Control **15**(6): 471.

http://www.sciencedirect.com/science/article/B6T6S-49H1M6C-2/2/187305c10f6039673a3f028749aa600f

The genetically modified (GM) Roundup Ready(R) soybean event GTS 40-3-2 contains the bacterial gene 5-enol-pyruvylshikimate-3-phosphate synthase. A 534 bp rearrangement of the DNA in the 3' region flanking the functional insert likely occurred as a consequence of the insertion event. The structure of the DNA surrounding the insertion has not been fully characterized. A semi-nested PCR method identified the rearranged soybean DNA in samples of raw, partially processed and highly processed food at a level of 0.01%. The 534 bp rearranged segment contained a contiguous portion at least 238 bp long that was also identified in non-GM soybeans. Specific combinations of semi-nested PCR primers differentiated GM and non-GM soybean DNA. These studies confirm that the rearranged DNA originates from the soybean genome and does not involve the introduction of non-soybean genetic material.

Wurz, A., A. Bluth, et al. (1999). "Quantitative analysis of genetically modified organisms (GMO) in processed food by PCR-based methods." Food Control **10**(6): 385.

http://www.sciencedirect.com/science/article/B6T6S-3Y2F99V-9/2/2c585067a6abe9ad8619ede46b04143f

Two different PCR-based approaches for the quantitative analysis of genetically modified organism (GMO) - components in foods are presented using Soybean derived samples as an example. The first method - a double competitive PCR - is well suited to determine threshold levels of GMO content in food. The other - PCR on-line measurement - is suited to determine ratios of transgenic versus non-transgenic component. Both methods provide a means to alleviate the problems of standardisation encountered with simple qualitative PCR approaches and will allow to cope with threshold levels for GMO, once issued by legislative bodies.

Food Microbiology (2)

Carminati, D., A. Perrone, et al. (2004). "Characterization of Listeria monocytogenes strains isolated from Gorgonzola cheese rinds." Food Microbiology **21**(6): 801.

http://www.sciencedirect.com/science/article/B6WFP-4D3W9F4-P/2/37e9778ec2e71dd1ce8c100ff726fb70

Listeria monocytogenes is a foodborne pathogen frequently present in ripened soft cheeses. Forty-three strains of L. monocytogenes isolated from the rind of ripened Gorgonzola cheeses produced in 24 different dairy plants were characterized by biotyping, serotyping, and molecular typing. Biotyping was performed by studying two phenotypes closely associated with virulence, such as hemolytic and phospholipase C activities. Traditional typing techniques did not allow a discrimination among the 43 strains studied. All strains showed a good hemolytic activity on blood agar, and only slight differences were observed when titration of hemolytic activity of culture supernatants was performed. Also phospholipase activities were quite similar for all the strains. Concerning serotyping, all strains belonged to serotype 1/2a. The molecular characterization was performed by RAPD-PCR. Combined cluster analysis following PCR amplification experiments allowed to group L. monocytogenes strains into few distinguishable profiles. At a level of similarity of 80%, the 43 strains were grouped into only 5 composite profile groups. Although isolated in 24 different plants, the presence of a few closely related strains demonstrated a possible relationship between these cheese isolates; a special ability of these strains to adapt to Gorgonzola cheese processing environment could be suggested.

Normanno, G., A. Parisi, et al. (2004). "Typing of Escherichia coli O157 strains isolated from fresh sausage." Food Microbiology **21**(1): 79.

http://www.sciencedirect.com/science/article/B6WFP-49XWVF1-C/2/9fbd0bc0819cd0b74e434cc9cd98c0b7

E. coli O157 is a foodborne pathogen responsible for serious human illnesses, such as hemorrhagic colitic and hemolytic uremic syndrome. Ground beef products are among the foods that are more commonly contaminated, and the strains isolated have been frequently found to carry virulence factors of this pathotype. This paper reports the results of serotyping assays and of investigations performed to screen for virulence factors of 10 E. coli O157 strains isolated from fresh sausages purchased at retail meat outlets in various provinces of Apulia (southern Italy). The presence of verocytotoxins was assessed on VERO cells and ELISA tests. Multiplex PCR assays were performed for the eae, stx1, stx2 and hlyA genes. Six of the 10 strains examined presented the H7 antigen and all of them proved to be potentially pathogenic due to the presence of individual or multiple virulence factors.

Food Research International (1)

Giraffa, G., P. De Vecchi, et al. (1997). "Population dynamics of thermophilic lactobacilli in mixed starter whey cultures." Food Research International **30**(2): 137.

http://www.sciencedirect.com/science/article/B6T6V-3RYCKHY-6/2/40d09d1b973e0141e32e949044ccb92b

The starter used in the production of Parmesan cheese consists of mixed-strain whey cultures of thermophilic rod lactic acid bacteria, where several strains of each species can be found. The present work was undertaken to demonstrate the effects on the strain composition of interactions among individual lactobacilli grown together in cheese whey. Three strains belonging to Lactobacillus helveticus and Lactobacillus delbrueckii subsp. bulgaricus and subsp. lactis were cultured in Parmesan cheese whey and counted by a molecular approach based on Random

Amplified Polymorphic DNA (RAPD) fingerprinting. Results showed interactions among the three lactobacilli as confirmed by comparison of growth kinetics and pH behaviour of the respective strains in mixed-strain culture with single-strain cultures. The present work underlines the importance of noting strain-to-strain interactions among thermophilic lactobacilli in whey co-cultures.

Forensic Science International (86)

Aler, M., A. Salas, et al. (2001). "Y-chromosome STR haplotypes from a Western Mediterranean population sample." Forensic Science International **119**(2): 254.

http://www.sciencedirect.com/science/article/B6T6W-433P7K2-F/2/539a2c9218681e8027a3aad5976a73c4

Nine Y-chromosome STRs were investigated in a male population sample from the Western Mediterranean region of Valencia (Eastern Spain). Complete nine Y-chromosomal STRs haplotypes were obtained in 140 individuals, among which 113 different haplotypes were observed. The most common haplotype was shared by 5% of the sample, while 99 haplotypes were unique. The gene diversity was 0.9892 and the discrimination capacity was 0.8071. Significant population differences were observed with respect to other Iberian populations, such as the Basques and Northern Portugueses.

Andersen, J. F., M. J. Greenhalgh, et al. (1996). "Further validation of a multiplex STR system for use in routine forensic identity testing." Forensic Science International **78**(1): 47.

http://www.sciencedirect.com/science/article/B6T6W-3VXHJC1-7/2/173826338826962eece9411f3f5276ad

A polymerase chain reaction- (PCR) based short tandem repeat (STR) system has recently been developed for use in routine forensic identity testing [1]. The methodology involves the simultaneous amplification of alleles at four loci on different chromosomes, followed by the fluorescent detection of products using an automated DNA sequencer. The adoption of this technology into operational casework offers several advantages over systems currently in use, particularly the ability to obtain results from very old or small samples, reduced operator time when compared with conventional DNA (single locus probe) analysis and the potential for automation. Validation studies were incorporated into the development work on this system [2,11]. The scope of these studies has been extended by further investigation carried out in this laboratory to test the reliability of the system under normal operational procedures. It was demonstrated that the precision of size determination was sufficient for the discrimination of alleles and size windows for allelic designation were established. A collaborative exercise carried out in conjunction with two independent laboratories demonstrated the robustness of allelic designation. Having tested both the DNA quantification and amplification techniques against DNA samples from a wide range of animal and microbial species, it was confirmed that results are only obtained from higher primate DNA. The PCR methodology was tested with both simulated and real casework samples (over 250 in total). Reportable results were obtained from most items yielding extracted DNA. Approximately 20% of the casework items from which no grouping (ABO, PGM) nor SLP results were obtained, gave reportable STR results. A method for the routine

purification of DNA extracts which failed to amplify was established and validated for use in forensic casework. The STR multiplex system developed by Kimpton et al. [1] proved robust and reliable when tested under the operational procedures in place in this laboratory.

Ballard, D. J., C. Phillips, et al. "A study of mutation rates and the characterisation of intermediate, null and duplicated alleles for 13 Y chromosome STRs." Forensic Science International **In Press, Corrected Proof** http://www.sciencedirect.com/science/article/B6T6W-4FBM1R7-2/2/d8d15fe56eb1841d584ff32547418831

Previously reported Y chromosome STR haplotype databases for three UK population groups, plus additionally analysed samples, have been scrutinised for the presence of non-standard (intermediate, null and duplicated) alleles. These alleles have been characterised by sequencing, some showing changes in the repeat structure, and the frequencies reported. Mutation rates for each of the 13 STRs have been calculated when analysis of father-son pairs has been possible. An example illustrating the use of non-standard alleles in a large family tree is outlined.

Balogh, M. K., J. Burger, et al. (2003). "STR genotyping and mtDNA sequencing of latent fingerprint on paper." Forensic Science International **137**(2-3): 188.

http://www.sciencedirect.com/science/article/B6T6W-49JPRN4-2/2/7c9ec9869e7780040c40aee8df04d325

A systematic study was conducted to investigate whether DNA can be successfully extracted from latent fingerprints deposited on ordinary paper and analysed using short tandem repeat profiling and mitochondrial DNA sequencing. In order to evaluate the performance of latent fingerprint analysis in a criminal case, experiments with varying conditions were carried out to improve our understanding of low copy number (LCN) DNA typing. After optimising the extraction methods to achieve increased sensitivity, the examination of touched paper can routinely yield the STR profile of the individual who has touched it. A fingerprint can therefore be considered as a potential source of DNA for genetic identification. Nevertheless, the findings of our "after enhancement experiment" (using chemically or physically pre-treated fingerprints), and our "mixture experiment" (using fingerprints from three to four people on the same sheet of paper) help to define the limitations of the low copy number PCR technique in forensic casework.

Barros, F., M. V. Lareu, et al. (1992). "Detection of polymorphisms of human DNA after polymerase chain reaction by miniaturized SDS-PAGE." Forensic Science International **55**(1): 27.

http://www.sciencedirect.com/science/article/B6T6W-4C06NK5-N0/2/89fa89f31d0a4747221e74ac64c29f12

PCR followed by SDS-PAGE in miniaturized non-denaturing gels permits in some cases the identification of single base pair substitutions in small DNA fragments and therefore, the study of human DNA polymorphisms. The usefulness of the system in forensic science is investigated by typing the HLA-DQA1 locus and the VNTR recognized with the probe pMCT118 (locus D1S80) and it shows to be advantageous over previously published methods for typing the MCT118 system, whereas in HLA-DQA1 typing for forensic casework, both dot-blot with ASO probes and this method could be complementary.

Benecke, M. (1998). "Random amplified polymorphic DNA (RAPD) typing of necrophageous insects (diptera, coleoptera) in criminal forensic studies: validation and use in practice." Forensic Science International **98**(3): 157.

http://www.sciencedirect.com/science/article/B6T6W-3VCVFB4-4/2/8b70a7b135d67f81566eaf7138b91c44

To permit quick identification of arthropods, random amplified polymorphic DNA typing (RAPD) was used to support classical morphological and medico-legal analysis of maggots on a human corpse. The method was employed to determine if maggots which were found on the inside of a body bag were identical (a) with maggots found on the outside of the bag, and (b) pupae found on the floor under the corpse. Pre-mixed RAPD reaction beads together with semiautomatic computer aided analysis of the PCR products are shown to discriminate between closely related necrophageous insect species (flies and beetles) found on corpses. From the 11 RAPD primers used, one alone was sufficient in resolving a practical forensic situation. This is the first report of a forensic application of RAPD DNA typing.

Berger, B., H. Niederstatter, et al. (2003). "Molecular characterization and Austrian Caucasian population data of the multi-copy Y-chromosomal STR DYS464." Forensic Science International **137**(2-3): 221.

http://www.sciencedirect.com/science/article/B6T6W-49M0RB7-1/2/2944c505fd2699be5f92325aafe26423

DYS464 is a multi-copy STR system with four positions on the Y-chromosome (DYS464a, b, c, and d) which was recently identified and characterized [Forensic Sci. Int. 130 (2002) 97]. The aims of our study were to perform a population study, to estimate the mutation rate and an extensive sequence analysis in order to confirm the nomenclature. Fourteen different alleles were found in an Austrian population sample with an allele length varying from 9 to 19 repeats. All alleles were cloned and sequenced. Alleles 9-19 showed the general repeat structure (CCTT)n. (CCTT)2. (CCTT)3. (CCTT)4. (CCTT)2. (CCTT)2. The nomenclature is based on the number of repeated units of the variable (CCTT)n-stretch only. In 13% of the samples intermediate alleles, namely 14.3A, 14.3B and 15.3 were detected. In these alleles the variable repeat block is interrupted by a CTT motif (14.3A: (CCTT)3CTT(CCTT)11; 14.3B and 15.3: (CCTT)7CTT(CCTT)7/8). A comparison with GenBank entries revealed the existence of a length variant due to a deletion of one cytosine in the 5' flanking region of the first repeat block. We designed an alternative forward primer to circumvent possible ambiguities in the allele designation. A total of 54 different genotypes were identified in 135 men corresponding to a discrimination capacity (DC) of 40% and a gene diversity (GD) of 0.97. These values are much higher than those of other Y-chromosomal short tandem repeats (Y-STRs). DYS464 has the same haplotype diversity (HD) as the combination of the five Y-STR loci with the lowest gene diversities of the Y-STR core set. On the other hand, a combination of the three most diverse loci (DYS464, DYS385 and DYS390) has the same capacity to distinguish between paternal lineages than the complete minimal haplotype (minHT) consisting of eight Y-STR loci. In our population sample the addition of DYS464 to the minHT increases the number of different haplotypes from 110 to 122. The mutation-rate estimate based on the 70 meioses analyzed amounts to 2.86 x 10-2 (95% confidence interval 3.5 x 10-3 to 9.95 x 10-2). This value is approximately 10 times higher than the average mutation-rate estimate for Y-STRs.

Budowle, B., L. T. Nhari, et al. (1997). "Zimbabwe black population data on the six short tandem repeat loci - CSF1PO, TPOX, THO1, D3S1358, VWA and FGA." Forensic Science International **90**(3): 215.

http://www.sciencedirect.com/science/article/B6T6W-3TKKV2W-7/2/b20ab6944fcf140e81aa537cdfb2c211

Allele frequencies for six tetrameric short tandem repeat (STR) loci CSF1PO, TPOX, THO1, D3S1358, VWA, and FGA were determined in a Black African sample population from Zimbabwe. All loci are highly polymorphic and meet Hardy-Weinberg expectations. An inter-class correlation test analysis detected only one departure from independence out of 15 pair-wise comparisons of the six loci (i.e., CSF1PO/VWA loci, P=0.026). The allele frequency data at four of the six STR loci in the Black African sample population are similar to African American data.

Butler, J. M., A. E. Decker, et al. "Allele frequencies for 27 Y-STR loci with U.S. Caucasian, African American, and Hispanic samples." Forensic Science International **In Press, Corrected Proof** http://www.sciencedirect.com/science/article/B6T6W-4FTWJJT-1/2/61453fd9dfaabaf15b20d01a8e9cba77

A total of 263 U.S. Caucasians, 260 African Americans and 140 U.S. Hispanics or a subset of 31 Caucasians, 32 African Americans, and 32 Hispanics were typed for 27 Y-chromosome short tandem repeat (Y-STR) markers: DYS444, DYS446, DYS449, DYS463, DYS485, DYS490, DYS495, DYS504, DYS505, DYS508, DYS520, DYS522, DYS525, DYS532, DYS533, DYS534, DYS540, DYS556, DYS557, DYS570, DYS575, DYS576, DYS594, DYS632, DYS635, DYS641, and DYS643. Allele frequencies for each locus are reported along with nomenclature based on sequence analysis.

Butler, J. M., R. Schoske, et al. (2002). "A novel multiplex for simultaneous amplification of 20 Y chromosome STR markers." Forensic Science International **129**(1): 10.

http://www.sciencedirect.com/science/article/B6T6W-46PBY9D-1/2/03112525820610b42482749bb32cf259

A multiplex polymerase chain reaction (PCR) assay capable of simultaneously amplifying 20 Y chromosome short tandem repeat (STR) markers has been developed to aid human identity testing and male population studies. These markers include all of the Y STRs that make up the "extended haplotype" used in Europe (DYS19, DYS385, DYS389I/II, DYS390, DYS391, DYS392, DYS393, and YCAII) plus additional polymorphic Y STRs (DYS437, DYS438, DYS439, DYS447, DYS448, DYS388, DYS426, GATA A7.1, and GATA H4). Primers for the markers DYS385, DYS389, and YCAII target duplicated regions of the Y chromosome and thus can provide two polymorphic peaks for each respective primer set. This Y STR 20plex, which utilizes 34 different PCR primers, is the first to include a simultaneous amplification of all the markers within the European "minimal" and "extended" haplotypes. Relative primer positions are compared between the newly developed primers described here and previously published ones. Efforts were made to avoid X chromosome homology in the primer design as well as close packing of PCR product size ranges in order to keep all alleles less than 350 bp through careful examination of known allele ranges. Haplotype comparisons between the 20plex and a commercially available kit found excellent agreement across the 76 samples in the Y chromosome consortium panel.

Cabrero, C., A. Diez, et al. (1995). "Allele frequency distribution of four PCR-amplified loci in the Spanish population." Forensic Science International **71**(2): 153.

http://www.sciencedirect.com/science/article/B6T6W-4007G86-

9/2/95e2c0cc62b13222cc68f6e4d545eaa8

The allele frequency distributions of four VNTR loci amplified by PCR have been studied in a population of 205 individuals from Spain. The loci analysed are D1S80 and three STRs: HUMTH01, HUMFES/FPS and HUMACTBF2 (SE33). The former was visualized in Metaphor agarose gels, and the STRs in sequencing polyacrylamide gels under denaturing conditions which could separate alleles with differences of a single base. This is of particular importance in the HUMTH01 locus, a tetrameric STR in which two alleles (9.3 and 10) were detected differing in a single base. Furthermore, HUMACTBP2 has at least 30 alleles, some of which may vary by as little as one base. At this locus a variation in the allele mobility was observed, depending on the electrophoretic conditions. For this reason, there should be careful consideration before this marker is accepted and validated as a common interlaboratory system. This paper does not include any comparison of the frequencies obtained for this locus with other recent studies. For the rest of the loci, the frequencies found have been compared with other published population studies; they show a degree of difference, particularly in the D1S80 locus. Finally, the systems were tested for Hardy-Weinberg equilibrium, and some statistical parameters of forensic interest were calculated.

Carracedo, A., E. D'Aloja, et al. (1998). "Reproducibility of mtDNA analysis between laboratories: a report of the European DNA profiling group (EDNAP)." Forensic Science International **97**(2-3): 165.

http://www.sciencedirect.com/science/article/B6T6W-3V5MP79-B/2/658ce23da9e6bc695d7e15123f10d6c9

The aim of this collaborative exercise was to determine whether uniformity of mtDNA sequencing results could be achieved among different EDNAP laboratories. Laboratories were asked to sequence mtDNAHV1 region (16024-16365) from three bloodstains, proceeding in accordance with the protocol and strategies currently used in each individual laboratory. Cycle sequencing was used by 11 laboratories and solid phase single stranded sequencing was used by one laboratory. Different PCR strategies and PCR conditions were used by the different laboratories. Three laboratories used semi-nested PCR, two nested PCR, three direct amplification of HV1 and four amplification of overlapping fragments covering the HV1 region. Despite the diversity of methodologies used, all the laboratories reported the same results. The successful result of this exercise shows that PCR based mtDNA typing by automated sequencing is a valid, robust and reliable means of forensic identification despite the different strategies and methodologies used by the different laboratories.

Corach, D., L. Filgueira Risso, et al. (2001). "Routine Y-STR typing in forensic casework." Forensic Science International **118**(2-3): 131.

http://www.sciencedirect.com/science/article/B6T6W-42SXF3V-7/2/1a7d7ddc5834d937aa3ef19dd4b62c71

In the field of molecular diagnosis, forensic casework analysis is one of the most demanding investigations, due to its social impact. Optimization of DNA typing multiplex reactions with identical cycling conditions as those required by autosomal short tandem repeats (STR) multiplex reduces errors, and saves time and reagents. Previously, we validated a five Y-STRs set, all of them generating single band patterns. This work reports the optimization of combined multiplexes, a triplex (DYS19, DYS390 and DYS391) and a duplex (DYS392 and DYS393), that can be amplified in identical cycling conditions as those required by commercially available multiplex autosomal STR kits. In addition both Y chromosome multiplexes can be combined for

co-injection on a capillary electrophoresis based automated sequencer. Statistical attributes of the haplotypes of the five Y-STR investigated were evaluated in unrelated males from different metropolitan areas of Argentina. This system was successfully used for investigating more than 350 forensic routine cases in our country.

Corte-Real, F., L. Souto, et al. (1999). "Population distribution of six PCR-amplified loci in Madeira Archipelago (Portugal)." Forensic Science International **100**(1-2): 93.

http://www.sciencedirect.com/science/article/B6T6W-3W4GJ8F-8/2/8ec1deeb8afa0fa7879943b64fc9db7b

Frequency data of the short tandem repeat (STR) loci HUMTH01, HUMVWA31/A, HUMF13A1, HUMFES/FPS, D12S391 and HUMFIBRA/FGA were determined in blood stains obtained from a population of unrelated individuals from the Madeira Archipelago. The observed genotype distribution showed no significant deviation from the Hardy-Weinberg equilibrium and there was no evidence for association of alleles among the six loci. Population data showed a combined discrimination power of 0.9999998 and a chance of exclusion of 0.99597. The frequencies are similar to those of other compared caucasian populations but significant differences were found between the Madeira population and Japanese, Chinese, Greenland Eskimos and Quechua Amerindians. The six loci studied, together proved to be highly discriminating and valuable for forensic cases.

Cotton, E. A., R. F. Allsop, et al. (2000). "Validation of the AMPFlSTR(R) SGM Plus(TM) system for use in forensic casework." Forensic Science International **112**(2-3): 151.

http://www.sciencedirect.com/science/article/B6T6W-40XNW8K-7/2/8ffdb40d8e75c5a5b8330e34e6040c3c

The AMPFlSTR(R) SGM Plus(TM) system is a commercially available STR multiplex produced by Applied Biosystems, a division of Perkin Elmer, Foster City, California, USA that supersedes SGM. The multiplex contains the six SGM loci, amelogenin and four additional loci. These additional loci are D3S1358, D19S433, D16S539 and D2S1338. Consequently, the match probability is significantly improved (conservatively quoted as 1 in 109 for reporting a full profile match). The system was subjected to validation. For example, ageing and degradation studies demonstrated semen stains to be the most stable evidence type, whereas buccal scrapes were the least stable. An apparent rise in the sensitivity increases the chance of obtaining successful results from the more difficult samples submitted for analysis. Two of the new loci (D3S1358 and D19S433) are low molecular weight (between 100 and 150 base pairs); this improved the success rates of the degraded samples where high molecular weight loci may drop out. Of 26 non-primates tested, four gave results that appeared as single peaks and were unlikely to cause interpretation problems. None of the 19 micro-organisms tested gave discernible results. Extensive casework and simulated casework studies demonstrated that SGM and SGM plus results were comparable. There was one example of a null allele (primer binding site mutation) recorded at the HUMFIBRA locus (in both systems). However, a concordance study of 1000 samples using both SGM and SGM plus did not demonstrate any differences in typing.

Cowland, J. B., H. O. Madsen, et al. (1995). "HLA-DQA1 typing in Danes by two polymerase chain reaction (PCR) based methods." Forensic Science International **73**(1): 1.

http://www.sciencedirect.com/science/article/B6T6W-4575ST7-1/2/f175b9c09b7a6e29b6542e4c9638a6d9

A total of 280 persons were HLA-DQA1 typed by two different polymerase chain reaction (PCR) based methods: (i) a reverse dot-blot (RDB) method, which can differentiate between six alleles, and (ii) a combined PCR-restriction fragment length polymorphism (PCR-RFLP) and allele-specific amplification (ASA) method, which together recognise eight alleles. In 146 unrelated Danish individuals, the HLA-DQA1 alleles were in Hardy-Weinberg equilibrium. For identity testing, the power of discrimination (PD) of HLA-DQA1 was 0.932 with the RDB method and 0.942 with the PCR-RFLP/ASA method. For paternity testing, the theoretical chance of exclusion in HLA-DQA1 of non-fathers was 0.634 with the RDB method and 0.660 with the PCR-RFLP/ASA method.

De Leo, D., S. Turrina, et al. (2001). "Italian population data for D1S1656, D3S1358, D8S1132, D10S2325, VWA, FES/FPS, and F13A01." Forensic Science International **123**(1): 71.

http://www.sciencedirect.com/science/article/B6T6W-44HXKBT-D/2/09c9e2584575efb397a75a2829fe0161

Allele frequencies for seven STRs loci were obtained from a sample of 215 unrelated healthy Italian individuals.

Dixon, L. A., C. M. Murray, et al. "Validation of a 21-locus autosomal SNP multiplex for forensic identification purposes." Forensic Science International **In Press, Corrected Proof** http://www.sciencedirect.com/science/article/B6T6W-4F973F7-2/2/74a72bf87098798c8c5afedd463c7a88

A single nucleotide polymorphism (SNP) multiplex has been developed to analyse highly degraded and low copy number (LCN) DNA template, i.e. Hb) and the drop-out threshold (Ht) defined as the maximum peak height of a surviving heterozygous allele, where its partner may have dropped out. The discrimination power of the system is estimated at 1 in 4.5 million, using a White Caucasian population database. Comparisons using artificially degraded samples profiled with both the SNP multiplex and AMPFISTR[trademark] SGM plus[trademark] (Applied Biosystems) demonstrated a greater likelihood of obtaining a profile using SNPs for certain sample types. Saliva stains degraded for 147 days generated an 81% complete SNP profile whilst short tandem repeats (STRs) were only 18% complete; similarly blood degraded for 243 days produced full SNP profiles but only 9% with STRs. Reproducibility studies showed concordance between SNP profiles for different sample types, such as blood, saliva, semen and hairs, for the same individual, both within and between different DNA extracts.

Eichmann, C., B. Berger, et al. "Estimating the probability of identity in a random dog population using 15 highly polymorphic canine STR markers." Forensic Science International **In Press, Corrected Proof** http://www.sciencedirect.com/science/article/B6T6W-4D5KSGG-4/2/bcc2ae3c1e6c289a203d44c3b17e096e

Dog DNA-profiling is becoming an important supplementary technology for the investigation of accident and crime, as dogs are intensely integrated in human social life. We investigated 15 highly polymorphic canine STR markers and two sex-related markers of 131 randomly selected dogs from the area around Innsbruck, Tyrol, Austria, which were co-amplified in three PCR

multiplex reactions (ZUBECA6, FH2132, FH2087Ua, ZUBECA4, WILMSTF, PEZ15, PEZ6, FH2611, FH2087Ub, FH2054, PEZ12, PEZ2, FH2010, FH2079 and VWF.X). Linkage testing for our set of marker suggested no evidence for linkage between the loci. Heterozygosity (HET), polymorphism information content (PIC) and the probability of identity (P(ID)theoretical, P(ID)unbiased, P(ID)sib) were calculated for each marker. The HET(exp)-values of the 15 markers lie between 0.6 (VWF.X) and 0.9 (ZUBECA6), P(ID)sib-values were found to range between 0.49 (VWF.X) and 0.28 (ZUBECA6). Moreover, the P(ID)sib was computed for sets of loci by sequentially adding single loci to estimate the information content and the usefulness of the selected marker sets for the identification of dogs. The estimated P(ID)sib value of all 15 markers amounted to 8.5 X 10-8. The presented estimations turned out to be a helpful approach for a reasonable choice of markers for the individualisation of dogs.

Grimes, E. A., P. J. Noake, et al. (2001). "Sequence polymorphism in the human melanocortin 1 receptor gene as an indicator of the red hair phenotype." Forensic Science International **122**(2-3): 124.

http://www.sciencedirect.com/science/article/B6T6W-4471K5D-7/2/8a71d833ae42ad553861b96d67973ded

We describe a minisequencing protocol for screening DNA samples for the presence of 12 mutations in the human melanocortin 1 receptor gene (MC1R), eight of which are associated with the red hair phenotype. A minisequencing profile which shows homozygosity for one of these mutations or the presence of two different mutations would strongly indicate that the sample donor is red haired. The absence of any red hair causing mutations would indicate that the sample donor does not have red hair. We report the frequencies of MC1R variants in the British red haired population.

Gupta, S. K., S. K. Verma, et al. "Molecular insight into a wildlife crime: the case of a peafowl slaughter." Forensic Science International **In Press, Corrected Proof**
http://www.sciencedirect.com/science/article/B6T6W-4FBM1R7-3/2/9ae4665f18b74d054e14b085a742137a

We report a recent case in which a wildlife warden had suspected that some people had killed and cooked a peacock. Cooked meat, intestine of bird and the wooden block used for chopping were seized from the site of crime and forwarded to our laboratory for DNA testing. Mitochondrial cytochrome b sequence analysis revealed that the cooked meat and remnants of the bird were of a chicken, but the DNA obtained from the wooden block was of an Indian Peafowl (Peacock) testifying that the wooden chopping block was used to chop the meat of an endangered bird, thus bringing to light a wildlife crime.

Gyllensten, U. B., A. Josefsson, et al. (1992). "DNA typing of forensic material with mixed genotypes using allele-specific enzymatic amplification (polymerase chain reaction)." Forensic Science International **52**(2): 149.

http://www.sciencedirect.com/science/article/B6T6W-4C35YFD-HT/2/17aa4f92e18100a009c0dc2faf543c23

Biological material in forensic casework frequently contains a mixture of genotypes, with a predominance of material from the victim and only trace amounts from the person committing the crime. Physical separation of the two genotypes or preferential lysis of different cell types may

sometimes be possible. However, it is often difficult to achieve complete separation due to the lysis of cells or lack of material. We have developed an enzymatic amplification system for the HLA DQA1 locus, that will allow the presence of individual alleles in a sample with mixed genotypes to be determined, independent of their initial proportion in the sample. This system permits the identification of an allele representing less than 10-4 of the background genotype. Use of polymerase chain reaction (PCR) with general primers allows only alleles representing more than about 1% to be detected, while the allele-specific amplification represents up to a 1000-fold increase in sensitivity. This method was applied to a rape case and a combined rape and murder case; in both cases the biological evidential materials contained a mixture of alleles from the victim and the rapist. Allele-specific PCR revealed the presence of alleles identical to those of the suspect using DNA from a vaginal swab taken after a rape incident, whereas by using general primers in the PCR only trace amounts of alleles other than those of the victim were found. Similarly, allele-specific amplification of DNA from vaginal swabs from the murder case revealed the presence of alleles identical to those of the suspect, while standard PCR only indicated the presence of genetic material from the victim.

Hall, A. and J. Ballantyne (2003). "Novel Y-STR typing strategies reveal the genetic profile of the semen donor in extended interval post-coital cervicovaginal samples." Forensic Science International **136**(1-3): 58.

http://www.sciencedirect.com/science/article/B6T6W-495680J-4/2/6334b6a80183df468bad52f350e821f2

For a variety of reasons, some victims of sexual assault provide vaginal samples more than 24-36 h after the incident. In these cases, the ability to obtain an autosomal STR profile of the semen donor from the living victim diminishes rapidly as the post-coital interval is extended. We have used a number of carefully selected Y-STR loci in a variety of multiplex or monoplex formats to extend the post-coital interval from which a genetic profile of the semen donor can be obtained. The proposed Y-STR typing strategies enable the routine detection of the male donor Y-STR haplotype in cervicovaginal samples recovered up to 4 days post-coitus. We attribute our success to a number of factors that significantly improve the sensitivity and specificity of the analysis. Firstly, we utilize a subset of Y-STR loci that have been carefully selected for their superior performance under stressed conditions in both multiplex and monoplex formats. Specifically these loci function with low copy number templates in the presence of a vast excess of potentially confounding female DNA. Secondly, sperm and non-sperm DNA is co-extracted without a differential extraction process to prevent the unnecessary loss of the small number of structurally fragile sperm remaining in the cervicovaginal tract several days after intercourse. Thirdly, low copy number detection is facilitated by increasing the cycle number to 34-35 cycles and by the ability to input up to 450 ng of co-extracted sperm/non-sperm DNA into the PCR reaction without the appearance of confounding female artifacts. Lastly, the proper collection of post-coital cervicovaginal samples, instead of the lower or mid-vaginal tract samples often taken, is required for optimal recovery of sperm for analysis. In this report we demonstrate that our previously described 19 Y-STR loci systems (MPI and MPII) permit a reliable high resolution haplotype determination of the semen donor in cervicovaginal samples taken up to 48 h after intercourse. However, as the post-coital interval is extended further, dramatic loss of signal is observed and haplotype determination of the male donor is no longer possible with MPI and MPII. Nonetheless, subsets of these 19 loci (MPA and MPB) have been developed specifically to detect the male haplotype in samples recovered 4 days after intercourse. Thus, it is possible to derive an 11-19 locus Y-STR profile of the semen donor in cervicovaginal samples recovered 2-4 days after intercourse.

Hayashi, K., Y. Hanaoka, et al. (2000). "An autopsy case of Klinefelter's syndrome suspected and its DNA

analysis." Forensic Science International **113**(1-3): 119.

http://www.sciencedirect.com/science/article/B6T6W-414N4VW-S/2/508645c54ca673448fa0bb9bb441a564

We experienced an autopsy case, small testes and tall stature, which suggested Klinefelter's syndrome. DNA analysis was performed to confirm the genetic abnormality. Case History: A 28-year-old man who was single and lived with his parents. He suddenly lost his consciousness in a sitting room and died. Autopsy findings: He was 176 cm in height and 57 kg in weight. The post-mortem hypostasis was red-purple on his back, and rigor mortis was strong in each joint of the whole body. The heart weighted 340 g, in which dark red fluidal blood (300 ml) without coagulation was contained. The testes were smaller than normal adult male (left and right testes with epididymides weighted 8.1 g and 6.0 g, respectively). As a results of pathological examination, clumped Leydig cells, sclerotic and hyalined tubules were observed. Some germ cells with spermatozoid were also present. DNA Analysis: Generally, Klinefelter's syndrome is determined by karyotype analysis and/or the detection of sex chromatin. However, in this case, karyotype analysis and the detection of sex chromatin could not be demonstrated, because the blood which was collected in the autopsy became too old. Therefore, we tried sex determination and STR analysis (HPRT, HUMARA and DXS 1470) using DNA extracted from stored blood materials. Consequently, in the sex determination, no different situation was found in the X- and Y-specific bands from normal male's and as results of STR analyses, we could not corroborate the Klinefelter's syndrome.

Hu, S. P., D. Q. Wu, et al. "Genetic profile of 15 STR loci in the Min Nan population in Southeast China." Forensic Science International **In Press, Corrected Proof**
http://www.sciencedirect.com/science/article/B6T6W-4DS98WW-5/2/9407a44be7c975ae413a2dea390b71d5

Genetic profile of 15 short tandem repeats (STR) loci were determined in a Chinese Han population from the Min Nan mountainous area, Southeast China.

Hu, S. P., X. J. Yu, et al. (2005). "Analysis of STR polymorphisms in the Chao Shan population in South China." Forensic Science International **147**(1): 93.

http://www.sciencedirect.com/science/article/B6T6W-4CJCWWD-2/2/26765b7bffb969b0ee73b623b854ceb0

Chao Shao area is a littoral under the jurisdiction of Guangdong province, abutting on Fujian. Historically, the area was relatively isolated from other parts of China until 1990s, when it started to take a small number of immigrants from other regions. People residing in this area speak in unique dialect and have distinct lifestyle. Allele frequencies for the 15 short tandem repeats (STR) loci included in the AmpFLSTR(R) Identifiler(TM) kit were obtained from a sample of 144 unrelated Chinese born and living in the Chao Shan area, South China.

Inagaki, S., Y. Yamamoto, et al. (2004). "A new 39-plex analysis method for SNPs including 15 blood group loci." Forensic Science International **144**(1): 45.

http://www.sciencedirect.com/science/article/B6T6W-4CB676S-2/2/465e03547345e3086acf82727f5561cc

A novel 39-plex typing system for single nucleotide polymorphisms (SNPs) has been developed. This multiplex approach has the advantage of being able to type 38 autosomal SNPs and one sex-discriminating base exchange site on the X and Y chromosomes rapidly and simultaneously. The SNP loci on the autosomes, which we examined, contain 15 loci distributed on blood type genes: three on RhCE, two each on Km and Gc, and one each on Duffy, AcP1, Tf, MN, GPT, EsD, PI, and Kidd genes.Thirty-seven genomic DNA fragments containing a total of 38 SNPs and one sex-discriminating site were amplified in one multiplex PCR reaction. Following the reaction, single nucleotide primer extension reaction was performed by dividing these SNP loci into five groups. The SNP type of each of the 39 loci was determined at one time by capillary electrophoresis using the newly designed multi-injection method.The combined PD (power of discrimination) of this typing system was (1-1.1) x 10-14, and the MEC (mean exclusion chance) was 0.9990. We applied this system to forensic cases, including 16 paternity testing cases (13 non-exclusion and three exclusion cases) and one personal identification case. For the paternity testing cases, the highest Essen-Moller's W-value was 0.9999995. The pM (matching probability) of the personal identification case was 2.22 x 10-17. These data showed that this system was an excellent tool for use in forensic cases of paternity testing and personal identification.

Inoue, H., A. Kimura, et al. (2002). "Degradation profile of mRNA in a dead rat body: basic semi-quantification study." Forensic Science International **130**(2-3): 127.

http://www.sciencedirect.com/science/article/B6T6W-4760B5W-2/2/6394a1ed8f92679a701571fda2e0930c

To profile postmortem degradation of mRNA, total RNA was extracted, at given postmortem intervals, from the brain, lung, heart and liver of rats left at 20 [deg]C. In electrophoretic analysis, total RNA was most stable in the brain, moderately stable in the lung and heart, and most unstable in the liver. Northern blot analysis of total RNA extracts from the brain and liver of dead rats with a cDNA probe for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) showed that GAPDH mRNA degraded in a similar fashion to total RNA. Analysis of the postmortem degradation profile of GAPDH mRNA with real-time reverse transcriptase-polymerase chain reaction (real-time RT-PCR) gave results consistent with those above, indicating that real-time RT-PCR is reliable for estimation of the mRNA level in specimens from dead bodies. Real-time RT-PCR analysis showed that degradation rates of three housekeeping genes, GAPDH, [beta]-actin and hypoxanthine guanine phosphoribosyltransferase, in the brains of dead rats were similar. The degradation rate of interleukin-1[beta] (IL-1[beta]) mRNA induced by intravenous injection of LPS to rats was higher than that of GAPDH mRNA in the lung. In real-time RT-PCR analysis using GAPDH mRNA as an internal standard, the detection level of IL-1[beta] mRNA decreased in the postmortem interval. However, enhanced expression of IL-1[beta] was detected for at least 3 days postmortem.

Ishida, K., B.-L. Zhu, et al. (2000). "Novel approach to quantitative reverse transcription PCR assay of mRNA component in autopsy material using the TaqMan fluorogenic detection system: dynamics of pulmonary surfactant apoprotein A." Forensic Science International **113**(1-3): 127.

http://www.sciencedirect.com/science/article/B6T6W-414N4VW-T/2/52504b144e1c0014aa66630c5f85d1a8

A novel approach to quantitative reverse transcription (RT)-PCR assay of mRNA component using fluorescent TaqMan methodology and a new instrument (ABI Prism 7700 sequence detection system) was developed for autopsy materials. Pulmonary surfactant apoprotein A (SP-A) mRNA from a cadaveric lung was quantitated in real-time. The target SP-A gene and the endogenous reference of glyceraldehyde-3-phosphate (GAPDH) were amplified in the same tube,

and an amount of the target was normalized to the reference. This assay had a high reproducibility and discrimination even in forensic autopsy materials up to 96 h postmortem. Elevated SP-A expressions were determined in some cases. This system without post-PCR sample handling would be a very useful tool in pathological diagnosis and DNA analysis.

Juusola, J. and J. Ballantyne (2003). "Messenger RNA profiling: a prototype method to supplant conventional methods for body fluid identification." Forensic Science International **135**(2): 85.

http://www.sciencedirect.com/science/article/B6T6W-490H2DF-D/2/75c34372623631608763b4a1de77aaf9

Conventional methods of body fluid identification use a variety of labor-intensive, technologically diverse techniques that are performed in a series, not parallel, manner and are costly in terms of time and sample. Theoretically, the identification of a body fluid may be made by determining a sufficient number of mRNAs that are expressed exclusively in cells that collectively comprise that body fluid. Advantages of an mRNA-based approach, compared to conventional biochemical methods of analysis, include greater specificity, simultaneous and semi-automatic analysis through a common assay format, improved timeliness, decreased sample consumption and compatibility with DNA extraction methodologies.In this report, we demonstrate that RNA is stable in biological stains and can be recovered in sufficient quantity and quality for analysis. Messenger RNA from the housekeeping genes S15, [beta]-actin and GAPDH was detected in blood, semen and saliva stains using a sensitive reverse transcriptase-polymerase chain reaction assay (RT-PCR). Additionally, we have identified a number of candidate tissue-specific genes, statherin, histatin 3, PRB1, PRB2 and PRB3 that may be useful for the positive identification of saliva. Messenger RNAs from these genes were detectable in saliva stains but not in blood or semen stains. Collectively these findings constitute the basis of a prototype RNA based assay system that may eventually supplant conventional methods for body fluid identification.

Kimpton, C., P. Gill, et al. (1995). "Report on the second EDNAP collaborative STR exercise." Forensic Science International **71**(2): 137.

http://www.sciencedirect.com/science/article/B6T6W-4007G86-8/2/98573d921ca74aae07ac2b905cd89d25

The European DNA Profiling Group (EDNAP) has previously carried out collaborative exercises to determine which STR systems will produce results that can be reproduced by different laboratories. The first EDNAP exercise involving STR systems focused on different types of loci: a simple locus with six common alleles (HUMTH01) and a complex locus with >35 alleles (ACTBP2). Generally the simpler STR system was found to be readily amenable for use across a wide range of different technologies, whereas a more complex locus presented difficulties. The second EDNAP STR exercise was intended to take the process of investigation a stage further. Some laboratories are developing automation, coupled with fluorescent methods of detection and multiplex applications, whereas others use manual methods involving visual detection techniques such as silver staining. The purpose of this exercise was to determine whether loci amenable to multiplexing with automation (as a quadruplex reaction) could also be successfully used with manual methods, either by multiplexing in duplex reactions or alternatively by using just a single pair of PCR primers.

Klintschar, M. and P. Wiegand (2003). "Polymerase slippage in relation to the uniformity of tetrameric

repeat stretches." Forensic Science International **135**(2): 163.

http://www.sciencedirect.com/science/article/B6T6W-490RG6H-3/2/76b79844ae546d13cc1caee470765c54

The aim of the study was to test the hypothesis that polymerase slippage correlates to the length of repeat stretches consisting of uniform repeats against the alternative hypothesis that the total number of repeats is most relevant. Two short tetrameric short tandem repeats (STRs) with different repeat structures were investigated: D3S1545 containing only homogeneous (GATA)n repeat stretches and D7S1517 with compound repeat arrays of GAAA and CAAA repeats. Additionally two different polymerases (Herculase and AmpliTaq Gold) were used which gave comparable results. No correlation was found for the hypothesis "total repeat number against percent of stutter"; in contrast, the other hypothesis that the number of uniform repeats is relevant for the degree of stutter gave a strong positive correlation (0.82 for selected D7S1517 alleles) which confirmed the hypothesis that polymerase slippage correlates to the length of repeat stretches consisting of uniform repeats.

Krenke, B. E., L. Viculis, et al. (2005). "Validation of a male-specific, 12-locus fluorescent short tandem repeat (STR) multiplex." Forensic Science International **148**(1): 1.

http://www.sciencedirect.com/science/article/B6T6W-4D9R96Y-1/2/1feb186db95679e44bf07167cb29940d

Y chromosome-specific short tandem repeat (Y-STR) analysis has become another widely accepted tool for human identification. The PowerPlex[trademark] Y System is a fluorescent multiplex that includes the 12 loci: DYS19, DYS385a/b, DYS389I/II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438 and DYS439. This panel of markers incorporates the 9-locus European minimal haplotype (EMH) loci recommended by the International Y-STR User Group and the 11-locus set recommended by the Scientific Working Group on DNA Analysis Methods (SWGDAM). Described here are inter-laboratory results from 17 developmental validation studies of the PowerPlex[trademark] Y System and include the following results: (a) samples distributed between laboratories and commercial standards produced expected and reproducible haplotypes; (b) use of common amplification and detection instruments were successfully demonstrated; (c) full profiles were obtained with standard 30 and 32 cycle amplification protocols and cycle number (24-28 cycles) could be modified to match different substrates (such as direct amplification of FTA[trademark] paper); (d) complete profiles were observed with reaction volumes from 6.25 to 50 [mu]L; (e) minimal impact was observed with variation of enzyme concentration; (f) full haplotypes were observed with 0.5-2X primer concentrations; however, relative yield between loci varied with concentration; (g) reduction of magnesium to 1 mM (1.5 mM standard) resulted in minimal amplification, while only partial loss of yield was observed with 1.25 mM magnesium; (h) decreasing the annealing temperature by 2-4 [deg]C did not generate artifacts or locus dropout and most laboratories observed full amplification with the annealing temperature increased by 2 [deg]C and significant locus dropout with a 4 [deg]C increase in annealing temperature; (i) amplification of individual loci with primers used in the multiplex produced the same alleles as observed with the multiplex amplification; (j) all laboratories observed full amplification with [greater-than-or-equal]125 pg of male template with partial and/or complete profiles observed using 30-62.5 pg of DNA; (k) analysis of [less-than or equal to]500 ng of female DNA did not yield amplification products; (l) the minor male component of a male/female mixture was observed with [less-than or equal to]1200-fold excess female DNA with the majority of alleles still observed with 10,000-fold excess female; (m) male/male mixtures produced full profiles from the minor contributor with 10-20-fold excess of the major contributor; (n) average stutter for each locus; (o) precision of sizing were determined; (p) human-specificity studies displayed amplification products only with some primate samples; and (q) reanalysis of 102 non-probative casework samples from 65 cases produced results consistent with original findings and in some instances

additional identification of a minor male contributor to a male/female mixture was obtained. In general, the PowerPlex[trademark] Y System was shown to have the sensitivity, specificity and reliability required for forensic DNA analysis.

Lazaruk, K., J. Wallin, et al. (2001). "Sequence variation in humans and other primates at six short tandem repeat loci used in forensic identity testing." Forensic Science International **119**(1): 1.

http://www.sciencedirect.com/science/article/B6T6W-4326069-1/2/c61c7a1d022edde5948c569f19204150

A large number of alleles from the six different short tandem repeat (STR) loci FGA, D3S1358, vWA, CSF1PO, TPOX and TH01, used in human identity testing were sequenced to provide support for the robustness of fluorescent STR DNA typing by allele size. Sequence information for some of these loci (FGA, vWA, TH01) is an extension of published work, whereas no extensive sequence information is available with respect to the D3S1358, CSF1PO, and TPOX loci. Sequencing of alleles at each locus has provided quantitative data with respect to the true nucleotide length of common alleles, and of alleles that vary in length from the common alleles. All alleles that were identified as "off-ladder" alleles through fluorescent typing at these STR loci have proven to be true length variant alleles. Sequencing at the D3S1358 and CSF1PO loci allowed for the establishment of a common nomenclature for these loci. A correlation between percent stutter and the length of the core tandem repeat is demonstrated at the FGA locus. Alleles in which the core tandem repeat is interrupted by a repeat unit of different sequence have a reduced percent stutter. DNA samples from three non-human primates (chimpanzee, orangutan, and gorilla) were compared to the human sequences, and shown to differ markedly across loci with respect to their homology. The effects of primer binding site mutations on the amplification efficiency at a particular locus, and methods used to interpret amplification imbalance of heterozygous alleles at a locus is also addressed.

Lee, H. Y., M. J. Park, et al. (2005). "Selection of twenty-four highly informative SNP markers for human identification and paternity analysis in Koreans." Forensic Science International **148**(2-3): 107.

http://www.sciencedirect.com/science/article/B6T6W-4CPDJJR-4/2/ec2fcb493c196c921c064d03bef89fb5

A number of DNA marker types suitable for human identification and parentage testing have been developed, of which single nucleotide polymorphisms (SNPs) merit attention as they are abundant, genetically stable, and amenable to high-throughput automated analysis. In this regard, 24 highly informative SNP markers representing each 22 autosome and both sex chromosomes were selected, and the allele and genotype frequencies of these SNPs were determined in a group composed of 30 unrelated Koreans. Based on frequency data from this group, the estimated probability of identity (PI) and probability of paternity exclusion (PE) with 22 autosomal SNP loci were 1.905 x 10-10 and 98.9%, respectively. The SNPs in this study offer a small but highly accurate database that will be an essential reference for SNP-based forensic application in the future.

Legrand, B., P. d. Mazancourt, et al. (2002). "DNA genotyping of unbuffered formalin fixed paraffin embedded tissues." Forensic Science International **125**(2-3): 205.

http://www.sciencedirect.com/science/article/B6T6W-44W42N7-

1/2/a9f40e15fe26484df66816996544571c

Formalin-induced DNA degradation was studied at different fixation times (3, 7, 16 and 32 days) each on 10 formalin fixed paraffin embedded tissues (FFPET) stored for 15 years at room temperature.The four different extraction protocols used in this study showed that Chelex(R)100 extracts performed the best at 3 and 7 days of formalin fixation (DFF) (with regard to the quantity and the quality of the DNA). However, Qiamp extracts showed better results for long sized alleles, as well for single polymerase chain reaction (PCR) amplifications after 16 and 32 DFF, as for multiplex PCR at shorter fixation times. DNA degradation is expressed by the size of the amplified alleles, only 100 bp templates surviving after 32 DFF (AMG locus). Single locus amplifications (CD4 and FES/FPS alleles) performed better than multiplex PCR (ProfilerPlus), with nearly 100% positive results at 7 DFF. In both types of amplifications, the success rate decreased proportionally with the time of formalin fixation and, consequently, with the size of the required DNA template.

Leibelt, C., B. Budowle, et al. (2003). "Identification of a D8S1179 primer binding site mutation and the validation of a primer designed to recover null alleles." Forensic Science International **133**(3): 220.

http://www.sciencedirect.com/science/article/B6T6W-48KMJ25-2/2/d12c1f26e0d443658c1be592f8df46bd

A population study of Chamorros and Filipinos using short tandem repeat (STR) loci amplified with the AmpFlSTR(R) Profiler Plus(TM) PCR amplification kit demonstrated an excess of observed homozygosity at the D8S1179 locus. Use of a different set of D8S1179 primers to type the same samples did not demonstrate an excess of homozygosity and showed discordant genotypes at the D8S1179 locus. A single point mutation, G-to-A transition, 16 nucleotides from the 3' end of the reverse primer, was identified to cause allele dropout when using the AmpFlSTR(R) Profiler Plus(TM) primer set. An additional D8S1179 reverse primer specific for the variant was constructed resulting in the recovery of the null allele. The primer was included in the newly developed AmpFlSTR(R) Identifiler(TM) PCR amplification kit. No deleterious effects or non-specific peaks were observed in validation experiments evaluating primer concentration, Mg2+ concentration, annealing temperature and population samples.

Lessig, R., J. Edelmann, et al. (2000). "Population genetics of ACTBP2 (SE33) in Western Saxony (Germany)." Forensic Science International **113**(1-3): 39.

http://www.sciencedirect.com/science/article/B6T6W-414N4VW-9/2/a1ab814a1d7edcb5f8abb71b096f7693

In order to apply a useful STR system we performed a population study in Western Saxony (Germany). The allele distribution was investigated in a sample of 431 unrelated adults. In addition, 170 families from routine paternity cases were examined for the presence of meiotic mutations, and two mutations were observed.

Lessig, R., J. Edelmann, et al. (2001). "Population genetics of Y-chromosomal microsatellites in Baltic males." Forensic Science International **118**(2-3): 153.

http://www.sciencedirect.com/science/article/B6T6W-42SXF3V-

C/2/85c71972de3dd5e52a2fc14b421ddd36

Y-chromosomal microsatellites (STRs) are potentially useful in forensic practice but, in contrast to autosomal systems, large and diverse population databases are required in order to facilitate the statistical evaluation of donor-stain matches. Since appropriate data from the Baltic region have so far been lacking, blood samples were obtained from 430 males originating from one of the three Baltic states and these samples were genotyped using a previously described "extended core set" of nine Y-STR marker systems. Allele frequency distributions and discrimination indices were calculated, and the three populations were tested for genetic differences by means of analysis of molecular variance (AMOVA). A larger genetic difference became apparent between Estonian and both Lithuanian and Latvian males than between the latter two, non-Finno-Ugric speaking populations. The haplotype data reported here have been included into the Y-STR database maintained at the Institute of Legal Medicine, Humboldt University, Berlin.

Linacre, A. and J. Thorpe (1998). "Detection and identification of cannabis by DNA." Forensic Science International **91**(1): 71.

http://www.sciencedirect.com/science/article/B6T6W-3S2BKV5-8/2/46ff5449134702ff0235ef109ee34a80

The unambiguous identification of illicit substances, including Cannabis sativa, is a major concern of law enforcement agencies. Current methods of cannabis identification involve the use of techniques such as HPLC and GC to identify cannabinoids. A method for the identification of cannabis using DNA-specific primers has been developed and is described here. The nucleotide sequences between the trnL and trnF genes in the chloroplast of Cannabis sativa have been determined and Cannabis sativa-specific nucleotide sequences within the intergenic spacer between the trnL 3' exon and trnF gene identified. Primers, made to these sequences, have been tested on a range of different plant extracts but only give a PCR product in the presence of Cannabis sativa. The successful production of a PCR product using these primers identifies the presence of cannabis.

Lopez, A. M., S. Alvarez, et al. (2004). "Population data for 16 Y-chromosome STRs in four populations from Pyrenees (Spain)." Forensic Science International **140**(1): 125.

http://www.sciencedirect.com/science/article/B6T6W-4BFVP2F-1/2/3f8deb5c306426cbcfd39d381ff23c4f

Population frequencies for the eight Y-STR loci included in the "minimal haplotype" from Y-STR Haplotype Reference Database (YHRD) plus other eight Y-STRs (DYS434, DYS435, DYS436, DYS437, DYS438, DYS439, GATA H4 and GATA A10) were obtained for a sample of 133 males from four main geographical areas in the Pyrenees (Spain): Vall D'Aran (Lerida), Cerdanya (Gerona), Alt Urgell (Lerida) and Jacetania (Huesca). Haplotype diversities were estimated in the four populations.

Malgorn, Y. and R. Coquoz (1999). "DNA typing for identification of some species of Calliphoridae: An interest in forensic entomology." Forensic Science International **102**(2-3): 111.

http://www.sciencedirect.com/science/article/B6T6W-3XH31K5-4/2/1afec6d3e6ba60d6d20e6de7982edf1f

To determine precisely post mortem interval, larvae and puparium species found on a corpse have to be identified. Among more than 200 cases examined at the entomology department of the Institut de Recherche Criminelle de la Gendarmerie Nationale, two-thirds concerned corpses less than one month old. Therefore, insects from first and second screwworms are the most frequently found [1]. Some species commonly found in France, such as different Lucilia and Calliphora vicina Robineau-Desvoidy, are easily identifiable at an adult stage, but are almost impossible to differentiate at immature stages when only fragments of puparium or necrosed first instar larvae are available. For this reason, an easy and objective method of identification was thus searched by genetic analysis of these insects. Sequencing of partial gene of sub unit I of cytochrome oxydase has been used to predict restriction sites. Restriction enzyme cleavage of PCR products with Dde I allowed us to differentiate these species.

Manetto, G., F. Crivellente, et al. (1998). "A simplified approach to capillary electrophoretic separation of polymerase chain reaction fragments of forensic interest." Forensic Science International **92**(2-3): 259.

http://www.sciencedirect.com/science/article/B6T6W-3SHBM46-G/2/dce9e1ddceb23b374e74b8e7b83be020

We report a sensitive and low-cost capillary electrophoretic typing method and its application to a short tandem repeat system widely used in forensic identification (HUMCD4). Separations are carried out with internally coated 100 [mu]m fused-silica capillaries filled with a noncrosslinked gel sieving matrix based on 1.50-1.75 hydroxyethylcellulose. Detection was by laser-induced fluorescence detection. As running buffer, 100 mM Tris-borate with 0.1 mM EDTA, pH 8.7, was used. Samples were simply diluted (>=1:10 in water) prior to separation. Both electrokinetic injection and analytical separation were carried out at -180 V/cm. The method enabled separation of HUMCD4 alleles, with fair precision in terms of absolute and relative migration times (R.S.D.s of 0.22% and 0.04%, respectively, in intraday tests). The average accuracy of CD4 fragment sizing was 0.218 base pairs. These results confirmed the high suitability of capillary electrophoresis as a screening method for small-size DNA polymorphisms.

Minaguchi, K. and O. Takenaka (2000). "Structural variations of the VWA locus in humans and comparison with non-human primates." Forensic Science International **113**(1-3): 9.

http://www.sciencedirect.com/science/article/B6T6W-414N4VW-3/2/3ec5bac2569d7b3e6fd0d30176aed78a

The HUMVWA locus was examined in 160 samples from the Japanese population. A total of 142 fragments were sequenced, and the counterpart sequences were also determined in non-human primates. In humans, 10 different alleles were found; they could be grouped into seven allelic classes based on the total number of repeats. No variation was observed in the alleles 17, 18 and 19, which showed consensus sequence structures and in the allele 14, which showed a different structure. New variation was found in alleles 15, 16, and 20, which had differences occurred in a basic (TCTA)(TCTG)n repeat in the 5' side. The counterpart fragments were successfully amplified in three species (chimpanzees, gorilla, and orangutan) out of four kinds of anthropoids, three species (rhesus macaques, Japanese macaques, and green monkey) out of four kinds of old world monkeys, but not in one species of either new world monkey or prosimian. The sizes of the fragments distributed from 92 to 180 bp in non-human primates and showed allelic size differences in four species. The sequence of the 5' flanking region followed by primer sequences in humans and anthropoids, which consisted of 19 bp, was identical in all, but differed from that in old world monkeys. The basic repeat motifs of humans and anthropoids consisted of TCTA, TCTG, and TCCA but that of old world monkeys consisted of TCTG, TCCG and TCCA The

structures of humans and anthropoids were essentially similar, but with characteristic difference in each species. Differences in the allelic structures of old world monkeys were complex. Seven different alleles were observed in two rhesus and two Japanese macaques and one type of allele was observed in two green monkeys. Duplication of more than two repeat units of 4 bp was found in an allele of an old world monkey. These data illuminate interesting features of mutational changes in STRs during the long generations and also some insight into evolutional aspects of primates.

Mukaida, M., Y. Takada-Matuzaki, et al. (2003). "The identification of a victim using the DGGE method for trace deposits collected on adhesive film." Forensic Science International **132**(2): 157.

http://www.sciencedirect.com/science/article/B6T6W-47X1YJ0-5/2/547077a28619e5f5ace7aa67467f1854

The denaturant gradient gel electrophoresis (DGGE) method was used in order to simultaneously estimate the genotypes of different factors in a gel plate consisting of one sheet. A genotype analysis of the blood groups (MN, Duffy, Kidd type) and serotype (Gc system) was carried out. DNA samples were extracted from trace deposits which were transferred on adhesive film from a blood trace obtained from a car tire after a fatal car accident. The reference DNA was prepared from the victim's blood. The PCR amplification fragments were amplified from the gene which controlled each blood group. The primers were designed in order to analyze the genotypes with one to three base substitutions in the amplification product. The denaturant concentration limit of the gel for the DGGE method to detect each genotype of the blood groups (MN, Duffy, Kidd type and Gc system) and other conditions of electrophoresis were performed according to previously methods.The each genotype of the blood groups and the Gc system were all simultaneously distinguished in one plate.

Nellemann, L. J., J. Frederiksen, et al. (1996). "PCR typing of DNA fragments of the two short tandem repeat (STR) systems upstream of the human myelin basic protein (MBP) gene in Danes and Greenland Eskimos." Forensic Science International **78**(2): 139.

http://www.sciencedirect.com/science/article/B6T6W-3VXJ5D7-8/2/72d77128dc3bbb32e3cc03501acaa14a

DNA from the double short tandem repeat (STR) system MBP (locus 18q23-pter) was amplified by the polymerase chain reaction (PCR) and the two polymorphic repeat systems were separated by cutting with the restriction enzyme NlaIII. The lengths of the DNA fragments of the two MBP STR systems MBP-A and MBP-B were analyzed by vertical electrophoresis in polyacrylamide gels followed by silver staining. DNA samples from 112 unrelated Danes, 140 unrelated Greenland Eskimos, and 88 Danish mother/child pairs were analyzed. The distributions of MBP phenotypes were in Hardy-Weinberg equilibrium in both the Eskimo and Danish populations. Significant differences were observed between the distribution of fragments ('alleles') in Greenland Eskimos and in Danes. The allele MBP-A7 was considerably more frequent in Eskimos (0.2214) than in Danes (0.0775) and also the allele MBP-B9 was considerably more frequent in Eskimos (0.225) than in Danes (0.06). Strong gametic associations were found between fragments from the MBP-A and MBP-B series in both Danes and Eskimos. Some of the associations were different in Danes and Eskimos. In the 88 Danish mother/child pairs, the segregation of the MBP genotypes were in accordance with a genetic model of co-dominant inheritance and no mutation was found. Two MBP STR regions with irregular structures were sequenced. One fragment had a single base G to A transition at position 124 in the primer binding region between the MBP-A and MBP-B regions. In the other fragment, a deletion starting at position 117 and including the primer binding region between the MBP-A and MBP-B regions

was found.

Pai, C.-Y., L.-L. Hsieh, et al. "Mitochondrial DNA sequence alterations observed between blood and buccal cells within the same individuals having betel quid (BQ)-chewing habit." Forensic Science International **In Press, Corrected Proof** http://www.sciencedirect.com/science/article/B6T6W-4FM3X2K-1/2/4876a67e62196e10b06c0972b38da815

There are hundreds of millions of betel quid (BQ) lovers widely spreading around the world. Compositions in BQ may generate reactive oxygen species, which would induce DNA damage. However, oral epithelial cells as well as blood have often been used as reference samples in comparison with the mitochondrial DNA (mtDNA) sequence of hairs. The main purpose of this study was to investigate the extent of mtDNA sequence variation in regular BQ-chewers' oral epithelial cells, and thus to evaluate the forensic availability of the buccal cells from BQ-chewers using the mtDNA markers. The hypervariable segments I and II in the D-loop control region of mtDNA between paired samples of blood and buccal scrape cells from 75 non-BQ-chewers (to be a control group), 60 BQ-chewers, and 67 oral cancerous patients were DNA sequenced and compared. Among the three groups, the alteration rates of 1.3% (1 out of 75), 10% (6 out of 60), and 61% (41 out of 67) were identified from the control, BQ-chewers, and the cancerous group, respectively. In the cancerous group, as expected, high rate of DNA alteration between blood and buccal samples was found. In the BQ-chewers, one and five individuals had the length and point alterations, respectively. Interestingly, most of point alteration sites, e.g., mtDNA positions 153, 16189, 16093 identified from BQ-chewers, were also observed in previous literatures. As for the control subjects, one case with point alteration, and none with length alteration, was identified. For all the three groups, not only the oral cells but also the normal blood samples exhibited high frequency (>55%) of length heteroplasmy at poly-(C)n track. Statistical analyses revealed that significance was observed between the severity of mtDNA alteration in BQ-chewers' oral epithelial cells and the history of BQ-chewing ($p = 0.02$), with a tendency of positive association. Based on the guidelines by Carracedo et al., we suggest that the interpretation of mtDNA variations between criminal evidences and the oral epithelial cells (as a reference or known sample) from BQ-chewers should be performed with particular caution using the PCR-based mtDNA sequencing. Our findings would be valuable in mtDNA analysis of hair evidence, especially for those countries where the habit of BQ-chewing is popular.

Pai, C.-Y., L.-L. Hsieh, et al. (2002). "Allelic alterations at the STR markers in the buccal tissue cells of oral cancer patients and the oral epithelial cells of healthy betel quid-chewers: an evaluation of forensic applicability." Forensic Science International **129**(3): 158.

http://www.sciencedirect.com/science/article/B6T6W-46Y4PX6-3/2/2c1b0809e0cb7274bacabf1b18047373

Although cancerous specimens are usually not used in forensic DNA typing, they might be forcibly employed under certain instances. On the other hand, though the oral epithelial samples have been applied to forensic identification, the great popularity of betel quid (BQ)-chewing in Taiwan, which is known to be a risk factor leading to an oral cancer, makes this application questionable. The DNA stability of nine short tandem repeat (STR) markers (the AmpFlSTR(TM) kit) was first investigated and then used to evaluate the forensic appropriateness of the oral samples of both healthy BQ-chewers and the archived clinical specimens from oral cancer patients. The analyses were performed on buccal samples from 100 BQ-chewers and 100 oral cancer patients, as well as their paired peripheral blood samples, and a group of 100 non-BQ-chewers were used for the control. In the group of 100 oral cancer patients, two types of DNA instability were found. They were major allelic imbalance, and allelic alterations including the expansion, the contraction and the un-classified type (i.e. can not be confirmed as the expansion

or the contraction). The overall percentage of the cancerous subjects demonstrating DNA instability was 33% (five patients possessing both types of DNA instability). Both types of DNA instability showed a tendency of increasing with the severity of the pathological stage of oral cancer. Forty-four occurrences of major allelic imbalance were found from 21 cancer patients. The statistical result revealed that there was no significant difference in the allelic imbalanced occurrence among the nine STR loci. Allelic alterations were found in 17 patients, within which 12 individuals had the expansion, five had the contraction, and three were the un-classified type. Further, among these 17 patients, three were found to acquire multiple allelic alterations at multiple loci. In the group of 100 unrelated healthy BQ-chewers, two loci with major allelic imbalance were detected. However, the two imbalanced alleles were virtually half lost, and could still be recognized as heterozygous alleles. The statistical results of ANOVA, [chi]2, and Scheffe tests indicated that the means of allelic imbalance at the nine STR loci of the oral cancerous group revealed a significant difference from those in the control group. Our results suggest that oral cancer tissues cannot be used as references for forensic purposes using the PCR-based STR systems, whereas the oral swabs from healthy BQ-chewers can be employed, but should be done with caution.

Park, M. J., H. Y. Lee, et al. "Forensic evaluation and haplotypes of 19 Y-chromosomal STR loci in Koreans." Forensic Science International **In Press, Corrected Proof**
http://www.sciencedirect.com/science/article/B6T6W-4DDTM9F-1/2/e0d04308dd4a92a9d3cf7ea72dafcdd0

In this study, 19 Y-specific STR loci (DYS19, DYS389I/II, DYS390, DYS391, DYS392, DYS393, DYS385, DYS388, DYS434, DYS435, DYS436, DYS437, DYS438, DYS439, DYS446, DYS449, and DYS464) were analyzed in 301 unrelated Korean males by three multiplex PCR systems. The haplotype diversity using the classical set of Y-STRs (DYS19, DYS389I/II, DYS390, DYS391, DYS392, DYS393, and DYS385; multiplex I) was 0.9963. For the same individuals, the haplotype diversity value using the new set of highly informative Y-STRs (DYS385, DYS446, DYS449, and DYS464; multiplex III) was 0.9989, while that using the combined set of Y-STRs by adding DYS388 to the previously studied DYS434, DYS435, DYS436, DYS437, DYS438, and DYS439 (multiplex II) was 0.9509. A total of 297 different haplotypes were identified using the 19 Y-STR markers, of which 293 were unique and 4 were found twice. The overall haplotype diversity was 0.9999.The evaluation of the information of selected markers by combination of each marker with the minimal haplotype showed that DYS434, DYS435, DYS436, DYS437, and DYS438 do not significantly contribute to increment of haplotype diversity. However, respective conjunction of DYS464, DYS449, and DYS446 with the minimal haplotype considerably increased the haplotype diversity. Especially, DYS464 is expected to be the most useful marker that can be included in the expanded minimal haplotype. These results including the haplotype data at 19 Y-STR loci in the present study would provide useful information in forensic practice in a Korean population.

Parson, W., A. Brandstatter, et al. (2004). "The EDNAP mitochondrial DNA population database (EMPOP) collaborative exercises: organisation, results and perspectives." Forensic Science International **139**(2-3): 215.

http://www.sciencedirect.com/science/article/B6T6W-4BCXKDN-1/2/25266d22fe4c3fe540c647c2b51e10d3

This paper presents an overview of the organisation and the results of the collaborative exercises (CE) of the European DNA Profiling (EDNAP) Group's mitochondrial DNA population database project (EMPOP). The aim of the collaborative exercises was to determine whether uniformity of mtDNA sequencing results could be achieved among different laboratories. These were asked to sequence either the complete mtDNA control region or the two hypervariable regions HVI (16024-

16365) and HVII (73-340) from DNA extracts, buccal swabs or bloodstains, proceeding in accordance with the protocol and strategies used in each individual laboratory. The results of the collaborative exercises were employed to identify possible sources of errors that could arise during the analysis and interpretation of mtDNA profiles. These findings were taken as a basis to tentatively make suitable arrangements for the construction of a high quality mtDNA database. One hundred fifty mtDNA profiles were submitted to the evaluating laboratory, and disaccording profiles were classified into four groups corresponding to the source of error: clerical errors, sample mix-ups, contaminations and discrepancies with respect to the mtDNA nomenclature. Overall, 14 disaccording haplotypes (16 individual errors) were observed. The errors included 10 clerical errors, 3 interpretation problems, 2 cases of sample mix-up and 1 case of point heteroplasmic mixture, where the 2 sequencing reactions brought inconsistent base calls. This corresponds to an error rate of 10.7% in a virtual mtDNA database consisting of the collaborative exercise results. However, this estimate is still conservative compared to conclusions drawn by authors of meanwhile numerous publications critically reviewing published mtDNA population databases. Our results and earlier published concerns strongly emphasize the need for appropriate safety regulations when mtDNA profiles are compiled for database purposes in order to accomplish the high standard required for mtDNA databases that are used in the forensic context.

Pestoni, C., I. Munoz, et al. (1996). "Distribution of the AMPFLPs YNZ22, 3'APOB and COL2A1 in the population of Galicia (NW Spain)." Forensic Science International **80**(3): 175.

http://www.sciencedirect.com/science/article/B6T6W-3W0NG5K-1/2/baaa8764aa6e1cff77fd62200d050949

Two different electrophoretic methods were used for typing three amplified fragment length polymorphisms (AMPFLPs), (3'ApoB, YNZ22 and COL2A1) in a Galician (NW Spain) population sample. Because of the problems of anomalous mobility for the 3'ApoB system and the intermediate alleles found in the COL2A1 system, the use of automated sequencers and denaturing conditions is recommended for typing these two systems. Nevertheless, simple electrophoretic methods, such as the PhastSystem, can be used for YNZ22 typing. Although intermediate COL2A1 alleles can be distinguished with the sequencers, a binning approach was adopted for comparison purposes. The population sampled was in Hardy-Weinberg equilibrium for the three systems using an exact test. This type of statistical analysis is more appropriate when the number of alleles in a system is high. No significant differences with other Caucasian populations were found for the three systems studied. The characteristics of the polymorphisms, shown by 3'ApoB, YNZ22 and COL2A1, reflected in the statistical parameters studied, demonstrate that these AMPFLPs are of considerable interest for forensic purposes.

Poetsch, M., S. Seefeldt, et al. (2001). "Analysis of microsatellite polymorphism in red deer, roe deer, and fallow deer -- possible employment in forensic applications." Forensic Science International **116**(1): 1.

http://www.sciencedirect.com/science/article/B6T6W-41WJBSP-1/2/9d404c608cae2c4df291ae0cacb1902b

DNA microsatellites play a major role in population genetics, linkage mapping, and parentage studies of mammals. In addition, they may be used for forensic purposes, if an individual identification of a specific animal is necessary. Therefore, we tested a variety of microsatellite polymorphism derived from reindeer (Rangifer tarandus) by PCR and sequencing analysis for use in red deer (Cervus elaphus), roe deer (Capreolus capreolus) and fallow deer (Dama dama). Twelve of these microsatellites were selected for further analysis. In all these microsatellite

polymorphism short tandem repeats could be detected for one or all three species as shown by sequencing analysis. In red deer, more than two alleles were found in eight microsatellites, in roe deer more than two alleles could be demonstrated in seven microsatellites, whereas in fallow deer more than two alleles were found in only two microsatellite polymorphism. A comparison of sequences of PCR products from the three deer species with the sequences of reindeer revealed several differences between the four species. In six microsatellites -- selected because or their reliability in PCR and because of their polymorphic character -- we established a sequenced allelic ladder and give population data of all three species from 82 deer of the Northeast region of Germany (Vorpommern). Our results show the possibility to use microsatellite polymorphism in the identification of deer in forensic applications like poaching.

Poetsch, M., H. Wittig, et al. (2003). "Mitochondrial diversity of a northeast German population sample." Forensic Science International **137**(2-3): 125.

http://www.sciencedirect.com/science/article/B6T6W-49M0RB7-4/2/7bc3338551d0a88f59c2f9a9ccd239bb

Mitochondrial DNA sequences of the hypervariable regions HV I and HV II were analyzed in 300 unrelated individuals born and living in the northeast corner of Germany (Western Pomerania) to generate a database for forensic identification purposes in this region. Sequence polymorphism were detected using PCR and direct sequencing analysis. A total of 242 different haplotypes were found as determined by 147 variable positions. The most frequent haplotype (263G, 315.1C) was found in 10 individuals and is also the most common sequence in Europe. Three other haplotypes were shared by 5 individuals, 2 sequences by 4, 8 haplotypes by 3, 15 sequences by 2 persons, and 213 sequences were unique. The genetic diversity was estimated to be 0.99 and the probability of two random individuals showing identical mitochondrial DNA (mtDNA) haplotypes is 0.6%. A comparison with other studies from Germany showed only little differences in the distribution of haplogroups. Nevertheless, one frequent haplotype in northeast Germany (five unrelated individuals) could only rarely be found in other German and European regions. Our results may indicate that despite a high admixture proportion in the German population some regions could demonstrate certain characteristic features.

Potter, T. (2003). "Co-amplification of ENFSI-loci D3S1358, D8S1179 and D18S51: validation of new primer sequences and allelic distribution among 2874 individuals." Forensic Science International **138**(1-3): 104.

http://www.sciencedirect.com/science/article/B6T6W-49XPTY2-1/2/1d5ec2cc3e7c140b8b8b590d19847846

The present communication presents a new triplex PCR co-amplifying three loci (D3S1358, D8S1179 and D18S51) recommended for STR typing by the European Network of Forensic Science Institutes (ENFSI). Twenty-two different primers were tested to optimise the PCR. Four of the six primer sequences finally chosen were self selected, the fifth was a published one and the sixth derived from a commercially available multiplex kit. Using this PCR-setup, even minimum amounts of genomic DNA are sufficient to analyse the STR loci D3S1358, D8S1179 and D18S51 in parallel. Especially in forensic casework, where DNA is mostly limited and often contaminated with enzyme inhibitors, this new PCR proved to be very advantageous. To demonstrate the reliability, buccal swabs from 2874 persons were typed not only with the new triplex PCR but also with a commercially available multiplex kit.

Prinz, M., A. Ishii, et al. (2001). "Validation and casework application of a Y chromosome specific STR multiplex." Forensic Science International **120**(3): 177.

http://www.sciencedirect.com/science/article/B6T6W-43J6SDP-3/2/34bc0c5d01e35102d707acfce1699f7f

A series of validation experiments was performed for a Y chromosome specific STR multiplex system following the suggestions made by the Technical Working Group DNA Analysis Methods (TWGDAM). The multiplex PCR products were detected on Perkin-Elmer 373 and 377 automated sequencers using two labeling colors. No problems regarding the stability, robustness and sensitivity of the Y STR multiplex were observed. Mixture studies revealed a cut off rate similar to autosomal STRs for mixtures of male DNAs and no interference of any female admixture. The comparison of the Y STR results to the autosomal typing results for 56 nonprobative semen stains and swabs, showed a slightly higher success rate in detecting the semen donor's alleles for the Y STR multiplex. Two examples are shown to illustrate the usefulness of Y STR typing for DNA mixtures. In one case the Y STR results confirmed an isolated exclusion; in the other case, the interpretation of a mixture was clarified since the Y STR results proved the presence of DNA from at least two semen donors. Y STR typing is a valuable addition to the forensic DNA testing panel.

Pu, C.-E., C.-M. Hsieh, et al. (1999). "Genetic variation at nine STR loci in populations from the Philippines and Thailand living in Taiwan." Forensic Science International **106**(1): 1.

http://www.sciencedirect.com/science/article/B6T6W-3Y80FF4-1/2/f8dfc11570d0ed16f58ae92ae3f0c0e8

In order to apply a set of nine STR loci and the amelogenin locus in forensic testing, we have performed a population study on individuals from the Philippines and Thailand living in Taiwan (273 Philippine and 146 Thai individuals were typed by commercially available kits and an automated sequencer). A total of 73 alleles for all systems for both populations could be observed in these two populations. No new intermediate fragments were found. Allele frequencies showed no deviation from Hardy-Weinberg equilibrium. The mean exclusion power (MEP) ranged from 0.327 (TPOX) to 0.706 (FGA), the discriminating power (DP) ranged from 0.790 (TPOX) to 0.963 (FGA) for Philippinos, MEP ranged from 0.247 (TPOX) to 0.723 (FGA), DP ranged from 0.761 (TPOX) to 0.968 (FGA) for Thais, the combined MEP is >0.9988 and the combined DP is >0.9999999993 for both Philippinos and Thais.

Pu, C.-E., F.-C. Wu, et al. (1998). "DNA short tandem repeat profiling of Chinese population in Taiwan determined by using an automated sequencer." Forensic Science International **97**(1): 47.

http://www.sciencedirect.com/science/article/B6T6W-3V8RCR5-M/2/d92fb8b0bd253ff09371d22dadffb96f

We performed a population study on a Taiwan population using a set of nine short tandem repeat (STR) loci and the amelogenin locus. Allele and genotype frequencies of the STR systems D3S1358, vWA, FGA, TH01, TPOX, CSF1PO, D5S818, D13S317 and D7S820 were determined by polymerase chain reaction followed by automated sequencer analysis. A total of 80 alleles could be observed, using all systems, in a population of 500 individuals. No new intermediate fragments were found in these systems. Allele frequencies showed no deviation from the Hardy-Weinberg equilibrium. The mean exclusion power (MEP) ranged from 0.294 for TPOX to 0.711 for FGA (the combined MEP was >0.999), the discriminating power (DP) ranged from 0.782 for

TPOX to 0.964 for FGA, (the combined DP was >0.99999999949).

Quintans, B., V. Alvarez-Iglesias, et al. (2004). "Typing of mitochondrial DNA coding region SNPs of forensic and anthropological interest using SNaPshot minisequencing." Forensic Science International **140**(2-3): 251.

http://www.sciencedirect.com/science/article/B6T6W-4BRBK4G-C/2/1b41081564f6f10e7dd4185a81bc408d

The development of new methodologies for high-throughput SNP analysis is one of the most stimulating areas in genetic research. Here, we describe a rapid and robust assay to simultaneously genotype 17 mitochondrial DNA (mtDNA) coding region SNPs by minisequencing using SNaPshot. SNaPshot is a methodology based on a single base extension of an unlabeled oligonucleotide with labeled dideoxy terminators. The set of SNPs implemented in this multiplexed SNaPshot reaction allow us to allocate common mitochondrial West Eurasian haplotypes into their corresponding branch in the mtDNA skeleton, with special focus on those haplogroups lacking unambiguous diagnostic positions in the first and second hypervariable regions (HVS-I/II; by far, the most common segments analyzed by sequencing). Particularly interesting is the set of SNPs that subdivide haplogroup H; the most frequent haplogroup in Europe (40-50%) and one of the most poorly characterized phylogenetically in the HVS-I/II region. In addition, the polymorphic positions selected for this multiplex reaction increase considerably the discrimination power of current mitochondrial analysis in the forensic field and can also be used as a rapid screening tool prior to full sequencing analysis. The method has been validated in a sample of 266 individuals and shows high accuracy and robustness avoiding both the use of alternative time-consuming classical strategies (i.e. RFLP typing) and the need for high quantities of DNA template.

Ricaut, F.-X., C. Keyser-Tracqui, et al. "STR-genotyping from human medieval tooth and bone samples." Forensic Science International **In Press, Corrected Proof**
http://www.sciencedirect.com/science/article/B6T6W-4D490H0-D/2/73d906b63376bb8e4d5f5bf288280985

We extracted the DNA contained in samples of bones and teeth from 10 skeletons excavated from the Gravette site (400-1000 AD, south of France). Ancient DNA was analysed by autosomal short tandem repeats (STRs). The DNA present in these ancient remains appeared very degraded, but nevertheless, better conserved in tooth than in bone samples. Moreover, we showed that the DNA extracted from ancient dental pulp was not exempt from polymerase chain reaction (PCR) inhibitors, which could result from extreme DNA fragmentation. An adapted protocol with a supplementary step of purification removed this inhibition.

Saitoh, H., S. Ueda, et al. (1998). "The different mobility of complementary strands depends on the proportion AC/GT." Forensic Science International **91**(2): 81.

http://www.sciencedirect.com/science/article/B6T6W-3S2BKV5-9/2/1a8de0c1626090fbd9299fb83fa5bf60

The electrophoretic mobility of DNA fragments on denaturing polyacrylamide gel depends on various factors. One of these is the base composition of a single-stranded DNA (ssDNA). We confirmed that one strand and its complementary strand of polymerase chain reaction (PCR)

products migrated with different mobilities in all alleles detected at 12 out of the 13 short tandem repeat (STR) loci studied. The mobility differences between complementary strands (MD) were also observed regardless of end-polishing with Pfu DNA polymerase. MD was therefore not influenced by additional nucleotides to each strand of the PCR products. We then analyzed the relation between MD and the base composition using one representative allele at each of the 13 loci. The results indicated that MD was affected by the adenine plus cytosine (AC) content in the ssDNA and was proportional to the values of the AC content divided by the guanine plus thymine (GT) content in the AC-rich strand (the proportion AC/GT). When the proportion AC/GT was well-balanced, MD decreased. The same tendency was observed even in the end-polished strands. In this study, the electrophoretic mobility of an ssDNA on denaturing polyacrylamide gels was shown to depend on the proportion AC/GT. Unless the same side of the PCR products is labelled in the context of a PCR-based STR typing, distinct alleles may be mistaken for identical ones because of the different mobility of complementary strands. Accordingly, the labelled strand should be described if only one strand of the PCR products is detected. When using an allelic ladder marker as a size standard, the labelled side should be unified between STR alleles and the allelic ladder alleles.

Sajantila, A., M. Strom, et al. (1991). "The polymerase chain reaction and post-mortem forensic identity testing: Application of amplified D1S80 and HLA-DQ[alpha] loci to the identification of fire victims." Forensic Science International **51**(1): 23.

http://www.sciencedirect.com/science/article/B6T6W-4C35YMH-MV/2/1392b361f0734a2c8efdc3275422a886

The application of DNA typing methods after amplification by the polymerase chain reaction (PCR) of DNA derived from body tissues from charred fire victims was investigated. A total of 26 different tissue specimens from ten extensively burned individuals were analyzed. The samples included femoral muscle, psoas muscle, bone marrow and blood. The post-mortem period varied from 38 to 183 h. After amplifying the DNA by PCR from the various tissues, the D1S80 locus was analyzed with a high resolution polyacrylamide gel electrophoresis technique followed by silver staining and the alleles of the HLA-DQ[alpha] locus were detected by using a reverse dot blot format. All samples could be typed for both loci and the genotypes were consistent in the various tissues from each individual. A parentage test was performed in two cases and Mendelian inheritance of the alleles for both loci was observed.

Sanchez, J. J., C. Borsting, et al. (2003). "Multiplex PCR and minisequencing of SNPs--a model with 35 Y chromosome SNPs." Forensic Science International **137**(1): 74.

http://www.sciencedirect.com/science/article/B6T6W-49D6YWB-1/2/54c45c15778ca4940344bfeb24a0f967

We have developed a robust single nucleotide polymorphism (SNPs) typing assay with co-amplification of 25 DNA-fragments and the detection of 35 human Y chromosome SNPs. The sizes of the PCR products ranged from 79 to 186 base pairs. PCR primers were designed to have a theoretical Tm of 60+/-5 [deg]C at a salt concentration of 180 mM. The sizes of the primers ranged from 19 to 34 nucleotides. The concentration of amplification primers was adjusted to obtain balanced amounts of PCR products in 8 mM MgCl2. For routine purposes, 1 ng of genomic DNA was amplified and the lower limit was approximately 100 pg DNA. The minisequencing reactions were performed simultaneously for all 35 SNPs with fluorescently labelled dideoxynucleotides. The size of the minisequencing primers ranged from 19 to 106 nucleotides. The minisequencing reactions were analysed by capillary electrophoresis and multicolour fluorescence detection. Female DNA did not influence the results of Y chromosome

SNP typing when added in concentrations more than 300 times the concentrations of male DNA. The frequencies of the 35 SNPs were determined in 194 male Danes. The gene diversity of the SNPs ranged from 0.01 to 0.5.

Sanchez, J. J., M. Brion, et al. (2004). "Duplications of the Y-chromosome specific loci P25 and 92R7 and forensic implications." Forensic Science International **140**(2-3): 241.

http://www.sciencedirect.com/science/article/B6T6W-4BRBK4G-B/2/383e68afd08b05f7b3a86c12500b6677

In the present study, we demonstrate that two commonly used Y-chromosome single nucleotide polymorphisms (SNPs), P25 and 92R7, are paralogous sequence variants (PSVs) originating from segmental duplications and that at least one of the sequence variants in each group of loci is polymorphic. Several methodologies were used in order to detect the SNP alleles and the PSVs of the loci. All results obtained with the various typing techniques supported the conclusion. The allele distributions of the binary markers were analysed in more than 600 males with seven different haplogroups. For P25, the ancestral allele C was found in several samples from different haplogroups. The derived allele A was always present with an additional C variant. Haplogroup P was defined by the derived allele A at the 92R7 locus. However, the ancestral allele G was always associated with an A variant due to the duplication.

Santos, S. M. M., B. Budowle, et al. (1996). "Portuguese population data on the six short tandem repeat loci -- CSF1PO, TPOX, THO1, D3S1358, VWA and FGA." Forensic Science International **83**(3): 229.

http://www.sciencedirect.com/science/article/B6T6W-3RG55N8-10/2/3a061c80e5e170fe3f5726d4f1aefd81

Allele frequencies for six tetrameric short tandem repeat (STR) loci CSF1PO, TPOX, THO1, D3S1358, VWA and FGA were determined in a Caucasian population sample from Portugal. All loci are highly polymorphic and meet Hardy-Weinberg expectations. There is little evidence for association of alleles among the six loci. The three loci D3S1358, VWA and FGA are more polymorphic and, hence, are more informative than the loci CSF1PO, TPOX, and THO1. However, all six loci would be useful for human identification applications. The STR allelic frequency data are similar to other Caucasian data.

Schoske, R., P. M. Vallone, et al. (2004). "High-throughput Y-STR typing of U.S. populations with 27 regions of the Y chromosome using two multiplex PCR assays." Forensic Science International **139**(2-3): 107.

http://www.sciencedirect.com/science/article/B6T6W-4B4HKB1-2/2/51235e3cf3404bb7fddd0d2061d63407

Two Y-chromosome short tandem repeat (STR) multiplex polymerase chain reaction (PCR) assays were used to generate haplotypes for 19 single copy and 3 multi-copy Y-STRs. A total of 27 PCR products were examined in each sample using the following loci: DYS19, DYS385 a/b, DYS388, DYS389I/II, DYS390, DYS391, DYS392, DYS393, DYS426, DYS437, DYS438, DYS439, DYS447, DYS448, DYS450, DYS456, DYS458, DYS460, DYS464 a/b/c/d, H4, and YCAII a/b. The first multiplex is the Y-STR 20plex previously described by Butler et al. [Forensic

Sci. Int. 129 (2002) 10]. The second multiplex is a novel Y-STR 11plex and includes DYS385 a/b, DYS447, DYS448 and the new markers DYS450, DYS456, DYS458, and DYS464 a/b/c/d. These two multiplexes were tested on 647 males from three United States population sample sets: 260 African Americans, 244 Caucasians, and 143 Hispanics. Haplotype comparisons between common loci included in the 20plex and 11plex assays as well as commercially available kits found excellent agreement across a sampling of the population samples. The multi-copy loci DYS464, DYS385, and YCAII were the most polymorphic followed by the following single copy Y-STRs: DYS458, DYS390, DYS447, DYS389II, DYS448, and DYS456. Samples containing the most common type in the European database could be well resolved with additional markers beyond the minimal haplotype loci.

Shepherd, C., S. Harbison, et al. (2004). "Y STR haplotype data for New Zealand population groups using the Y-Plex(TM) 6 kit." Forensic Science International **145**(1): 69.

http://www.sciencedirect.com/science/article/B6T6W-4CG2KCF-1/2/ab50b93ec782b0fd39bc037227083c7b

Allele and haplotype frequencies were obtained for the six Y STR loci DYS19, DYS389II, DYS390, DYS391, DYS393 and DYS385 in the New Zealand population. Ninety-two different haplotypes were found. The Maori population had a specific haplotype that occurred in over 30% of the population. The Pacific Island population exhibited a triple repeat at the DYS385 locus in 26% of individuals, something rarely observed in other population groups.

Shigeta, Y., Y. Yamamoto, et al. (1999). "Polymorphism of the D12S391 microsatellite in a Japanese population sample." Forensic Science International **102**(1): 61.

http://www.sciencedirect.com/science/article/B6T6W-3WSMFJX-7/2/924b5addb152bd8d260f93a1664259d2

Using the polymerase chain reaction (PCR), we studied the short tandem repeat (STR) polymorphism observed at the D12S391 locus. In 350 Japanese examined, 14 different alleles ranging from 209 bp to 261 bp were detected. Allele 18 (221 bp) showed the highest frequency at 0.30. Observed and expected values of respective genotypes satisfied the Hardy-Weinberg equilibrium ([chi]2=24.08, P=0.24, df=20). In addition, 18 additional sequence structures (suballeles), were detected in this study. Within the suballeles, sequence variants, in which the initial repeat of (AGAT) was replaced with (AGGT), was found in five samples. It was found that the analysis of single-strand conformation polymorphism (SSCP) before sequence analysis was useful for distinguishing these suballeles.

Shindo, S. and N. Yoshioka "Polymorphisms of the cholecystokinin gene promoter region in suicide victims in Japan." Forensic Science International **In Press, Corrected Proof**
http://www.sciencedirect.com/science/article/B6T6W-4DVBVJG-6/2/7c2d296501ecc6b6d58700a0e0fd84fe

Cholecystokinin (CCK), a neurotransmitter in the central nervous system (CNS), co-exists in a large portion of A10 dopamine neurons to exert some effect on dopamine behavior. The aim of this study was to determine whether any association exists between the genotype of CCK gene promoter regions (-45C/T and -196G/A) and suicidal behavior. Genotypes and allele frequencies of CCK -45C/T and -196G/A were analyzed using polymerase chain reaction (PCR) followed by

single-strand conformational polymorphism (SSCP) analysis on the genomic DNA from selected suicide victims (N = 154) and from control subjects (N = 328). Statistical analysis was performed using the Mantel-Haesnzel [chi]2-test and multiple logistic regression analysis with distinction of gender. An association between CCK -196G/A polymorphism and suicidal behavior in Japanese males was confirmed by statistical analysis (Odds ratio: 3.462, 95% CI: 1.128-10.626, P = 0.038 by multiple logistic regression analysis). However, a significant association between CCK -196G/A polymorphism and suicidal behavior was not discovered in females. The polymorphism of the CCK gene promoter region was found to represent a susceptibility factor for suicidal behavior in Japanese males.

Spitaleri, S., D. Piscitello, et al. (2004). "Experimental procedures comparing the activity of different Taq polymerases." Forensic Science International **146**(Supplement 1): S167.

http://www.sciencedirect.com/science/article/B6T6W-4DKKFD7-6/2/acc148e818469e40ccb1ae9848bd9a12

Forensic investigations involve several scientific branches among which biological analyses are much more frequently requested as a consequence of their importance and great versatility towards most of the traces found on the crime scene.Biological analyses are lead in subsequent steps: extraction, amplification and STR typing of the specimens collected on the crime scene. All of these techniques have been modified from original protocols according to the kind of sample to process. A critical point in our analysis is trying to amplify small amounts of DNA extracted from decomposed tissues or objects, small biological traces have been left on, with high fidelity and account. That's why we have decided to settle on an experimental procedure aimed to find the best DNA polymerase according to our purposes.We have tested different Taq polymerases on the same known DNA sample at several dilutions and have compared quality and amount of amplified DNA in order to appreciate the amplifying capability of each enzyme. These data have been analyzed as a function of the technical properties of each engineered Taq polymerase and results are shown in details.

Sterlinko, H., I. Z. Pajnic, et al. (2001). "Human Y-specific STR haplotypes in a Slovenian population sample." Forensic Science International **120**(3): 226.

http://www.sciencedirect.com/science/article/B6T6W-43J6SDP-B/2/db732b101c582180f48c83ef935898a8

The allele distribution of the systems DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS385 and YCAII were investigated in a sample of 121 unrelated males from Slovenia

Susukida, R., A. Kido, et al. (1998). "GPT genotyping by polymerase chain reaction and restriction fragment length polymorphism analysis." Forensic Science International **98**(3): 185.

http://www.sciencedirect.com/science/article/B6T6W-3VCVFB4-7/2/a6a3f14dd78d5b150d81b628312a50fd

Polymorphism of GPT was investigated in blood samples from 241 unrelated Japanese individuals by PCR-RFLP analysis. Three common types were identified which agreed with those obtained by starch gel electrophoresis. The allele frequencies were GPT*1=0.604 and GPT*2=0.396. The population data fitted the Hardy-Weinberg law. The PCR-RFLP patterns of

GPT were also demonstrated in semen, and the types coincided with those in the corresponding blood. The present method permitted GPT genotyping in bloodstains and semen stains stored for 20 years. The GPT system determined by PCR-RFLP can still be a useful supplement in medicolegal individualization of biological stains.

Thomson, J. A., V. Pilotti, et al. (1999). "Validation of short tandem repeat analysis for the investigation of cases of disputed paternity." Forensic Science International **100**(1-2): 1.

http://www.sciencedirect.com/science/article/B6T6W-3W4GJ8F-1/2/1579960d8bccea259b30a666a7c1470e

This study details validation of two separate multiplex STR systems for use in paternity investigations. These are the Second Generation Multiplex (SGM) developed by the UK Forensic Science Service and the PowerPlex(TM)1 multiplex commercially available from Promega Inc. (Madison, WI, USA). These multiplexes contain 12 different STR systems (two are duplicated in the two systems). Population databases from Caucasian, Asian and Afro-Caribbean populations have been compiled for all loci. In all but two of the 36 STR/ethnic group combinations, no evidence was obtained to indicate inconsistency with Hardy-Weinberg (HW) proportions. Empirical and theoretical approaches have been taken to validate these systems for paternity testing. Samples from 121 cases of disputed paternity were analysed using established Single Locus Probe (SLP) tests currently in use, and also using the two multiplex STR systems. Results of all three test systems were compared and no non-conformities in the conclusions were observed, although four examples of apparent germ line mutations in the STR systems were identified. The data was analysed to give information on expected paternity indices and exclusion rates for these STR systems. The 12 systems combined comprise a highly discriminating test suitable for paternity testing. 99.96% of non-fathers are excluded from paternity on two or more STR systems. Where no exclusion is found, Paternity Index (PI) values of >10000 are expected in >96% of cases.

Thymann, M., L. J. Nellemann, et al. (1993). "Analysis of the locus D1S80 by amplified fragment length polymorphism technique (AMP-FLP). Frequency distribution in Danes. Intra and inter laboratory reproducibility of the technique." Forensic Science International **60**(1-2): 47.

http://www.sciencedirect.com/science/article/B6T6W-4C35XSJ-4B/2/fa4980c1700fc9e0b791ef7cf34735f6

DNA from the locus D1S80 was amplified by polymerase chain reaction (PCR) and analyzed by electrophoresis in vertical polyacrylamide gels followed by silverstaining. DNA samples from 119 unrelated Danes and 97 mother/child pairs were examined. The amplified fragment length polymorphism (AMP-FLP) analysis of the D1S80 locus demonstrated 21 alleles and a heterozygosity of 77%. Of the 231 possible phenotypes, 57 were observed. All mother/child pairs shared at least one D1S80 allele. The D1S80 typing results in 70 Danes were compared to the results obtained on the same samples in another laboratory and the results were concordant in all cases.

Tomita, M., T. Nohno, et al. (2002). "Hypervariable locus of the 3'-flanking region of the neurotensin receptor gene: an effective region for personal identification in forensic practice." Forensic Science International **127**(1-2): 119.

http://www.sciencedirect.com/science/article/B6T6W-45TTS82-1/2/8954cec78d06bc8736eca45720651de2

We examined the complex short tandem repeat (STR) locus at the 3'-flanking region of the neurotensin receptor (NTR) gene. The polymorphism of this locus was first reported as a simple tetranucleotide repeat variation by Le et al. [1], but it also offers a surprisingly informative variation, that permits reliable individual identification by two complementary strategies: fluorescent-labelled polymerase chain reaction (PCR)/electrophoresis and direct sequencing of the PCR products. We determined the alleles in 203 Japanese by fluorescent-labelled PCR/electrophoresis. Determination was based on their length with a reliability of +/-1 bp, and the frequency of each allele was very low. Sequencing analysis further grouped these alleles in detail. Sequencing demonstrated that the locus varied by six repetitive units and three insertion/deletion positions of nucleotide fragments. We detected multiple alleles having different structures even in the same allele length. We found structural differences in homozygous alleles having the same base pair size. We also determined that apparently homozygous alleles were heterozygous from sequencing electropherograms showing an overlap of nucleotides or +/-1 bp difference. These results indicate that this locus is structurally hypervariable in addition to having allelic length variations, promising a great advance in individual identification in forensic practice.

Tsai, L. C., C. Y. Lin, et al. (2001). "Sequence polymorphism of mitochondrial D-loop DNA in the Taiwanese Han population." Forensic Science International **119**(2): 239.

http://www.sciencedirect.com/science/article/B6T6W-433P7K2-C/2/b7bfd747c1a62d36a651677af06540cc

In order to demonstrate the sequence diversity of mitochondrial D-loop DNA in the Taiwanese Han population, we established a database of 155 unrelated individuals. For each individual, the complete 980 bp DNA region from the 5' end of HVI to 3' end of HVII segment was sequenced. In these 155 sequence data, 149 different haplotypes were observed, amongst these haplotypes, 144 were unique, 4 were found in 2 individuals and 1 was found in 3 individuals. When compare to the Anderson sequence, 144 transitions, 24 transversions, 5 insertions and 5 deletions were found. Eight positions exhibited more than one polymorphic sequence, six exhibited two variants while two exhibited three variants. Over the 1024 bp that was analysed, pairwise differences between the sequences were 11.35+/-3.53 bp. The sequence and nucleotide diversity were 0.9994 and 0.0116, respectively. The probability of two individuals randomly matching over the entire control region was 0.007. The diversity in the mitochondrial D-loop indicates the value of this locus for casework within Taiwan.

Tzen, C.-Y., T.-Y. Wu, et al. (2001). "Sequence polymorphism in the coding region of mitochondrial genome encompassing position 8389-8865." Forensic Science International **120**(3): 204.

http://www.sciencedirect.com/science/article/B6T6W-43J6SDP-6/2/bd82097f2cd12ccc4703ac8d7c816823

Analysis of the polymorphic sequences in mitochondrial DNA (mtDNA) has been widely applied to forensic tests and anthropology studies. However, these polymorphic data in human have thus far been derived from the displacement-loop and intergenic regions only. Here, we report the identification of clustered polymorphic sites in the mitochondria coding region encompassing position 8389-8865. The DNA sequences of 119 unrelated Chinese were determined by PCR amplification and direct sequencing. The results showed that heteroplasmy was found in five individuals, 39 sites were noted in this 477 bp region, and 41 haplotypes were identified. The

probability of identity and allelic diversity were estimated as 0.1265 and 0.8809, respectively. The results suggest that sequence polymorphism from position 8389-8865 in human mtDNA can be used as a marker for identity investigation.

Vallone, P. M., A. E. Decker, et al. (2005). "Allele frequencies for 70 autosomal SNP loci with U.S. Caucasian, African-American, and Hispanic samples." Forensic Science International **149**(2-3): 279.

http://www.sciencedirect.com/science/article/B6T6W-4DCWHF2-1/2/0afca869ac369cc6f8c7c8257a3e6f14

189 samples from 3 different U.S. sample groups Caucasian (74), African American (71) and Hispanic (44) were typed for 70 autosomal genetic markers. These 70 markers are bi-allelic (C/T) short nucleotide polymorphisms (SNPs). For each sample, the 70 SNP markers were typed in 11 unique 6-plexes and a single 4-plex PCR. A total of 10 of the 210 tests (70 loci X 3 populations) for Hardy-Weinberg equilibrium indicated a statistically significant result. In order to evaluate the minimum number of SNP loci needed to distinguish all 189 samples from one another, we ranked the loci according to their levels of observed heterozygosity and p-values obtained upon testing for Hardy-Weinberg equilibrium. The top 12 loci according to these ranking criteria were tabulated along with the number of unique genotypes observed when combining subsequent SNP markers. The 12 selected SNPs possessed an observed heterozygosity of >0.45 in all three populations examined and thus would be expected to exhibit more differences between samples. All of the 189 samples in this study were individualized with a subset of 12 SNP loci. However, it is likely that the addition of more than 12 SNP loci will be required to resolve larger sets of unrelated individuals from one another. By way of comparison, in these same 189 individuals all but one pair is resolved from one another with three of the traditional short tandem repeat (STR) loci possessing the highest heterozygosity values (D2S1338, D18S51, and FGA) run with the Identifiler kit. The final pair of unrelated samples could be resolved with the combination of 4 STR loci: D2S1338, D18S51, FGA, and VWA.

Vauhkonen, H., M. Hedman, et al. (2004). "Evaluation of gastrointestinal cancer tissues as a source of genetic information for forensic investigations by using STRs." Forensic Science International **139**(2-3): 159.

http://www.sciencedirect.com/science/article/B6T6W-4B7YFXJ-2/2/d6b45079de49e01390a399b643b30fe5

Malignant tissue samples may sometimes be the only source of biological material for forensic investigations, including identification of individuals or paternity testing. However, in use of such samples, uncertainties due to microsatellite instability (MSI) and loss of heterozygosity (LOH) often associated with neoplasias may be encountered. In this study, we have analysed the applicability of autosomal tetranucleotide short tandem repeat (STR) markers, which are routinely used in forensic analysis, to gain genetic information. MSI and LOH were analysed in 41 surgically removed gastrointestinal cancer specimens and the adjascent non-cancerous tissue marginals. The cancer specimens showed great variability in their genetic phenotypes due to MSI or LOH, with only 32% being microsatellite-stable. Of the 15 autosomal STR loci analysed, only TH01 had no MSI-type alteration in these samples. The loci most frequently affected by MSI were D8S1179, D21S11, D18S51 and D19S433 (MSI in 15-17% of cases). LOH-type alterations were observed at all of the loci, including the amelogenin locus used for sex determination. The highest LOH frequency was found at locus D18S51 (27%). The genetic alterations at the marker loci may indicate false homozygosity or heterozygosity, and false gender may result from erroneous deduction of DNA profiles. Therefore, typing of autosomal STRs from malignant tissues in

forensic settings warrants careful interpretation of MSI and LOH results together with microscopic analysis of a tissue specimen. Results by two commercially available and widely used forensic DNA profiling kits used here were comparable.

Verma, S. K., K. Prasad, et al. (2003). "Was elusive carnivore a panther? DNA typing of faeces reveals the mystery." Forensic Science International **137**(1): 16.

http://www.sciencedirect.com/science/article/B6T6W-49FR99N-1/2/a9bbdc43974f1a9059492e99f0846a49

In this study, we report the findings of a recent case in which the officials of an Indian zoo claimed that an animal, possibly a carnivore, is periodically visiting the zoo from a nearby vast forest area and causing panic in zoo and nearby villages. They collected some elusive faecal material from the vicinity of an herbivore enclosure. Looking to the pugmarks found in that area and faecal matter ceased, the officials could not decide whether it was a lioness, a tiger or a panther. We resolved this mystery by DNA-based analysis of the faecal material, using our recently developed novel universal primers to amplify and sequence a specific fragment of mitochondrial cytochrome b gene. The findings of the DNA-based analyses were confirmed after few days when the zoo officials trapped the animal of same species as suggested in our report. The potential of our procedure to investigate the cases related to wildlife offence is discussed.

von Wurmb, N., D. Meissner, et al. (2001). "Influence of cyanoacrylate on the efficiency of forensic PCRs." Forensic Science International **124**(1): 11.

http://www.sciencedirect.com/science/article/B6T6W-44KTHV6-3/2/c2ffc382235c57151e8dd2f1b42cfa27

Cyanoacrylate ester (CA) is commonly used by criminalists to detect latent fingerprints on smooth surfaces. We investigated whether this treatment has an influence on a subsequent DNA typing of biological stains, and on the efficiency of three different forensic PCRs (mtDNA, Y-STR determination and the Profiler Plus kit).Using fluorescence labeled primers and an automated detection system, we could show that the presence of CA led to weaker PCR products. Depending on the DNA extraction method the amplification results were significantly weaker compared to untreated controls. To simulate forensic cases we prepared blood and saliva stains on glass slides, extracted the DNA using two different methods and compared the signal intensities of the amplified DNA fragments. Depending on the extraction methods, the presence of CA significantly hampered the amplification of DNA from small stains whereas there was virtually no difference comparing the amplification results of DNA extracted from bigger stains.

Wallman, J. F. and S. C. Donnellan (2001). "The utility of mitochondrial DNA sequences for the identification of forensically important blowflies (Diptera: Calliphoridae) in southeastern Australia." Forensic Science International **120**(1-2): 60.

http://www.sciencedirect.com/science/article/B6T6W-43G30BC-C/2/8861b39c20f2ef5421f565668b955627

The applicability of mitochondrial DNA (mtDNA) sequencing was investigated for the identification of the following forensically important species of blowflies from southeastern Australia: Calliphora albifrontalis, C. augur, C. dubia, C. hilli hilli, C. maritima, C. stygia, C. vicina, Chrysomya

rufifacies, Ch. varipes and Onesia tibialis. All breed in carrion except O. tibialis, which is an earthworm parasitoid. Emphasis was placed on Calliphora species because they predominate among the carrion-breeding blowfly fauna of southern Australia and their immatures are difficult to identify morphologically. A partial sequence of the mitochondrial COII gene was determined for all species and for COI for C. albifrontalis, C. augur, C. dubia and C. stygia only. Five other species of blowflies, Chrysomya albiceps, Ch. rufifacies, Protophormia terraenovae, Lucilia illustris and L. sericata, for which sequence data were already available, were also included. Analysis of the COI and COII sequences revealed abundant phylogenetically informative nucleotide substitutions that could identify blowfly species to species group. In contrast, because of the low level of sequence divergence of sister species, the data could not distinguish among taxa from the same species group, i.e. the species within the C. augur and C. stygia groups. The molecular data support the existing species group separation of the taxa within Calliphora. Because of the speed and accuracy of current nucleotide sequencing technology and the abundant apomorphic substitutions available from mtDNA sequences, this approach, with the analysis of additional taxa and genes, is likely to enable the reliable identification of carrion-breeding blowflies in Australia.

Walsh, S. J., S. L. Robinson, et al. (2003). "Characterisation of variant alleles at the HumD21S11 locus implies unique Australasian genotypes and re-classification of nomenclature guidelines." Forensic Science International **135**(1): 35.

http://www.sciencedirect.com/science/article/B6T6W-48S35HX-6/2/9e2841515bf5607a5c4e05db68041b08

Several variant alleles of the HumD21S11 locus have only been reported in Australasian population samples. Fifteen such alleles were observed in Caucasian and Australian Aborigine sub-population databases compiled from residents of the state of Western Australia. Each variant was sequenced to authenticate the allelic designation and determine the structural conformation. Nine novel structural variants are described. The structure of the repeat region of these rare alleles combined with the STR designation brings aspects of the HumD21S11 nomenclature guidelines into question, in particular the designation of common incomplete repeats (or "0.2's"). The conformation of the sequences provides evidence in support of a genetic relationship between the Australian Aborigine and the Papuan people.

Yamamoto, T., K. Tamaki, et al. (1994). "DNA typing of the D1S8 (MS32) locus by rapid detection minisatellite variant repeat (MVR) mapping using polymerase chain reaction (PCR) assay." Forensic Science International **66**(1): 69.

http://www.sciencedirect.com/science/article/B6T6W-4C35Y8T-F7/2/4e9b173c6f2e35e19db09bdbd80b594c

The typing of the D1S8 (MS32) lucus using the minisatellite variant repeat (MVR) polymerase chain reaction (PCR) method was performed by visualising amplified DNA stained with ethidium bromide. The results from rapid detection MVR-PCR were compared with those from the original MVR-PCR using Southern blot hybridisation with a 32P-labelled probe. With genomic DNA extracted from blood samples of 40 healthy unrelated Japanese individuals, the first 41 codes, on average, were correctly determined by rapid detection MVR-PCR without band intensity information, compared with at least 60 codes typed by the original MVR-PCR. The rapid detection MVR-PCR method was applied to bloodstains to simulate forensic samples. On average, 39 code positions could be determined from DNA extracted from 3-month-old bloodstains of six persons. Rapid detection MVR-PCR is more convenient than the original MVR-PCR, furnishes much information with regard to personal identification, and should be applicable to forensic fields.

Yong, R. Y. Y., L. T. Aw, et al. (2004). "Allele frequencies of 15 STR loci of three main ethnic populations in Singapore using an in-house marker panel." Forensic Science International **141**(2-3): 175.

http://www.sciencedirect.com/science/article/B6T6W-4BT1TSW-2/2/899dd868237148297d8b45c39c7d104a

Allele frequency data for 15 Short Tandem Repeat (STR) loci was studied for the three main ethnic groups residing in Singapore, namely Chinese, Malay and Indian. An in-house STR marker panel was employed, consisting all 13 tetranucleaotide STR listed in CODIS (Combined DNA Index System, USA) and two pentanucleaotide STR, Penta D and Penta E. This represents a comprehensive report for allele distribution in the Singapore population for these 15 microsatellite markers commonly used in forensic science.

Zarrabeitia, M. T., T. Amigo, et al. (2002). "A new pentaplex system to study short tandem repeat markers of forensic interest on X chromosome." Forensic Science International **129**(2): 85.

http://www.sciencedirect.com/science/article/B6T6W-46SFFWP-1/2/f7f0fa8a065a62683233776ec71e22e2

A new method has been optimised to amplify five X chromosome short tandem repeat (STR) markers of interest in forensic medicine: human phosphoribosyl transferase (HPRTB), DXS101, androgen receptor (ARA), DXS7423 and DXS8377. Markers were conveniently amplified in a single PCR reaction with fluorochrome-labelled primers, which allowed the analysis of fragment sizes after injection into a capillary electrophoresis system. The most common alleles of each locus were sequenced and used in a control ladder to type unknown samples.

Zhu, B., X. Li, et al. "Y-STRs haplotypes of Chinese Mongol ethnic group using Y-PLEX[trademark] 12." Forensic Science International **In Press, Corrected Proof**
http://www.sciencedirect.com/science/article/B6T6W-4F924HP-3/2/eed5bc3ad266aaa14e0166bd7ec7475e

Eleven Y-chromosome STR loci (DYS19, DYS389 I, DYS389 II, DYS390, DYS391, DYS392, DYS393, DYS385a,b, DYS438, DYS439) have been co-amplified in 96 healthy unrelated males of Chinese Mongol ethnic group, in order to investigate allele and haplotype frequencies of them, evaluate their usefulness in forensic casework.

Free Radical Biology and Medicine (10)

Dumont, P., M. Burton, et al. (2000). "Induction of replicative senescence biomarkers by sublethal oxidative stresses in normal human fibroblast." Free Radical Biology and Medicine **28**(3): 361.

http://www.sciencedirect.com/science/article/B6T38-3YNXYM6-7/2/f2c1155892407643f6614809edd9f5bb

We tested the long-term effects of sublethal oxidative stresses on replicative senescence. WI-38 human diploid fibroblasts (HDFs) at early cumulative population doublings (CPDs) were exposed to five stresses with 30 [mu]M tert-butylhydroperoxide (t-BHP). After at least 2 d of recovery, the cells developed biomarkers of replicative senescence: loss of replicative potential, increase in senescence-associated [beta]-galactosidase activity, overexpression of p21Waf-1/SDI-1/Cip1, and inability to hyperphosphorylate pRb. The level of mRNAs overexpressed in senescent WI-38 or IMR-90 HDFs increased after five stresses with 30 [mu]M t-BHP or a single stress under 450 [mu]M H2O2. These corresponding genes include fibronectin, osteonectin, [alpha]1(I)-procollagen, apolipoprotein J, SM22, SS9, and GTP-[alpha] binding protein. The common 4977 bp mitochondrial DNA deletion was detected in WI-38 HDFs at late CPDs and at early CPDs after t-BHP stresses. In conclusion, sublethal oxidative stresses lead HDFs to a state close to replicative senescence.

Favreau, F., I. Petit-Paris, et al. (2004). "Cyclooxygenase 1-dependent production of F2-isoprostane and changes in redox status during warm renal ischemia-reperfusion." Free Radical Biology and Medicine **36**(8): 1034.

http://www.sciencedirect.com/science/article/B6T38-4BRP0NV-1/2/f234d961cf588248a1e3df832438ba53

The detrimental role of oxidative stress has been widely described in tissue damage caused by ischemia-reperfusion. A nonenzymatic, reactive oxygen species-related pathway has been suggested to produce 8-iso-prostaglandin F2[alpha] (8-iso-PGF2[alpha]), an epimer of prostaglandin F2[alpha] (PGF2[alpha]), which has been proposed as an indicator of oxidative stress. Using an in vivo ischemia-reperfusion model in rat kidneys, we investigated intrarenal accumulation of 8-iso-PGF2[alpha] and PGF2[alpha]. Both prostanoids accumulated in the ischemic kidney and disappeared upon reperfusion. In addition, a nonselective (acetylsalicylic acid) or selective cyclooxygenase (COX) 1 inhibitor (SC-560) completely abrogated the 8-iso-PGF2[alpha] and PGF2[alpha] formation in kidneys subjected to ischemia. COX2 inhibition had no effect on the production of these prostanoids. Therefore the two metabolites of arachidonic acid seemed to be produced via an enzymatic COX1-dependent pathway. Neither COX overexpression nor COX activation was detected. We also investigated renal glutathione, which is considered to be the major thiol-disulfide redox buffer of the tissue. Total and oxidized glutathione was decreased during the ischemic period, whereas no further decrease was seen for up to 60 min of reperfusion. These data demonstrate that a dramatic decrease in antioxidant defense was initiated during warm renal ischemia, whereas the 8-iso-PGF2[alpha] was related only to arachidonate conversion by COX1.

Fukagawa, N. K., M. Li, et al. (1999). "Aging and high concentrations of glucose potentiate injury to mitochondrial DNA." Free Radical Biology and Medicine **27**(11-12): 1437.

http://www.sciencedirect.com/science/article/B6T38-3Y6GVRK-18/2/ddc9d8639fdcecd15962cbb8eadfa3ed

Deletions of mitochondrial DNA (mtDNA) are associated with aging and several chronic diseases. We have reported heterogeneous mutations between base pair 8468 and 13446 in mtDNA, the region known as the "common" deletion, in muscle of older humans with impaired glucose tolerance or diabetes mellitus. To further characterize potential effects of age and glycemia on mtDNA integrity, we studied corpulent JCR:LA-cp rats that are characterized by insulin resistance, hyperinsulinemia, and hyperlipidemia, factors strongly associated with both aging and cardiovascular disease. In addition to skeletal muscle, we isolated vascular smooth muscle cells (VSMC) from aortas of 6-, 12-, and 17-month-old rats and exposed them to 5-, 25-, 62-, and 100-

mM glucose or a combination of hypoxanthine (100 [mu]M) and xanthine oxidase (0.025 U/ml) to generate reactive oxygen species in separate cultures. Long- and short-fragment and nested polymerase chain reaction was used to detect mutations in the common deletion region. The data demonstrate that aging and the cp genotype confer susceptibility to mtDNA deletions in vivo and that high glucose concentrations can induce mtDNA mutations in vitro. Accordingly, aging and glucose-related oxidative stress and possibly hyperinsulinemia may contribute to alterations in mitochondrial gene integrity and the cp genotype appears to increase the susceptibility of muscle to the age-related accumulation of mtDNA mutations.

Kashiba, M., J. Oka, et al. (2002). "Impaired ascorbic acid metabolism in streptozotocin-induced diabetic rats." Free Radical Biology and Medicine **33**(9): 1221.

http://www.sciencedirect.com/science/article/B6T38-46NWYSG-1/2/ac12f8a8a29df8d4a7963dcd8ab5e3dc

Ascorbic acid (AA) metabolism in streptozotocin (STZ)-induced diabetic rats was determined by examining urinary excretion, renal reabsorption, reductive regeneration, and biosynthesis of AA at 3 and 14 days after STZ administration. AA concentrations in the plasma, liver, and kidney of the diabetic rats were significantly lower than those of controls on d 3, and decreased further as the diabetic state continued. Hepatic AA regeneration significantly decreased in the diabetic rats on d 3 in spite of increased gene expressions of AA regenerating enzymes and was further reduced on d 14. Hepatic activity of L-gulono-gamma-lactone oxidase, a terminal enzyme of hepatic AA biosynthesis, also decreased significantly on d 3 and decreased further on d 14. Urinary excretion of AA was significantly increased on d 3, with an increase in urine volume but no change in gene expressions of renal AA transporters (SVCT1 and SVCT2). Urinary excretion of AA was normalized on d 14. The results suggest that impaired hepatic and renal regeneration, as well as increased urinary excretion and impaired hepatic biosynthesis of AA, contributed to the decrease in AA in plasma and tissues of STZ-induced diabetic rats.

Kennedy, A. R., J. H. Ware, et al. (2004). "Selenomethionine protects against adverse biological effects induced by space radiation." Free Radical Biology and Medicine **36**(2): 259.

http://www.sciencedirect.com/science/article/B6T38-4B3JMTS-1/2/daa42d85f1c962b5021bb1c0ab112f57

Ionizing radiation-induced adverse biological effects impose serious challenges to astronauts during extended space travel. Of particular concern is the radiation from highly energetic, heavy, charged particles known as HZE particles. The objective of the present study was to characterize HZE particle radiation-induced adverse biological effects and evaluate the effect of -selenomethionine (SeM) on the HZE particle radiation-induced adverse biological effects. The results showed that HZE particle radiation can increase oxidative stress, cytotoxicity, and cell transformation in vitro, and decrease the total antioxidant status in irradiated Sprague-Dawley rats. These adverse biological effects were all preventable by treatment with SeM, suggesting that SeM is potentially useful as a countermeasure against space radiation-induced adverse effects. Treatment with SeM was shown to enhance ATR and CHK2 gene expression in cultured human thyroid epithelial cells. As ionizing radiation is known to result in DNA damage and both ATR and CHK2 gene products are involved in DNA damage, it is possible that SeM may prevent HZE particle radiation-induced adverse biological effects by enhancing the DNA repair machinery in irradiated cells.

Lontz, W., A. Sirsjo, et al. (1995). "Increased mRNA expression of manganese superoxide dismutase in psoriasis skin lesions and in cultured human keratinocytes exposed to IL-1[beta] and TNF-[alpha]." Free Radical Biology and Medicine **18**(2): 349.

http://www.sciencedirect.com/science/article/B6T38-3Y6PRWS-20/2/fceb01988db94e5372e99fa8af6deb20

Because reactive oxygen species have been implicated in the pathogenesis of various hyperproliferative and inflammatory diseases, the mRNA expression of the antioxidant enzyme superoxide dismutase was studied in psoriatic skin tissue. By using reverse transcription-PCR we found similar expression of copper, zinc superoxide dismutase (CuZnSOD) in the involved vs. uninvolved psoriatic skin. In contrast, the level of the manganese superoxide dismutase (MnSOD) mRNA message was consistently higher in lesional psoriatic skin as compared to adjacent uninvolved skin and healthy control skin. Parallel investigation of those cytokines that are thought to be direct or indirect inducers of the MnSOD activity revealed an increased mRNA expression of IL-1[beta], TNF-[alpha], and GM-CSF in lesional psoriatic skin. To study if these cytokines exert a direct effect on dismutase expression in epidermal cells, human keratinocytes in culture were challenged with IL-1[beta], TNF-[alpha], and GM-CSF. It was found that IL-1[beta] and TNF-[alpha], but not GM-CSF, induced the mRNA expression of MnSOD, and an additive effect was demonstrated for the two former cytokines. Further, the expression of both CuZnSOD and MnSOD transcripts was similar in cultured keratinocytes maintained at low differentiation (low Ca2+ medium) and cells forced to terminal differentiation (by high Ca2+ medium). Our results indicate that the abnormal expression of MnSOD mRNA in lesional psoriatic skin is not directly linked to the pathologic state of keratinocyte differentiation in the skin. It seems more likely that the cutaneous overexpression of MnSOD in psoriatic epidermis represents a protective cellular response evoked by cytokines released from inflammatory cells invading the diseased skin.

Pirlich, M., C. Muller, et al. (2002). "Increased proteolysis after single-dose exposure with hepatotoxins in HepG2 cells." Free Radical Biology and Medicine **33**(2): 283.

http://www.sciencedirect.com/science/article/B6T38-4668X49-G/2/8af966e6ad94f360d0b8bd58b9f249a5

Chronic ethanol consumption is associated with increased protein oxidation and decreased proteolysis in the liver. We tested the hypothesis that even single-dose treatment with ethanol or bromotrichloromethane causes increased protein oxidation and a distinct proteolytic response in cultured hepatocytes. HepG2 cells were treated for 30 min with ethanol, H2O2 and bromotrichloromethane at various nontoxic concentrations. Protein degradation was measured in living cells using [35S]-methionine labeling. Protein oxidation, and 20S proteasome activity were measured in cell lysates. Oxidized proteins increased immediately after ethanol, H2O2, and bromotrichloromethane exposure, but a further significant increase 24-h after exposure was observed only following ethanol and bromotrichloromethane treatment. All three reagents caused a significant increase of the overall intracellular proteolysis at rather low concentrations, which could be suppressed by the proteasome inhibitor lactacystin. A decline of proteolysis observed at higher--subtoxic--concentrations was not related to decreased proteasome activity. Preincubation with ketoconazole or 4-methylpyrazole completely prevented the ethanol- and bromotrichloromethane-induced but not the H2O2-induced protein oxidation and proteolysis, suggesting strongly an enzyme-mediated generation of reactive oxygen species. In conclusion single-dose exposure with ethanol or haloalkanes causes increased protein oxidation followed by an increased proteasome-dependent protein degradation in human liver cells.

Ricciarelli, R., C. d'Abramo, et al. (2004). "CD36 overexpression in human brain correlates with [beta]-

amyloid deposition but not with Alzheimer's disease." Free Radical Biology and Medicine **36**(8): 1018.

http://www.sciencedirect.com/science/article/B6T38-4BS08TP-4/2/f0c5f92276cbcd476ca827326ad52af9

Scavenger receptors recently have been related to Alzheimer's disease, although it is still unclear whether they contribute to the pathogenesis of the disease or reflect an inflammatory response to the deposition of amyloid [beta]-protein (A[beta]). In this study we demonstrate that CD36, a class B scavenger receptor, is highly expressed in the cerebral cortex of Alzheimer's disease patients and cognitively normal aged subjects with diffuse amyloid plaques compared with age-matched amyloid-free control brains. Moreover, in vitro experiments indicated that A[beta] is able to induce CD36 expression in neuronal cells after 24 h treatment. The interaction between CD36 and A[beta] has been reported to trigger oxidant production by macrophages and microglia. In line with this observation, we found an increased presence of nitrated proteins in brains showing A[beta] loads and CD36 overexpression, independent of the occurrence of Alzheimer's disease pathologic features.

Suliman, H. B., M. S. Carraway, et al. (2002). "Rapid mtDNA deletion by oxidants in rat liver mitochondria after hemin exposure." Free Radical Biology and Medicine **32**(3): 246.

http://www.sciencedirect.com/science/article/B6T38-451MV67-6/2/042b5622b9da28e7f61475899a32770b

The amounts of superoxide and hydrogen peroxide generated by mitochondria under physiological conditions can be enhanced by cellular stress. This study tested the hypothesis that the response to hemin-induced stress, which includes heme oxygenase-1 (HO-1) induction, predisposes to oxidative damage of mitochondrial DNA (mtDNA). Hepatic mitochondria from control, hemin-, and CO-exposed rats were incubated with tert-butyl hydroperoxide (tert-BH) or the NO donor 1,2,3,4-oxatriazolium, 5-amino-3- (3,4-dichlorophenyl)-chloride (GEA 3162). Mitochondrial total and oxidized glutathione (GSH and GSSG), total and free iron, and 8-oxo-7, 8-dihydro-2'-deoxyguanosine (8-OHdG) were determined with and without oxidants. As expected, oxidation by tert-BH induced significant GSH depletion and increased amounts of free iron and 8-OhdG. Oxidant exposure rapidly produced a large mtDNA deletion involving the coding regions for cytochrome c oxidase (COX 1) and NADH dehydrogenase (ND1 and ND2). Hemin and CO greatly exacerbated susceptibility to the deletion of mtDNA by tert-BH, and this was attenuated by preincubation with GSH methyl ester. Analysis of mitochondria-associated proteins Bax and Bcl-xl in hemin- and CO-exposed rats showed significant responses, revealing interactions with apoptotic pathways. Thus, hemin-induced mitochondrial events sensitize a specific region of the mitochondrial genome to deletion, which is related to depletion of GSH and is not explained by effects of CO. This mtDNA damage is associated with altered expression of mitochondrial cell death proteins, thereby suggesting a novel mechanism for systemic or environmental pro-oxidants to influence apoptosis.

Tolle, A., M. Schlame, et al. "Vitamin E differentially regulates the expression of peroxiredoxin-1 and -6 in alveolar type II cells." Free Radical Biology and Medicine **In Press, Uncorrected Proof**
http://www.sciencedirect.com/science/article/B6T38-4FM9KJW-1/2/3c9846725a287ca0b39af7c786d085c4

Vitamin E is the primary lipophilic antioxidant in mammals. Lack of vitamin E may lead to an increase of cytotoxic phospholipid-peroxidation products (PL-Ox). We could previously show that

alimentary vitamin E-depletion in rats did not change the concentrations of dienes, hydroperoxides, and platelet-activating factor-related oxidation products in alveolar type II cells (TII cells). We hypothesized that vitamin E deficiency increases the activity of enzymes involved in the degradation of PL-Ox. Degradation of PL-Ox may be catalyzed by phospholipase A2, PAF-acetylhydrolase, or peroxiredoxins (Prx's). Alimentary vitamin E deficiency in rats increased the expression of Prx-1 at the mRNA and protein levels and the formation of Prx-SO3, but it did not change the expression of Prx-6 or the activity of phospholipase A2 and PAF-acetylhydrolase in TII cells. H2O2-induced oxidative stress in isolated TII cells activated protein kinase C[alpha] (PKC[alpha]) and increased the expression of Prx-1 and Prx-6. Inhibition of PKC[alpha] in isolated TII cells by long-time incubation with PMA inhibited PKC[alpha] and Prx-1 but not Prx-6. We concluded that the expression of Prx-1 and -6 is selectively regulated in TII cells; PKC[alpha] regulates the expression of Prx-1 but not Prx-6. Prx-6 expression may be closely linked to lipid peroxidation.

Fungal Genetics and Biology (8)

Abramovitch, R. B., G. Yang, et al. (2002). "The ukb1 gene encodes a putative protein kinase required for bud site selection and pathogenicity in Ustilago maydis." Fungal Genetics and Biology **37**(1): 98.

http://www.sciencedirect.com/science/article/B6WFV-46P3W8J-C/2/9b5a34e997f3537ef29dc92daaaf45ba

Hong, S. G., R. A. Cramer, et al. (2005). "Alt a 1 allergen homologs from Alternaria and related taxa: analysis of phylogenetic content and secondary structure." Fungal Genetics and Biology **42**(2): 119.

http://www.sciencedirect.com/science/article/B6WFV-4DW98YY-3/2/265f1d644ab17c87ad9265abaf2be78f

A gene for the Alternaria major allergen, Alt a 1, was amplified from 52 species of Alternaria and related genera, and sequence information was used for phylogenetic study. Alt a 1 gene sequences evolved 3.8 times faster and contained 3.5 times more parsimony-informative sites than glyceraldehyde-3-phosphate dehydrogenase (gpd) sequences. Analyses of Alt a 1 gene and gpd exon sequences strongly supported grouping of Alternaria spp. and related taxa into several species-groups described in previous studies, especially the infectoria, alternata, porri, brassicicola, and radicina species-groups and the Embellisia group. The sonchi species-group was newly suggested in this study. Monophyly of the Nimbya group was moderately supported, and monophyly of the Ulocladium group was weakly supported. Relationships among species-groups and among closely related species of the same species-group were not fully resolved. However, higher resolution could be obtained using Alt a 1 sequences or a combined dataset than using gpd sequences alone. Despite high levels of variation in amino acid sequences, results of in silico prediction of protein secondary structure for Alt a 1 demonstrated a high degree of structural similarity for most of the species suggesting a conservation of function.

Kutil, B. L., G. Liu, et al. (2004). "Contig assembly and microsynteny analysis using a bacterial artificial

chromosome library for Epichlo" height="11" width="9"> festucae, a mutualistic fungal endophyte of grasses." Fungal Genetics and Biology **41**(1): 23.

http://www.sciencedirect.com/science/article/B6WFV-49TRKD3-1/2/56463dc6d856569b5f84383b5d0457cb

We constructed and characterized a bacterial artificial chromosome (BAC) library for Epichlo" height="11" width="9"> festucae, a genetically tractable fungal plant mutualist. The 6144 clone library with an average insert size of 87 kb represents at least 18-fold coverage of the 29 Mb genome. We used the library to assemble a 110 kb contig spanning the putative ornithine decarboxylase (odc) ortholog and subsequently expanded it to 228 kb with a single walking step in each direction. Furthermore, we evaluated conservation of microsynteny between E. festucae and some model filamentous fungi by comparing sequence available from a 43 kb region at the end of one BAC to publicly available fungal genome sequences. Orthologs to the 13 contiguous open reading frames (ORFs) identified in E. festucae are syntenic in Neurospora crassa and Magnaporthe grisea occurring in small sets of two, three or four colinear ORFs. This library is a valuable resource for research into traits important for the development and maintenance of a plant-fungus mutualistic symbiosis.

Martin, M. P., D. H. Coucheron, et al. (2003). "Structural features and evolutionary considerations of group IB introns in SSU rDNA of the lichen fungus Teloschistes." Fungal Genetics and Biology **40**(3): 252.

http://www.sciencedirect.com/science/article/B6WFV-49MDYXV-3/2/4b840452dc2cad662b694e6db23ee1fb

Different species of the lichen-forming ascomycete fungus Teloschistes were found to contain group IB introns at position S1506 in the small subunit ribosomal RNA gene. We have characterized the structural organization and phylogeny of the Teloschistes introns Tco.S1506, Tla.S1506, and Tvi.S1506. Common features to all the introns are a small size, a compact RNA structure, and an atypical catalytic ribozyme core sequence motif. Variations in intron sizes, due to sequence extensions in the P1 and P8 loop segments, were observed in different species and isolates. Phylogenetic analyses based on the ITS1-5.8S-ITS2 region as well as the introns show that the Teloschistes S1506 introns represent a distinct evolutionary isolated cluster among the nuclear group I introns. Furthermore, introns from different lineages of Teloschistes villosus appear not strictly vertically inherited probably due to horizontal transfer in one of the lineages.

O'Donnell, K., T. J. Ward, et al. (2004). "Genealogical concordance between the mating type locus and seven other nuclear genes supports formal recognition of nine phylogenetically distinct species within the Fusarium graminearum clade." Fungal Genetics and Biology **41**(6): 600.

http://www.sciencedirect.com/science/article/B6WFV-4C4WXRJ-1/2/ccdf2640e0859cbeade1089bae502fba

Species limits were investigated within the Fusarium graminearum clade (Fg clade) through phylogenetic analyses of DNA sequences from portions of 11 nuclear genes including the mating-type (MAT) locus. Nine phylogenetically distinct species were resolved within the Fg clade, and they all possess contiguous MAT1-1 and MAT1-2 idiomorphs consistent with a homothallic reproductive mode. In contrast, only one of the two MAT idiomorphs was found in five other species, four of which were putatively asexual, and the other was heterothallic. Molecular evolutionary analyses indicate the MAT genes are under strong purifying selection and that they

are functionally constrained, even in species for which a sexual state is unknown. The phylogeny supports a monophyletic and apomorphic origin of homothallism within this clade. Morphological analyses demonstrate that a combination of conidial characters could be used to differentiate three species and three species pairs. Species rank is formally proposed for the eight unnamed species within the Fg clade using fixed nucleotide characters.Index Descriptors: Fusarium head blight; Mating-type; Histone H3; Homothallic; Heterothallic; Gene trees; Species trees; Species limits; Phylogeny; Reciprocal monophyly

Pratt, R. J. and R. Aramayo (2002). "Improving the efficiency of gene replacements in Neurospora crassa: a first step towards a large-scale functional genomics project." Fungal Genetics and Biology **37**(1): 56.

http://www.sciencedirect.com/science/article/B6WFV-46P3W8J-7/2/47512785770fe0db07710ac5a0f896d1

Ramussen, J. P., A. H. Taylor, et al. (2004). "Guest, a transposable element belonging to the Tc1/mariner superfamily is an ancient invader of Neurospora genomes." Fungal Genetics and Biology **41**(1): 52.

http://www.sciencedirect.com/science/article/B6WFV-49NVFHF-4/2/1c7267dcf57c35f50cf7502799c29cae

Guest is a transposable element of the Tc1/mariner superfamily with 30-40 bp terminal inverted repeats and a TA dinucleotide target site duplication. Guest was originally discovered in the St. Lawrence 74A laboratory strain of the filamentous fungus Neurospora crassa. In this report, Guest iterations subcloned from a cosmid library of the Oakridge 74A strain were used to design PCR primers that permitted the detection of Guest in wild isolates of N. crassa. Guest is present in N. crassa as multiple copies ranging between 100 bp and 2.4 kb and is present in the mating type locus of several Neurospora species. Bioinformatic analysis of the entire N. crassa genome (Oakridge 74A strain) detected 48 Guest iterations. All iterations appeared to have been inactivated either by repeat-induced point mutation or sequence deletion, with the majority being remnants less than 400 bp in length. The possible involvement of Guest in the evolution of the variable region that flanks the mating type idiomorphs in several Neurospora species is discussed.

Spiering, M. J., H. H. Wilkinson, et al. (2002). "Expressed sequence tags and genes associated with loline alkaloid expression by the fungal endophyte Neotyphodium uncinatum." Fungal Genetics and Biology **36**(3): 242.

http://www.sciencedirect.com/science/article/B6WFV-46BMVNH-9/2/7d449928b69e5b073d6755cd937d2115

Genetic Toxicology and Environmental Mutagenesis (11)

Andreassen, A., L. Mollersen, et al. (2002). "One dose of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) or 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) induces tumours in Min/+ mice by truncation mutations or LOH in the Apc gene." Mutation Research/Genetic Toxicology and Environmental Mutagenesis **517**(1-2): 157.

http://www.sciencedirect.com/science/article/B6T2D-45KT0KY-3/2/8c513c577d359ce72d07305926844f43

The C57BL/6J-Min/+ (multiple intestinal neoplasia) mouse has a heterozygous nonsense ApcMin (adenomatous polyposis coli) mutation, and numerous adenomas spontaneously develop in the intestine. Neonatal exposure of Min/+ mice to the food carcinogens 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) or 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) (one injection of 50 mg/kg) increased the number of small intestinal tumours about three- and two-fold, respectively. The number of colonic tumours was only increased in males. We examined whether the wild-type Apc allele was affected in intestinal tumours induced by either PhIP or IQ. In spontaneously formed and in IQ-induced small intestinal and colonic tumours from these mice, the main mechanism for tumour induction was loss of wild-type Apc allele, i.e. loss of heterozygosity (LOH). In contrast to the IQ-induced (84% LOH) and spontaneously (88% LOH) formed tumours, only 55% of the PhIP-induced small intestinal tumours from males showed LOH. Tumours that apparently had retained the wild-type Apc allele were further analysed for the presence of truncated Apc proteins by the in vitro synthesised protein (IVSP) assay. Truncated Apc proteins, indicating truncation mutations in exon 15 of the Apc gene, were detected in two of the 12 PhIP-induced tumours in segment 2 (codons 686-1217), and two of five IQ-induced tumours, one in segment 2 and the other in segment 3 (codons 1099-1693). Three of these four mutations, all in segment 2 of the Apc gene, were confirmed by sequencing. The PhIP-induced mutations were detected at codon 1125 (C deletion) and 1130 (G-T transversion), and the IQ-induced mutation was at codon 956 (C-T transition). Importantly, no truncated proteins were detected in tumours from unexposed mice with apparently retained wild-type Apc allele. These results show that one injection of either PhIP or IQ induces intestinal tumours in the Min/+ mice by inactivation of the wild-type Apc allele either by causing LOH or truncation mutations.

Chen, J. B., V. N. Dobrovolsky, et al. (1999). "Development of a mouse cell line containing the [Phi]X174 am3 allele as a target for detecting mutation." Mutation Research/Genetic Toxicology and Environmental Mutagenesis **444**(2): 347.

http://www.sciencedirect.com/science/article/B6T2D-3XNJV81-8/2/2d3846166dcb3891bb56e76c9d1be2db

Transgenic mice containing multiple copies of the [Phi]X174 am3 allele are being developed as a model for detecting tissue-specific in vivo mutation. In order to derive an analogous system for measuring am3 mutation in vitro, cells were cultured from 15-day-old C57Bl/6J mouse embryos that were homozygous for the transgene and these cells were transfected with a plasmid expressing the SV40 large T-antigen. Two G418-resistant colonies were isolated from this culture and expanded to continuously proliferating cell lines (PX-1 and PX-2). Line PX-2 was treated with up to 1.0 mg/ml of N-ethyl-N-nitrosourea (ENU), assayed for survival by cloning efficiency after overnight culture, and assayed for am3 mutations after 5 days of culture. Survival decreased to 31% at the highest dose of ENU, while mutant frequency increased with dose from approximately 2 x 10-7 in the untreated cells to 13 x 10-7 in cultures treated with 0.6 mg/ml of ENU. PX-2 cells also were treated with 0 and 0.6 mg/ml of ENU and mutant frequency assays were performed after 5, 24, 48 and 72 h of growth. The mutant frequency in the treated culture increased to 20 x 10-7 at 48 h and remained approximately the same at 72 h. These results indicate that PX-2 cells should be a useful resource for developing the in vivo am3 mutant assay and for evaluating the sensitivity of the am3 allele to various classes of mutagens.

Furitsu, K., H. Ryo, et al. (2005). "Microsatellite mutations show no increases in the children of the Chernobyl liquidators." Mutation Research/Genetic Toxicology and Environmental Mutagenesis **581**(1-2): 69.

http://www.sciencedirect.com/science/article/B6T2D-4F3FF7P-2/2/a20da8250e65071616364173fde26981

We performed a study on Belarusian "liquidators", exploring whether increase in the frequencies of germline mutations at microsatellite loci could be found in their progeny. The liquidators, mostly young males, were those involved (during 1986 and 1987) in clean-up operations in the radioactively contaminated area following the Chernobyl nuclear power plant accident in 1986. Many liquidators fathered children during the clean-up period and after the work had been terminated. The numbers of families studied were 64 (liquidators) and 66 (controls). A total of 72 loci (31 autosomal, one X-linked and 40 Y-linked) were used. DNA was isolated from peripheral blood lymphocytes and the microsatellite loci were amplified by the polymerase chain reaction with fluorescence-labelled primers. Mutations were detected as variations in the length of the loci.At the Y-linked loci, the mutation rates (expressed as number of mutations among the total number of loci for the individuals included) are 2.9 X 10-3 (4/1392) and 2.1 X 10-3 (3/1458) in the children of exposed and control parents, respectively. This difference is not statistically significant. At the autosomal loci, the corresponding estimates are 5.9 X 10-3 (11/1862; exposed group) and 8.5 X 10-3 (18/2108; control). Again, the difference is not significant. The possibility that the Belarusian population might have been unexpectedly exposed to some chemical contaminants in the environment appears unlikely in view of the finding that the spontaneous mutation rates at the same set of loci in several non-Belarusian populations were similar to those in Belarus. The estimated mean radiation dose to the liquidators was small, being about 39 mSv, and this might be one reason why no increases in mutation rates due to radiation could be found.

Fuscoe, J. C., G. W. Knapp, et al. (1998). "The frequency of illegitimate V(D)J recombinase-mediated mutations in children treated with etoposide-containing antileukemic therapy." Mutation Research/Genetic Toxicology and Environmental Mutagenesis **419**(1-3): 107.

http://www.sciencedirect.com/science/article/B6T2D-3V4J6JF-C/2/3b0c7dc9c94c477b26dd88c6a3a7dac2

Etoposide is among the most widely used anti-cancer drugs. Its use, however, has been associated with increased risk of secondary acute myeloid leukemia (AML) which is characterized by chromosomal translocations suggesting involvement of recombination-associated motifs at the breakpoints. A PCR-based assay was developed to quantitate the frequency of two illegitimate V(D)J recombinase-mediated genomic rearrangements--a 20-kb deletion in the hprt gene and the bcl2/IgH translocation (t(14;18)) found in non-Hodgkin's lymphoma. We examined both lymphocyte and non-lymphocyte blood cell DNA of children with acute lymphoblastic leukemia (ALL) for changes in the frequencies of these biomarkers during etoposide therapy to determine the level of illegitimate V(D)J recombination changes during therapy. A low level of t(14;18) was found in the lymphocytes before etoposide treatment, which was significantly reduced during etoposide therapy. In before-etoposide samples, no t(14;18) were found among 7.72 x 107 non-lymphocytes; during treatment none were found among 1.87 x 108 non-lymphocytes. Deletions were not found before etoposide treatment in either the lymphocytes (6.67 x 107) or non-lymphocytes (5.43 x 107) and were non-significantly elevated during etoposide therapy (1 in 1.4 x 108 lymphocytes and 1 in 1.39 x 108 non-lymphocytes). It is interesting to note the one patient with an hprt deletion mutation in non-lymphocytes; V(D)J recombination is not normally found in this cell type, but is the cell type from which AML derives. Several patients had clones of t(14;18)-bearing cells as determined by DNA sequence analysis. These results suggest that this

etoposide-based chemotherapy was ineffective in producing genomic rearrangements mediated by illegitimate V(D)J recombination in these patients.

Harrington-Brock, K., D. D. Collard, et al. (2003). "Bromate induces loss of heterozygosity in the Thymidine kinase gene of L5178Y/Tk+/--3.7.2C mouse lymphoma cells." Mutation Research/Genetic Toxicology and Environmental Mutagenesis **537**(1): 21.

http://www.sciencedirect.com/science/article/B6T2D-48BM66N-1/2/c5423a539d8917faa72bdf37549e8e5e

Potassium bromate (KBrO3) induces DNA damage and tumors in mice and rats, but is a relatively weak mutagen in microbial assays and the in vitro mammalian Hprt assay. Concern that there may be a human health risk associated with bromate, a disinfectant by-product of ozonation, has accompanied the increasing use of ozonation as an alternative to chlorination for treatment of drinking water. In this study, we have evaluated the mutagenicity of KBrO3 and sodium bromate (NaBrO3) in the Tk gene of mouse lymphoma cells. In contrast to the weak mutagenic activity seen in the previous studies, bromate induced a mutant frequency of over 100 x 10-6 at 0.6 mM with minimal cytotoxicity (70-80% survival) and over 1300 x 10-6 at 3 mM (~10% survival). The increase in the Tk mutant frequency was primarily due to the induction of small colony of Tk mutants. Loss of heterozygosity (LOH) analysis of 384 mutants from control and 2.7 mM KBrO3-treated cells showed that almost all (99%) bromate-induced mutants resulted from LOH, whereas in the control cultures 77% of the Tk mutants were LOH. Our results suggest that bromate is a potent mutagen in the Tk gene of mouse lymphoma cells, and the mechanism of action primarily involves LOH. The ability of the mouse lymphoma assay to detect a wider array of mutational events than the microbial or V79 Hprt assays may account for the potent mutagenic response.

McKinzie, P. B. and B. L. Parsons (2002). "Detection of rare K-ras codon 12 mutations using allele-specific competitive blocker PCR." Mutation Research/Genetic Toxicology and Environmental Mutagenesis **517**(1-2): 209.

http://www.sciencedirect.com/science/article/B6T2D-45NGSX6-1/2/72c28f66445465d05bc80de191c6bac6

Allele-specific competitive blocker PCR (ACB-PCR) is a sensitive allele-specific amplification method in which preferential amplification of the mutant allele occurs by using a primer that has more mismatches to the wild-type allele than to the mutant allele (mutant-specific primer, MSP). Additionally, a non-extendable primer with more mismatches to the mutant allele than to the wild-type allele (blocker primer, BP) competes with the MSP for binding to the wild-type allele, thereby reducing background amplification from the wild-type allele. ACB-PCR primer design is largely dependent upon the basepair substitution being measured, making it unclear if this method is broadly applicable. In an earlier study, an H-ras codon 61 CAA->AAA mutation had been detected by ACB-PCR at a sensitivity of 10-5. In this study, ACB-PCR was applied to two human K-ras codon 12 mutations: GGT->GTT and GGT->GAT. The method was optimized by systematically altering the concentrations of Perfect Match PCR Enhancer, MSP, BP, and dNTPs. For each mutation, mutant fractions as low as 10-5 were detected, indicating that this assay can be used on a variety of base substitution mutations. In addition, the results suggest that the 3'-terminal mismatches between the MSP and wild-type allele may be used to predict the ACB-PCR conditions that will be appropriate for the detection of other base substitution mutations. The range of concentrations for each of these components is narrow, making this method relatively easy to apply to additional mutational targets.

Mollersen, L., R. Vikse, et al. (2004). "Adenomatous polyposis coli truncation mutations in 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP)-induced intestinal tumours of multiple intestinal neoplasia mice." Mutation Research/Genetic Toxicology and Environmental Mutagenesis **557**(1): 29.

http://www.sciencedirect.com/science/article/B6T2D-4B7YPB6-1/2/4dbb27ff0716869ef6f5221f1f4c8b3f

The heterocyclic amine 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) induces intestinal tumours in C57BL/6J-multiple intestinal neoplasia (Min)/+ mice. The main mechanism for PhIP-induced tumour induction in Min/+ mice is loss of the wild-type adenomatous polyposis coli (Apc) allele, i.e. loss of heterozygosity (LOH). In this study, single injections of either 10, 17.5 or 25 mg/kg PhIP on days 3-6 after birth all increased the mean number of small intestinal tumours two to three-fold, from 37.7 in controls to 124.8 in the PhIP-treated Min/+ mice. In total, we analysed 292 small intestinal tumours and 253 of these had LOH. The frequency of LOH in the Apc gene was 88, 93, 83 and 84% in tumours of 0, 10, 17.5 and 25 mg/kg PhIP-treated mice, respectively. Therefore, these lower doses of PhIP did not reduce the frequency of LOH, as found in our previous study with a single injection of 50 mg/kg PhIP (Mutat. Res. 1-2 (2002) 157). In the second part of this study, we wanted to characterise Apc truncation mutations from tumour samples apparently retaining the Apc wild-type allele from this and two previous experiments with PhIP-exposed Min/+ mice. In the first half of exon 15 in Apc, we verified 25 mutations from 804 tumour samples of PhIP-treated mice. Of these were 60% G->T transversions, and 16% G deletions, indicating that these are the predominant types of PhIP-induced truncation mutations in the Apc gene in Min/+ mice. Most of the mutations were located between codon 989 and 1156 corresponding to the first part of the [beta]-catenin binding region. We also identified two Apc truncation mutations from 606 spontaneously formed intestinal tumours from untreated Min/+ mice, one C->T transition and one T insertion, which were different from those induced by PhIP.

Ryu, J.-C., J.-Y. Youn, et al. (1999). "Mutation spectrum of 4-nitroquinoline N-oxide in the lacI transgenic Big Blue(R) Rat2 cell line." Mutation Research/Genetic Toxicology and Environmental Mutagenesis **445**(1): 127.

http://www.sciencedirect.com/science/article/B6T2D-3XMGP4W-D/2/cbfa31dae84ebe5df75801eb78f059b1

This paper describes the spectrum of mutations induced by 4-nitroquinoline N-oxide (4-NQO) in the lacI target gene of the transgenic Big Blue(R) Rat2 cell line. There are only a few report for the mutational spectrum of 4-NQO in a mammalian system although its biological and genetic effects have been well studied. Big Blue(R) Rat2 cells were treated with 0.03125, 0.0625 or 0.125 [mu]g/ml of 4-NQO, the highest concentration giving 85% survival. Our results indicated that the mutant frequency (MF) induced by 4-NQO was dose-dependent with increases from three- to seven-fold. The DNA sequence analysis of lacI mutants from the control and 4-NQO treatment groups revealed an obvious difference in the spectra of mutations. In spontaneous mutants, transition (60%) mutations, especially G:C->A:T transition (45%), were most frequent. However, the major type of base substitution after treatment of 4-NQO was transversions (68.8%), especially G:C->T:A (43.8%), while only 25% of mutants were transitions. These results are consistent with those produced by 4-NQO in other systems and the transgenic assay system will be a powerful tool to postulate more accurately the mechanism of chemical carcinogenesis involved.

Teixeira, J. P., J. Gaspar, et al. (2002). "Aromatic DNA adduct levels in coke oven workers: correlation with polymorphisms in genes GSTP1, GSTM1, GSTT1 and CYP1A1." Mutation Research/Genetic Toxicology and Environmental Mutagenesis **517**(1-2): 147.

http://www.sciencedirect.com/science/article/B6T2D-45JYJTV-2/2/b66717cb9b7e22b72f08dbff71080293

The aim of this study was to use DNA adducts levels, detected by 32P-postlabelling, as a biomarker to assess human exposure to polycyclic aromatic hydrocarbons (PAHs) from a coke oven plant and explore the possible association between CYP1A1 MspI, GSTP1, GSTM1 and GSTT1 genotypes, and smoking status on bulky DNA adduct formation. A large amount of inter-individual variation in adduct level was observed among workers with the same job and the same smoking habits. No significant differences were observed in DNA adduct levels between the coke oven workers and control group. Smokers in the control group had significantly higher DNA adducts than the non-smokers in the same group (35.13+/-21.11 versus 11.18+/-8.00, per 108 nucleotides, P=0.003). In this group, the correlation between the level of DNA adducts and the cigarettes smoked was strongly significant (r=0.70, P8 nucleotides, P=0.03). These results suggests that tobacco smoke could behave as a confounding factor for evaluation of DNA adducts arising from occupational exposure. The levels of DNA adducts in smokers not occupationally exposed to PAHs is dependent on the polymorphisms CYP1A1 MspI in the 3' non-coding region (49.04+/-22.18 versus 25.85+/-15.87, per 108 nucleotides, PGST genotypes studied.

Zhang, L., R. B. Hayes, et al. (2004). "Lack of increased genetic damage in 1,3-butadiene-exposed Chinese workers studied in relation to EPHX1 and GST genotypes." Mutation Research/Genetic Toxicology and Environmental Mutagenesis **558**(1-2): 63.

http://www.sciencedirect.com/science/article/B6T2D-4BG45X0-1/2/f0ed3b1cc57d8ec4a2aa3fb870da4b5e

1,3-Butadiene (BD) is an important industrial chemical and pollutant. Its ability to induce genetic damage and cause hematological malignancies in humans is controversial. We have examined chromosome damage by fluorescence in situ hybridization (FISH) and mutations in the HPRT gene in the blood of Chinese workers exposed to BD. Peripheral blood samples were collected and cultured from 39 workers exposed to BD (median level 2 ppm, 6 h time-weighted average) and 38 matched controls in Yanshan, China. No difference in the level of aneuploidy or structural changes in chromosomes 1, 7, 8, and 12 was detected in metaphase cells from exposed subjects in comparison with matched controls, nor was there an increase in the frequency of HPRT mutations in the BD-exposed workers. Because genetic polymorphisms in glutathione S-transferase (GST) enzymes and microsomal epoxide hydrolase (EPHX1) may affect the genotoxic effects of BD and its metabolites, we also related chromosome alterations and gene mutations to GSTT1, GSTM1 and EPHX1 genotypes. Overall, there was no effect of variants in these genotypes on numerical or structural changes in chromosomes 1, 7, 8 and 12 or on HPRT mutant frequency in relation to BD exposure, but the GST genotypes did influence background levels of both hyperdiploidy and HPRT mutant frequency. In conclusion, our data show no increase in chromosomal aberrations or HPRT mutations among workers exposed to BD, even in potentially susceptible genetic subgroups. The study is, however, quite small and the levels of BD exposure are not extremely high, but our findings in China do support those from a similar study conducted in the Czech Republic. Together, these studies suggest that low levels of occupational BD exposure do not pose a significant risk of genetic damage.

Zhang, Y., D. G. Monckton, et al. (2002). "Detection of radiation and cyclophosphamide-induced

mutations in individual mouse sperm at a human expanded trinucleotide repeat locus transgene." Mutation Research/Genetic Toxicology and Environmental Mutagenesis **516**(1-2): 121.

http://www.sciencedirect.com/science/article/B6T2D-45H93HP-1/2/ac9e1b1989d6a6d159aa45fe0791717b

A method to measure the germline mutations induced by cancer treatment in humans is needed. To establish such a method we used a transgenic mouse model consisting of a human DNA repeat locus that has a high spontaneous mutation frequency as a biomarker. Alterations in repeat number were measured in individual sperm from mice hemizygous for an expanded (CTG)162 human myotonic dystrophy type 1 (DM1) microsatellite repeat using single genome-equivalent (g.e.) PCR and detection by a DNA fragment analyzer. Mutation frequencies were measured in DNA from sperm from controls and sperm derived from stem spermatogonia, differentiating spermatogonia, and spermatocytes exposed to radiation and from spermatocytes of mice treated with cyclophosphamide. There was no increase above control levels in mutations, scored as >1 repeat changes, in any of the treated groups. However, moderately large deletion mutants (between 9 and 20 repeat changes) were observed at frequencies of 2.2% when spermatocytes were treated with cyclophosphamide and, 1.8 and 2.5% when spermatocytes and stem cells, respectively, were treated with radiation, which were significantly higher than the frequency of 0.3% in controls. Thus, radiation and cyclophosphamide induced deletions in the expanded DM1 trinucleotide repeat. PCR artifacts were characterized in sperm DNA from controls and from mice treated with radiation; all artifacts involved losses of more than 20 DM1 repeats, and surprisingly the artifact frequency was higher in treated sperm than in control sperm. The radiation-induced increase in the frequency of PCR artifacts might reflect alterations in sperm DNA that destabilize the genome not only during PCR amplification but also during early embryonic development.

Geoderma (1)

Johnson, M. J., K. Y. Lee, et al. (2003). "DNA fingerprinting reveals links among agricultural crops, soil properties, and the composition of soil microbial communities." Geoderma **114**(3-4): 279.

http://www.sciencedirect.com/science/article/B6V67-47YPP75-3/2/dc591b0e0e66d99656adb82f9ab2447c

Rapid methods for characterizing soil microbial communities are essential to assess responses to perturbations and to improved management practices. This study compared the composition of microbial communities in 47 agricultural soil and adjacent land use samples collected in the San Joaquin Valley, CA. Microbial communities were characterized by DNA fingerprinting of the Intergenic Transcribed Spacer (ITS) region, using primers universal for bacteria or eucarya. Bacterial DNA fingerprints were more complex (containing 25-30 bands) than were eucaryotic fingerprints (8-15 bands). Field replicates from within an agricultural field were more similar to one another than samples collected in different fields under the same crop type or in close proximity to one another. Microbial communities in almond, grape, and tomato soils across different locations were more similar to one another than communities in cotton and safflower soils. Bacterial DNA fingerprints were significantly correlated with soil electrical conductivity, soil texture, inorganic carbon, and nitrogen content but not with pH and organic carbon content. The grouping of soil samples based on their soil reflectance properties was similar to the grouping based on the bacterial ITS analysis. Despite similarities among communities under some crops

and at some locations, there is tremendous unexplained diversity within agricultural soil microbial communities. More extensive sampling is needed to better understand the driving forces underlying microbial community composition.

Glycobiology (2)

Ahmed, H., S.-J. Du, et al. (2004). "Biochemical and molecular characterization of galectins from zebrafish (Danio rerio): notochord-specific expression of a prototype galectin during early embryogenesis." Glycobiology **14**(3): 219-232.

http://glycob.oupjournals.org/cgi/content/abstract/14/3/219

Galectins are a family of {beta}-galactoside-binding lectins that on synthesis are either translocated into the nucleus or released to the extracellular space. Their developmentally regulated expression, extracellular location, and affinity for extracellular components (such as laminin and fibronectin) suggest a role in embryonic development, but so far this has not been unequivocally established. Zebrafish constitute an ideal model for developmental studies because of their external fertilization, transparent embryos, rapid growth, and availability of a large collection of mutants. As a first step in addressing the biological roles in zebrafish embryogenesis, we identified and characterized members of the three galectin types: three protogalectins (Drgal1-L1, Drgal1-L2, Drgal1-L3), one chimera galectin (Drgal3), and one tandem-repeat galectin (Drgal9-L1). Like mammalian prototype galectin-1, Drgal1-L2 preferentially binds to N-acetyllactosamine. Genomic structure of Drgal1-L2 revealed four exons, with the exon-intron boundaries conserved with the mammalian galectin-1. Interestingly, this gene also encodes an alternatively spliced form of Drgal1-L2 that lacks eight amino acids near the carbohydrate-binding domain. Zebrafish galectins exhibited distinct patterns of temporal expression during embryo development. Drgal1-L2 is expressed postbud stage, and its expression is strikingly specific to the notochord. In contrast, Drgal1-L1 is expressed maternally in the oocytes. Drgal1-L3, Drgal3, and Drgal9-L1 are expressed both maternally and zygotically, ubiquitously in the adult tissues. The distinct temporal and spatial patterns of expression of members of the zebrafish galectin repertoire suggest that each may play distinct biological roles during early embryogenesis.

Kakiuchi, M., N. Okino, et al. (2002). "Purification, characterization, and cDNA cloning of {alpha}-N-acetylgalactosamine-specific lectin from starfish, Asterina pectinifera." Glycobiology **12**(2): 85-94.

http://glycob.oupjournals.org/cgi/content/abstract/12/2/85

We report here the purification, characterization, and cDNA cloning of a novel N-acetylgalactosamine-specific lectin from starfish, Asterina pectinifera. The purified lectin showed 19-kDa, 41-kDa, and 60-kDa protein bands on SDS-PAGE, possibly corresponding to a monomer, homodimer, and homotrimer. Interestingly, on 4-20% native PAGE the lectin showed at least nine protein bands, among which oligomers containing six to nine subunits had potent hemagglutination activity for sheep erythrocytes. The hemagglutination activity of the lectin was specifically inhibited by N-acetylgalactosamine, Tn antigen, and blood group A trisaccharide, but not by N-acetylglucosamine, galactose, galactosamine, or blood group B trisaccharide. The specificity of the lectin was further examined using various glycosphingolipids and biotin-labeled

lectin. The lectin was found to bind to Gb5Cer, but not Gb4Cer, Gb3Cer, GM1a, GM2, or asialo-GM2, indicating that the lectin specifically binds to the terminal {alpha}-GalNAc at the nonreducing end. The hemagglutination activity of the lectin was completely abolished by chelation with EDTA or EGTA and completely restored by the addition of CaCl2. cDNA cloning of the lectin showed that the protein is composed of 168 amino acids, including a signal sequence of 18 residues, and possesses the typical C-type lectin motif. These findings indicate that the protein is a C-type lectin. The recombinant lectin, produced in a soluble form by Escherichia coli, showed binding activity for asialomucin in the presence of Ca2+ but no hemagglutination.

Immuno-analyse & Biologie Specialisee (1)

Bessieres, M. H., S. Cassaing, et al. (2002). "Apport des techniques de biologie moleculaire dans le diagnostic prenatal de la toxoplasmose congenitale: Value of polymerase chain reaction test on amniotic fluid for the prenatal diagnosis of congenital toxoplasmosis." Immuno-analyse & Biologie Specialisee **17**(6): 358.

http://www.sciencedirect.com/science/article/B6VM6-47CMYCH-2/2/c4229b276798ccf9fdecb2b782425ccb

ResumeLe propos de cette etude est de rapporter les performances des techniques d'amplification genique (test PCR) sur le liquide amniotique pour le diagnostic prenatal de la toxoplasmose congenitale. Une amniocentese a ete realisee chez 261 patientes atteintes de toxoplasmose pendant leur grossesse. La recherche du toxoplasme dans le liquide amniotique a ete effectuee par PCR et inoculation a la souris. Parmi les 60 cas de toxoplasmose congenitale, 56 (21 %) ont ete depistes par ce diagnostic. La sensibilite de la PCR (90 %) est superieure a celle de l'inoculation a la souris (70 %). Les deux methodes ont une specificite et une valeur predictive positive de 100 %. La valeur predictive negative est de 94 % pour la PCR et 83 % pour l'inoculation. Tous les cas diagnostiques par le bilan prenatal ont ete confirmes par le suivi serologique de l'enfant. Le test PCR pratique sur le liquide amniotique est une methode simple, rapide et d'un grand apport pour le diagnostic prenatal d'une toxoplasmose congenitale. Il permet d'instituer un diagnostic et un traitement precoce des foetus infectes. Cet examen associe aux echographies limite le nombre d'interruptions de grossesse.

Industrial Crops and Products(1)

Kaufman, B., S. Richards, et al. (1999). "DNA isolation method for high polysaccharide Lesquerella species." Industrial Crops and Products **9**(2): 111.

http://www.sciencedirect.com/science/article/B6T77-3V8CHK5-4/2/f4dca4097b73657b0251a4473dc7e349

A prerequisite for molecular genetic studies is the ability to isolate DNA. In species of the genus Lesquerella, high polysaccharide content makes this basic requirement difficult to achieve. The

carbohydrates and the nucleic acids coprecipitate during sample preparation and form large pellets where the DNA is trapped in gum and is no longer retrievable. A DNA isolation method was developed to solve this problem. After breaking down the cell walls, the cell nuclei are separated from the cytoplasmatic and intracellular fluids by centrifugation in a viscous medium. This preparatory step separates the nuclei that contain the desired DNA from other cellular compartments containing the problem-causing carbohydrates. The DNA is then isolated from the nuclei without interference. High quality DNA was obtained and used successfully for restriction endonuclease digestion and polymerase chain reaction amplifications.

Infect. Immun. (65)

Altet, L., O. Francino, et al. (2002). "Mapping and Sequencing of the Canine NRAMP1 Gene and Identification of Mutations in Leishmaniasis-Susceptible Dogs." Infect. Immun. **70**(6): 2763-2771.

http://iai.asm.org/cgi/content/abstract/70/6/2763

The NRAMP1 gene (Slc11a1) encodes an ion transporter protein involved in the control of intraphagosomal replication of parasites and in macrophage activation. It has been described in mice as the determinant of natural resistance or susceptibility to infection with antigenically unrelated pathogens, including Leishmania. Our aims were to sequence and map the canine Slc11a1 gene and to identify mutations that may be associated with resistance or susceptibility to Leishmania infection. The canine Slc11a1 gene has been mapped to dog chromosome CFA37 and covers 9 kb, including a 700-bp promoter region, 15 exons, and a polymorphic microsatellite in intron 1. It encodes a 547-amino-acid protein that has over 87% identity with the Slc11a1 proteins of different mammalian species. A case-control study with 33 resistant and 84 susceptible dogs showed an association between allele 145 of the microsatellite and susceptible dogs. Sequence variant analysis was performed by direct sequencing of the cDNA and the promoter region of four unrelated beagles experimentally infected with Leishmania infantum to search for possible functional mutations. Two of the dogs were classified as susceptible and the other two were classified as resistant based on their immune responses. Two important mutations were found in susceptible dogs: a G-rich region in the promoter that was common to both animals and a complete deletion of exon 11, which encodes the consensus transport motif of the protein, in the unique susceptible dog that needed an additional and prolonged treatment to avoid continuous relapses. A study with a larger dog population would be required to prove the association of these sequence variants with disease susceptibility.

Autret, N., C. Raynaud, et al. (2003). "Identification of the agr Locus of Listeria monocytogenes: Role in Bacterial Virulence." Infect. Immun. **71**(8): 4463-4471.

http://iai.asm.org/cgi/content/abstract/71/8/4463

Listeria monocytogenes is a gram-positive facultative intracellular food-borne pathogen that can cause severe infections in humans and animals. We have recently adapted signature-tagged transposon mutagenesis (STM) to identify genes involved in the virulence of L. monocytogenes. A new round of STM allowed us to identify a new locus encoding a protein homologous to AgrA, the well-studied response regulator of Staphylococcus aureus and part of a two-component system involved in bacterial virulence. The production of several secreted proteins was modified

in the agrA mutant of L. monocytogenes grown in broth, indicating that the agr locus influenced protein secretion. Inactivation of agrA did not affect the ability of the pathogen to invade and multiply in cells in vitro. However, the virulence of the agrA mutant was attenuated in the mouse (a 10-fold increase in the 50% lethal dose by the intravenous route), demonstrating for the first time a role for the agr locus in the virulence of L. monocytogenes.

Baranova, I., T. Vishnyakova, et al. (2002). "Lipopolysaccharide Down Regulates Both Scavenger Receptor B1 and ATP Binding Cassette Transporter A1 in RAW Cells." Infect. Immun. **70**(6): 2995-3003.

http://iai.asm.org/cgi/content/abstract/70/6/2995

Lipopolysaccharide (LPS) has recently been shown to facilitate macrophage foam cell formation and has been suggested to be a proatherogenic factor. The mechanism of LPS induced cholesterol accumulation, however, is unclear. In this report, using the macrophage-like RAW 264.7 cell line, we provide experimental evidence that LPS's proatherogenic effects may at least in part reflect altered cholesterol metabolism. Data presented demonstrate that in a dose-dependent manner, LPS is able to down regulate the mRNA expression of the two primary high-density lipoprotein (HDL) receptors, scavenger receptor B1 (SR-B1) and ATP binding cassette A1 (ABCA1), with a 50% inhibitory concentration of less than 0.2 ng/ml, as well as to decrease SR-B1 protein expression by 80%. We also found that LPS treatment resulted in a significant decrease (to 20% of the control level) of the specific 125I-HDL binding as well as in 50% inhibition of the HDL-mediated cholesterol efflux compared to untreated cells. In addition, we compared the potencies of various modified LPS preparations and demonstrated that the phosphorylated lipid A portion of LPS, which is highly conserved among gram-negative microorganisms, including Chlamydia, is primarily responsible for the effects of LPS on SR-B1 and ABCA1 expression. Inhibitors of NF-{kappa}B activation were observed to efficiently block the suppressive effect of LPS on SR-B1 and ABCA1, suggesting a mechanism involving NF-{kappa}B. These data indicate that the LPS effects on cholesterol metabolism may contribute to the proatherogenic properties of LPS.

Behrens, M., J. Sheikh, et al. (2002). "Regulation of the Overlapping pic/set Locus in Shigella flexneri and Enteroaggregative Escherichia coli." Infect. Immun. **70**(6): 2915-2925.

http://iai.asm.org/cgi/content/abstract/70/6/2915

Most strains of Shigella flexneri 2a and enteroaggregative Escherichia coli carry a highly conserved chromosomal locus which encodes a 109-kDa secreted mucinase (called Pic) and, on the opposite strand in overlapping fashion, an oligomeric enterotoxin called ShET1, encoded by the setA and setB genes. Here, we characterize the genetic regulation of these overlapping genes. Our data suggest that pic and the setBA loci are transcribed as complementary 4-kb mRNA species. The major pic promoter is maximally activated at 37{degrees}C in exponential growth phase. Our data suggest that the setB gene is transcribed from a promoter which lies more than 1.5 kb upstream of the setB structural gene; setA may be transcribed via readthrough of the setB transcript and possibly by its own promoter. The long leader of the setB gene provides a strong silencing effect on setB transcription. The signals which provide relief from setB silencing are not clear, but significant induction is observed in a continuous anaerobic culture of human fecal bacteria, suggesting that some complex characteristics of the human intestine act to lift repression of setB expression. Our studies provide the first insights into the mechanisms affecting expression of this unusual virulence locus.

Bennett, J. S., D. T. Griffiths, et al. (2005). "Genetic Diversity and Carriage Dynamics of Neisseria lactamica in Infants." Infect. Immun. **73**(4): 2424-2432.

http://iai.asm.org/cgi/content/abstract/73/4/2424

Neisseria lactamica, a harmless human commensal found predominantly in the upper respiratory tracts of infants, is closely related to Neisseria meningitidis, a pathogen of global significance. Colonization with N. lactamica may be responsible for the increase in immunity to meningococcal disease that occurs during childhood, when rates of meningococcal carriage are low. This observation has led to the suggestion that N. lactamica whole cells or components are potential constituents of novel meningococcal vaccines. However, the dynamics of carriage and population diversity of N. lactamica in children are poorly understood, presenting difficulties for the choice of representative isolates for use in vaccine development. This problem was addressed by the multilocus sequence typing of N. lactamica isolates from two longitudinal studies of bacterial carriage in infants. The studies comprised 100 and 216 subjects, with N. lactamica carriage monitored from age 4 weeks until age 96 weeks and from age 2 weeks until age 24 weeks, respectively. The maximum observed carriage rate was 44% at 56 weeks of age, with isolates obtained on multiple visits for the majority (54 of 75, 72%) of carriers. The N. lactamica isolates were genetically diverse, with 69 distinct genotypes recovered from the 75 infants. Carriage was generally long-lived, with an average rate of loss of under 1% per week during the 28 weeks following acquisition. Only 11 of the 75 infants carried more than one genotypically unique isolate during the course of the study. Some participants shared identical isolates with siblings, but none shared identical isolates with their parents. These findings have implications for the design of vaccines based on this organism.

Berberov, E. M., Y. Zhou, et al. (2004). "Relative Importance of Heat-Labile Enterotoxin in the Causation of Severe Diarrheal Disease in the Gnotobiotic Piglet Model by a Strain of Enterotoxigenic Escherichia coli That Produces Multiple Enterotoxins." Infect. Immun. **72**(7): 3914-3924.

http://iai.asm.org/cgi/content/abstract/72/7/3914

Enterotoxigenic Escherichia coli (ETEC) strains that produce multiple enterotoxins are important causes of severe dehydrating diarrhea in human beings and animals, but the relative importance of these enterotoxins in the pathogenesis is poorly understood. Gnotobiotic piglets were used to study the importance of heat-labile enterotoxin (LT) in infection with an ETEC strain that produces multiple enterotoxins. LT- ({Delta}eltAB) and complemented mutants of an F4+ LT+ STb+ EAST1+ ETEC strain were constructed, and the virulence of these strains was compared in gnotobiotic piglets expressing receptors for F4+ fimbria. Sixty percent of the piglets inoculated with the LT- mutant developed severe dehydrating diarrhea and septicemia compared to 100% of those inoculated with the nalidixic acid-resistant (Nalr) parent and 100% of those inoculated with the complemented mutant strain. Compared to piglets inoculated with the Nalr parent, the mean rate of weight loss (percent per hour) of those inoculated with the LT- mutant was 67% lower ($P < 0.05$) and that of those inoculated with the complemented strain was 36% higher ($P < 0.001$). Similarly, piglets inoculated with the LT- mutant had significant reductions in the mean bacterial colony count (CFU per gram) in the ileum; bacterial colonization scores (square millimeters) in the jejunum and ileum; and clinical pathology parameters of dehydration, electrolyte imbalance, and metabolic acidosis ($P < 0.05$). These results indicate the significance of LT to the development of severe dehydrating diarrhea and postdiarrheal septicemia in ETEC infections of swine and demonstrate that EAST1, LT, and STb may be concurrently expressed by porcine ETEC strains.

Boekema, B. K. H. L., J. P. M. Van Putten, et al. (2004). "Host Cell Contact-Induced Transcription of the Type IV Fimbria Gene Cluster of Actinobacillus pleuropneumoniae." Infect. Immun. **72**(2): 691-700.

http://iai.asm.org/cgi/content/abstract/72/2/691

Type IV pili (Tfp) of gram-negative species share many characteristics, including a common architecture and conserved biogenesis pathway. Much less is known about the regulation of Tfp expression in response to changing environmental conditions. We investigated the diversity of Tfp regulatory systems by searching for the molecular basis of the reported variable expression of the Tfp gene cluster of the pathogen Actinobacillus pleuropneumoniae. Despite the presence of an intact Tfp gene cluster consisting of four genes, apfABCD, no Tfp were formed under standard growth conditions. Sequence analysis of the predicted major subunit protein ApfA showed an atypical alanine residue at position -1 from the prepilin peptidase cleavage site in 42 strains. This alanine deviates from the consensus glycine at this position in Tfp from other species. Yet, cloning of the apfABCD genes under a constitutive promoter in A. pleuropneumoniae resulted in pilin and Tfp assembly. Tfp promoter-luxAB reporter gene fusions demonstrated that the Tfp promoter was intact but tightly regulated. Promoter activity varied with bacterial growth phase and was detected only when bacteria were grown in chemically defined medium. Infection experiments with cultured epithelial cells demonstrated that Tfp promoter activity was upregulated upon adherence of the pathogen to primary cultures of lung epithelial cells. Nonadherent bacteria in the culture supernatant exhibited virtually no promoter activity. A similar upregulation of Tfp promoter activity was observed in vivo during experimental infection of pigs. The host cell contact-induced and in vivo-upregulated Tfp promoter activity in A. pleuropneumoniae adds a new dimension to the diversity of Tfp regulation.

Cadavid, D., Y. Bai, et al. (2003). "Infection and Inflammation in Skeletal Muscle from Nonhuman Primates Infected with Different Genospecies of the Lyme Disease Spirochete Borrelia burgdorferi." Infect. Immun. **71**(12): 7087-7098.

http://iai.asm.org/cgi/content/abstract/71/12/7087

Lyme borreliosis is a multisystemic disease caused by various genospecies of the spirochete Borrelia burgdorferi. To investigate muscle involvement in the nonhuman primate (NHP) model of Lyme disease, 16 adult Macaca mulatta animals inoculated with strain N40 of B. burgdorferi sensu strictu by syringe or by tick bite or with strain Pbi of B. burgdorferi genospecies garinii by syringe were studied. Animals were necropsied while immunosuppressed on day 50 (two animals each inoculated with B. burgdorferi N40 by syringe and with B. garinii Pbi by syringe) or on day 90, 40 days after immunosuppression had been discontinued (four animals each inoculated with strain N40 by syringe, with strain N40 by tick bite, and with strain Pbi by syringe). Skeletal muscles removed at necropsy were studied by (i) microscopic examination of hematoxylin-eosin-stained sections for inflammation and tissue injury; (ii) immunohistochemical and digital image analyses for antibody and complement deposition and cellular inflammation; (iii) Western blot densitometry for the presence of antibodies; and (iv) reverse transcription-PCR for measurement of the spirochetal load or C1q (the first component of the complement cascade) synthesis. The results showed that N40 was more infectious for NHPs than Pbi. NHPs inoculated with N40 but not with Pbi developed myositis. The inflammation in skeletal muscle was more severe in NHPs inoculated with N40 by syringe than in those inoculated by tick bite. The predominant cells in the inflammatory infiltrate were T cells and plasma cells. The deposition of antibody and complement in inflamed muscles from N40-inoculated NHPs was significantly higher than that in Pbi-inoculated NHPs. The spirochetal load was very high in the two N40-inoculated NHPs examined while they were immunosuppressed but decreased to minimal levels in the NHPs when immunocompetence was restored. We conclude that myositis can be a prominent feature of Lyme borreliosis depending on the infecting organism and host immune status.

Casciotti, L., K. H. Ely, et al. (2002). "CD8+-T-Cell Immunity against Toxoplasma gondii Can Be Induced but Not Maintained in Mice Lacking Conventional CD4+ T Cells." Infect. Immun. **70**(2): 434-443.

http://iai.asm.org/cgi/content/abstract/70/2/434

T-cell immunity is critical for survival of hosts infected with Toxoplasma gondii. Among the cells in the T-cell population, CD8+ T cells are considered the major effector cells against this parasite. It is believed that CD4+ T cells may be crucial for induction of the CD8+-T-cell response against T. gondii. In the present study, CD4-/- mice were used to evaluate the role of conventional CD4+ T cells in the immune response against T. gondii infection. CD4-/- mice infected with T. gondii exhibited lower gamma interferon (IFN-{gamma}) messages in the majority of their tissues. As a result, mortality due to a hyperinflammatory response was prevented in these animals. Interestingly, T. gondii infection induced a normal antigen-specific CD8+-T-cell immune response in CD4-/- mice. No difference in generation of precursor cytotoxic T lymphocytes (pCTL) or in IFN-{gamma} production by the CD8+-T-cell populations from the knockout and wild-type animals was observed. However, the mutant mice were not able to sustain CD8+-T-cell immunity. At 180 days after infection, the CD8+-T-cell response in the knockout mice was depressed, as determined by pCTL and IFN-{gamma} assays. Loss of CD8+-T-cell immunity at this time was confirmed by adoptive transfer experiments. Purified CD8+ T cells from CD4-/- donors that had been immunized 180 days earlier failed to protect the recipient mice against a lethal infection. Our study demonstrated that although CD8+-T-cell immunity can be induced in the absence of conventional CD4+ T cells, it cannot be maintained without such cells.

Chu, D., R. D. Bungiro, et al. (2004). "Molecular Characterization of Ancylostoma ceylanicum Kunitz-Type Serine Protease Inhibitor: Evidence for a Role in Hookworm-Associated Growth Delay." Infect. Immun. **72**(4): 2214-2221.

http://iai.asm.org/cgi/content/abstract/72/4/2214

Hookworm infection is a major cause of iron deficiency anemia and malnutrition in developing countries. The Ancylostoma ceylanicum Kunitz-type inhibitor (AceKI) is a 7.9-kDa broad-spectrum inhibitor of trypsin, chymotrypsin, and pancreatic elastase that has previously been isolated from adult hookworms. Site-directed mutagenesis of the predicted P1 inhibitory reactive site amino acid confirmed the role of Met26 in mediating inhibition of the three target serine proteases. By using reverse transcription-PCR, it was demonstrated that the level of AceKI gene expression increased following activation of third-stage larvae with serum and that the highest level of expression was reached in the adult stage of the parasite. Immunohistochemistry studies performed with polyclonal immunoglobulin G raised against recombinant AceKI showed that the inhibitor localized to the subcuticle of the adult hookworm, suggesting that it has a potential in vivo role in neutralizing intestinal proteases at the surface of the parasite. Immunization with recombinant AceKI was shown to confer partial protection against hookworm-associated growth delay without a measurable effect on anemia. Taken together, the data suggest that AceKI plays a role in the pathogenesis of hookworm-associated malnutrition and growth delay, perhaps through inhibition of nutrient absorption in infected hosts.

Collyn, F., M.-A. Lety, et al. (2002). "Yersinia pseudotuberculosis Harbors a Type IV Pilus Gene Cluster That Contributes to Pathogenicity." Infect. Immun. **70**(11): 6196-6205.

http://iai.asm.org/cgi/content/abstract/70/11/6196

Fimbriae have been shown to play an essential role in the adhesion of pathogenic gram-negative bacteria to host cells. In the enteroinvasive bacterium Yersinia pseudotuberculosis, we characterized a previously unknown 11-kb chromosomal locus involved in the synthesis of type IV pili. The locus consists of 11 open reading frames forming a polycistronic unit and encoding putative Pil proteins, PilLMNOPQRSUVW. When introduced into Escherichia coli, the Y. pseudotuberculosis operon reconstituted bundles of filaments at a pole on the bacterial surface, demonstrating that the pil locus was functional in a heterogenous genetic background. Environmental factors regulated transcription of the Y. pseudotuberculosis operon; in particular, temperature, osmolarity, and oxygen tension were critical cues. Deletion of the type IV pilus gene cluster was associated with a reduction of Y. pseudotuberculosis pathogenicity for mice infected orally. Forty-one percent of Y. pseudotuberculosis strains isolated from human or animal sources harbored the type IV pilus locus. Therefore, the pil locus of Y. pseudotuberculosis might constitute an "adaptation island," permitting the microorganism to colonize a vast reservoir.

Crother, T. R., C. I. Champion, et al. (2003). "Antigenic Composition of Borrelia burgdorferi during Infection of SCID Mice." Infect. Immun. **71**(6): 3419-3428.

http://iai.asm.org/cgi/content/abstract/71/6/3419

The general concept that during infection of mice the Borrelia burgdorferi surface protein composition differs profoundly from that of tick-borne or in vitro-cultivated spirochetes is well established. Specific knowledge concerning the differences is limited because the small numbers of spirochetes present in tissue have not been amenable to direct compositional analysis. In this report we describe novel means for studying the antigenic composition of host-adapted Borrelia (HAB). The detergent Triton X-114 was used to extract the detergent-phase HAB proteins from mouse ears, ankles, knees, and hearts. Immunoblot analysis revealed a profile distinct from that of in vitro-cultivated Borrelia (IVCB). OspA and OspB were not found in the tissues of SCID mice 17 days after infection. The amounts of antigenic variation protein VlsE and the relative amounts of its transcripts were markedly increased in ear, ankle, and knee tissues but not in heart tissue. VlsE existed as isoforms having both different unit sizes and discrete lower molecular masses. The hydrophobic smaller forms of VlsE were also found in IVCB. The amounts of the surface protein (OspC) and the decorin binding protein (DbpA) were increased in ear, ankle, knee, and heart tissues, as were the relative amounts of their transcripts. Along with these findings regarding VlsE, OspC, and DbpA, two-dimensional immunoblot analysis with immune sera also revealed additional details of the antigenic composition of HAB extracted from ear, heart, and joint tissues. A variety of novel antigens, including antigens with molecular masses of 65 and 30 kDa, were found to be upregulated in mouse tissues. Extraction of hydrophobic B. burgdorferi antigens from tissue provides a powerful tool for determining the antigenic composition of HAB.

De Gregorio, E., C. Abrescia, et al. (2003). "Asymmetrical Distribution of Neisseria Miniature Insertion Sequence DNA Repeats among Pathogenic and Nonpathogenic Neisseria Strains." Infect. Immun. **71**(7): 4217-4221.

http://iai.asm.org/cgi/content/abstract/71/7/4217

Neisseria miniature insertion sequences (nemis) are miniature DNA insertion sequences found in Neisseria species. Out of 57 elements closely flanking cellular genes analyzed by PCR, most were conserved in Neisseria meningitidis but not in N. lactamica strains. Since mRNAs spanning nemis are processed by RNase III at hairpins formed by element termini, gene sets could selectively be regulated in meningococci at the posttranscriptional level.

Deng, M., T. J. Templeton, et al. (2002). "Cryptosporidium parvum Genes Containing Thrombospondin Type 1 Domains." Infect. Immun. **70**(12): 6987-6995.

http://iai.asm.org/cgi/content/abstract/70/12/6987

Cryptosporidium parvum is recognized as an enteropathogen of great worldwide medical and veterinary importance, yet understanding of its pathogenesis has been hampered in part by limited knowledge of the invasion machinery of this parasite. Recently, genes containing thrombospondin type 1 (TSP1) domains have been identified in several genera of apicomplexans, including thrombospondin-related adhesive proteins (TRAPs) that have been implicated as key molecules for parasite motility and adhesion onto host cell surfaces. Previously, a large-scale random survey of the C. parvum genome conducted in our laboratory revealed the presence of multiple genomic DNA sequences with a high degree of similarity to known apicomplexan TRAP genes. In the present study, TBLASTN screening of available C. parvum genomic sequences by using TSP1 domains as queries identified a total of 12 genes possessing TSP1-like domains. All genes have putative signal peptide sequences, one or more TSP1-like domains, plus additional extracellular protein modules such as Kringle, epidermal growth factor, and Apple domains. Two genes, putative paralogs CpTSP8 and CpTSP9, contain predicted introns near their amino termini, which were verified by comparing PCR products from cDNA versus genomic DNA templates. Reverse transcription-PCR analysis of transcript levels reveals that C. parvum TSP genes were developmentally regulated with distinct patterns of expression during in vitro infection. TRAPC1, CpTSP3, and CpTSP11 were expressed at high levels during both early and late stages of infection, whereas CpTSP2, CpTSP5, CpTSP6, CpTSP8, and CpTSP9 were maximally expressed during the late stages of infection. Only CpTSP4 was highly expressed solely at an early stage of infection.

Dons, L., E. Eriksson, et al. (2004). "Role of Flagellin and the Two-Component CheA/CheY System of Listeria monocytogenes in Host Cell Invasion and Virulence." Infect. Immun. **72**(6): 3237-3244.

http://iai.asm.org/cgi/content/abstract/72/6/3237

The flagellum protein flagellin of Listeria monocytogenes is encoded by the flaA gene. Immediately downstream of flaA, two genes, cheY and cheA, encoding products with homology to chemotaxis proteins of other bacteria, are located. In this study we constructed deletion mutants with mutations in flaA. cheY, and cheA to elucidate their role in the biology of infection with L. monocytogenes. The {Delta}cheY, {Delta}cheA, and double-mutant {Delta}cheYA mutants, but not {Delta}flaA mutant, were motile in liquid media. However, the {Delta}cheA mutant had impaired swarming and the {Delta}cheY and {Delta}cheYA mutants were unable to swarm on soft agar plates, suggesting that cheY and cheA genes encode proteins involved in chemotaxis. The {Delta}flaA, {Delta}cheY, {Delta}cheA, and {Delta}cheYA mutants (grown at 24{degrees}C) showed reduced association with and invasion of Caco-2 cells compared to the wild-type strain. However, spleens from intragastrically infected BALB/c and C57BL/6 mice showed larger and similar numbers of the {Delta}flaA and {Delta}cheYA mutants, respectively, compared to the wild-type controls. Such a discrepancy could be explained by the fact that tumor necrosis factor receptor p55 deficient mice showed dramatically exacerbated susceptibility to the wild-type but unchanged or only slightly increased levels of the {Delta}flaA or {Delta}cheYA mutant. In summary, we show that listerial flaA. cheY, and cheA gene products facilitate the initial contact with epithelial cells and contribute to effective invasion but that flaA could also be involved in the triggering of immune responses.

Dubnau, E., P. Fontan, et al. (2002). "Mycobacterium tuberculosis Genes Induced during Infection of Human Macrophages." Infect. Immun. **70**(6): 2787-2795.

http://iai.asm.org/cgi/content/abstract/70/6/2787

We identified Mycobacterium tuberculosis genes preferentially expressed during infection of human macrophages using a promoter trap adapted for this pathogen. inhA encodes an enoyl-acyl carrier protein reductase that is required for mycolic acid biosynthesis (A. Quemard et al., Biochemistry 34:8235-8241, 1995) and is a major target for isoniazid (INH) in mycobacterial species (A. Banerjee et al., Science 263:227-230, 1994). Since overexpression of inhA confers INH resistance in Mycobacterium smegmatis (Banerjee et al., Science 263:227-230, 1994), we designed a promoter trap based on this gene. A library of clones, containing small fragments of M. tuberculosis DNA cloned upstream of inhA in a plasmid vector, was electroporated into M. tuberculosis, and the resulting culture was used to infect the human monocytic THP-1 cell line. Selection was made for clones surviving INH treatment during infection but retaining INH sensitivity on plates. The DNA upstream of inhA was sequenced in each clone to identify the promoter driving inhA expression. Thirteen genes identified by this method were analyzed by quantitative reverse transcription-PCR (R. Manganelli et al., Mol. Microbiol. 31:715-724, 1999), and eight of them were found to be differentially expressed from cultures grown in macrophages compared with broth-grown cultures. Several of these genes are presumed to be involved in fatty acid metabolism; one potentially codes for a unique DNA binding protein, one codes for a possible potassium channel protein, and the others code for proteins of unknown function. Genes which are induced during infection are likely to be significant for survival and growth of the pathogen; our results lend support to the view that fatty acid metabolism is essential for the virulence of M. tuberculosis.

Eitel, J. and P. Dersch (2002). "The YadA Protein of Yersinia pseudotuberculosis Mediates High-Efficiency Uptake into Human Cells under Environmental Conditions in Which Invasin Is Repressed." Infect. Immun. **70**(9): 4880-4891.

http://iai.asm.org/cgi/content/abstract/70/9/4880

The YadA protein is a major adhesin of Yersinia pseudotuberculosis that promotes tight adhesion to mammalian cells by binding to extracellular matrix proteins. In this study, we first addressed the possibility of competitive interference of YadA and the major invasive factor invasin and found that expression of YadA in the presence of invasin affected neither the export nor the function of invasin in the outer membrane. Furthermore, expression of YadA promoted both bacterial adhesion and high-efficiency invasion entirely independently of invasin. Antibodies against fibronectin and {beta}1 integrins blocked invasion, indicating that invasion occurs via extracellular-matrix-dependent bridging between YadA and the host cell {beta}1 integrin receptors. Inhibitor studies also demonstrated that tyrosine and Ser/Thr kinases, as well as phosphatidylinositol 3-kinase, are involved in the uptake process. Further expression studies revealed that yadA is regulated in response to several environmental parameters, including temperature, ion and nutrient concentrations, and the bacterial growth phase. In complex medium, YadA production was generally repressed but could be induced by addition of Mg2+. Maximal expression of yadA was obtained in exponential-phase cells grown in minimal medium at 37{degrees}C, conditions under which the invasin gene is repressed. These results suggest that YadA of Y. pseudotuberculosis constitutes another independent high-level uptake pathway that might complement other cell entry mechanisms (e.g., invasin) at certain sites or stages during the infection process.

Feldmesser, M., A. Mednick, et al. (2002). "Antibody-Mediated Protection in Murine Cryptococcus

neoformans Infection Is Associated with Pleotrophic Effects on Cytokine and Leukocyte Responses." Infect. Immun. **70**(3): 1571-1580.

http://iai.asm.org/cgi/content/abstract/70/3/1571

Cryptococcus neoformans, an encapsulated yeast, is a common cause of life-threatening meningoencephalitis in immunosuppressed patients. We previously observed that administration of a monoclonal antibody (MAb) to the capsular polysaccharide to mice with pulmonary infection prolonged survival and enhanced granulomatous inflammation without reducing lung CFU. To understand the mechanism of MAb action, we studied leukocyte recruitment and cytokine profiles in lungs of A/JCr mice. B lymphocytes were the predominant cell type in lung infiltrates, comprising 15 to 30% of the leukocytes. Despite alterations in histological appearance, fluorescence-activated cell sorter analysis revealed no significant difference in total numbers of lung leukocytes in MAb-treated mice and controls. Differences in the immune response to C. neoformans between MAb-treated mice and controls included (i) an increase in the percentage of granulocytes among lung leukocytes on day 14, (ii) higher macrophage surface expression of CD86 on day 28, (iii) larger amounts of IL-10 in lung homogenates at day 7, (iv) a trend toward smaller amounts of gamma interferon mRNA and protein on day 7, and (v) a smaller increase in the levels of interleukin-4 mRNA and protein on day 7. Hence, the immune responses to C. neoformans infection in the presence and absence of specific antibody were qualitatively similar, and antibody administration was associated with several subtle quantitative differences in immune response parameters that could translate into enhanced survival. MAb may function partly by down-regulating the inflammatory response and reducing host damage. Our findings demonstrate unexpected complexity in the interaction between specific MAb and other components of the host immune response.

Fletcher, L. D., L. Bernfield, et al. (2004). "Vaccine Potential of the Neisseria meningitidis 2086 Lipoprotein." Infect. Immun. **72**(4): 2088-2100.

http://iai.asm.org/cgi/content/abstract/72/4/2088

A novel antigen that induces cross-reactive bactericidal antibodies against a number of Neisseria meningitidis strains is described. This antigen, a [~]28-kDa lipoprotein called LP2086, was first observed within a complex mixture of soluble outer membrane proteins (sOMPs) following a series of fractionation, protein purification, and proteomics steps. Approximately 95 different neisserial isolates tested positive by Western blotting and PCR screening methods for the presence of the protein and the gene encoding LP2086. The strains tested included isolates of N. meningitidis serogroups A, B, C, W135, and Y, Neisseria gonorrhoeae, and Neisseria lactamica. To better understand the microheterogeneity of this protein, the 2086 genes from 63 neisserial isolates were sequenced. Two different subfamilies of LP2086 were identified based on deduced amino acid sequence homology. A high degree of amino acid sequence similarity exists within each 2086 subfamily. The highest degree of genetic diversity was seen between the two subfamilies which share approximately 60 to 75% homology at the nucleic acid level. Flow cytometry (fluorescence-activated cell sorting) analyses and electron microscopy indicated that the LP2086 is localized on the outer surface of N. meningitidis. Antiserum produced against a single protein variant was capable of eliciting bactericidal activity against strains expressing different serosubtype antigens. Combining one recombinant lipidated 2086 (rLP2086) variant from each subfamily with two rPorA variants elicited bactericidal activity against all strains tested. The rLP2086 family of antigens are candidates worthy of further vaccine development.

Flieger, A., K. Rydzewski, et al. (2004). "Cloning and Characterization of the Gene Encoding the Major Cell-Associated Phospholipase A of Legionella pneumophila, plaB, Exhibiting Hemolytic Activity."

Infect. Immun. **72**(5): 2648-2658.

http://iai.asm.org/cgi/content/abstract/72/5/2648

Legionella pneumophila, the causative agent of Legionnaires' disease, is an intracellular pathogen of amoebae, macrophages, and epithelial cells. The pathology of Legionella infections involves alveolar cell destruction, and several proteins of L. pneumophila are known to contribute to this ability. By screening a genomic library of L. pneumophila, we found an additional L. pneumophila gene, plaB, which coded for a hemolytic activity and contained a lipase consensus motif in its deduced protein sequence. Moreover, Escherichia coli harboring the L. pneumophila plaB gene showed increased activity in releasing fatty acids predominantly from diacylphospho- and lysophospholipids, demonstrating that it encodes a phospholipase A. It has been reported that culture supernatants and cell lysates of L. pneumophila possess phospholipase A activity; however, only the major secreted lysophospholipase A PlaA has been investigated on the molecular level. We therefore generated isogenic L. pneumophila plaB mutants and tested those for hemolysis, lipolytic activities, and intracellular survival in amoebae and macrophages. Compared to wild-type L. pneumophila, the plaB mutant showed reduced hemolysis of human red blood cells and almost completely lost its cell-associated lipolytic activity. We conclude that L. pneumophila plaB is the gene encoding the major cell-associated phospholipase A, possibly contributing to bacterial cytotoxicity due to its hemolytic activity. On the other hand, in view of the fact that the plaB mutant multiplied like the wild type both in U937 macrophages and in Acanthamoeba castellanii amoebae, plaB is not essential for intracellular survival of the pathogen.

Garandeau, C., H. Reglier-Poupet, et al. (2002). "The Sortase SrtA of Listeria monocytogenes Is Involved in Processing of Internalin and in Virulence." Infect. Immun. **70**(3): 1382-1390.

http://iai.asm.org/cgi/content/abstract/70/3/1382

Listeria monocytogenes is an intracellular gram-positive human pathogen that invades eucaryotic cells. Among the surface-exposed proteins playing a role in this invasive process, internalin belongs to the family of LPXTG proteins, which are known to be covalently linked to the bacterial cell wall in gram-positive bacteria. Recently, it has been shown in Staphylococcus aureus that the covalent anchoring of protein A, a typical LPXTG protein, is due to a cysteine protease, named sortase, required for bacterial virulence. Here, we identified in silico from the genome of L. monocytogenes a gene, designated srtA, encoding a sortase homologue. The role of this previously unknown sortase was studied by constructing a sortase knockout mutant. Internalin was used as a reporter protein to study the effects of the srtA mutation on cell wall anchoring of this LPXTG protein in L. monocytogenes. We show that the srtA mutant (i) is affected in the display of internalin at the bacterial surface, (ii) is significantly less invasive in vitro, and (iii) is attenuated in its virulence in the mouse. These results demonstrate that srtA of L. monocytogenes acts as a sortase and plays a role in the pathogenicity.

Gat, O., I. Inbar, et al. (2003). "Use of a Promoter Trap System in Bacillus anthracis and Bacillus subtilis for the Development of Recombinant Protective Antigen-Based Vaccines." Infect. Immun. **71**(2): 801-813.

http://iai.asm.org/cgi/content/abstract/71/2/801

We have recently reported Bacillus anthracis attenuated live vaccine strains efficiently expressing recombinant protective antigen (rPA) and have shown a direct correlation between the level of

rPA secreted by these cells and efficacy (S. Cohen, I. Mendelson, Z. Altboum, D. Kobiler, E. Elhanany, T. Bino, M. Leitner, I. Inbar, H. Rosenberg, Y. Gozes, R. Barak, M. Fisher, C. Kronman, B. Velan, and A. Shafferman, Infect. Immun. 68:4549-4558, 2000). To isolate more potent Bacillus promoters for a further increase in the production of rPA, we developed a promoter trap system based on various gfp reporter genes adapted for use in both Bacillus subtilis and B. anthracis backgrounds. Accordingly, a B. anthracis library of 6,000 clones harboring plasmids with chromosomal B. anthracis DNA fragments inserted upstream from gfpuv was constructed. Based on fluorescence intensity, 57 clones carrying potentially strong promoters were identified, some of which were DNA sequenced. The most potent B. anthracis promoter identified (Pntr; 271 bp) was 500 times more potent than the native pagA promoter and 70 times more potent than the {alpha}-amylase promoter (Pamy). This very potent promoter was tested along with the other promoters (which are three, six, and eight times more potent than Pamy) for the ability to drive expression of rPA in either B. subtilis or B. anthracis. The number of cell-associated pre-PA molecules in B. anthracis was found to correlate well with the strength of the promoter. However, there appeared to be an upper limit to the amount of mature PA secreted into the medium, which did not exceed that driven by Pamy. Furthermore, the rPA constructs fused to the very potent promoters proved to be deleterious to the bacterial hosts and consequently led to genetic instability of the PA expression plasmid. Immunization with attenuated B. anthracis expressing rPA under the control of promoters more potent than Pamy was less efficient in eliciting anti-PA antibodies than that attained with Pamy. The results are consistent with the notion that overexpression of PA leads to severe secretion stress and have practical implications for the design of second-generation rPA-based vaccines.

Gilmore, R. D., Jr., A. M. Carpio, et al. (2003). "Molecular Characterization of the sucB Gene Encoding the Immunogenic Dihydrolipoamide Succinyltransferase Protein of Bartonella vinsonii subsp. berkhoffii and Bartonella quintana." Infect. Immun. **71**(8): 4818-4822.

http://iai.asm.org/cgi/content/abstract/71/8/4818

Members of the genus Bartonella have historically been connected with human disease, such as cat scratch disease, trench fever, and Carrion's disease, and recently have been recognized as emerging pathogens causing other clinical manifestations in humans. However, because little is known about the antigens that elicit antibody production in response to Bartonella infections, this project was undertaken to identify and molecularly characterize these immunogens. Immunologic screening of a Bartonella vinsonii subsp. berkhoffii genomic expression library with anti-Bartonella antibodies led to the identification of the sucB gene, which encodes the enzyme dihydrolipoamide succinyltransferase. Antiserum from a mouse experimentally infected with live Bartonella was reactive against recombinant SucB, indicating the mounting of an anti-SucB response following infection. Antigenic cross-reactivity was observed with antiserum against other Bartonella spp. Antibodies against Coxiella burnetti, Francisella tularensis, and Rickettsia typhi also reacted with our recombinant Bartonella SucB. Potential SucB antigenic cross-reactivity presents a challenge to the development of serodiagnostic tests for other intracellular pathogens that cause diseases such as Q fever, rickettsioses, brucelloses, tularemia, and other bartonelloses.

Giuliani, M. M., L. Santini, et al. (2005). "The Region Comprising Amino Acids 100 to 255 of Neisseria meningitidis Lipoprotein GNA 1870 Elicits Bactericidal Antibodies." Infect. Immun. **73**(2): 1151-1160.

http://iai.asm.org/cgi/content/abstract/73/2/1151

GNA 1870 is a novel surface-exposed lipoprotein, identified by genome analysis of Neisseria meningitidis strain MC58, which induces bactericidal antibodies. Three sequence variants of the

protein were shown to be sufficient to induce bactericidal antibodies against a panel of strains representative of the diversity of serogroup B meningococci. Here, we studied the antigenic and immunogenic properties of GNA 1870, which for convenience was divided into domains A, B, and C. The immune responses of mice immunized with each of the three variants were tested using overlapping peptides scanning the entire protein length and using recombinant fragments. We found that while most of the linear epitopes are located in the A domain, the bactericidal antibodies are directed against conformational epitopes located in the BC domain. This was also confirmed by the isolation of a bactericidal murine monoclonal antibody, which failed to recognize linear peptides on the A, B, and C domains separately but recognized a conformational epitope formed only by the combination of the B and C domains. Arginine in position 204 was identified as important for binding of the monoclonal antibody. The identification of the region containing bactericidal epitopes is an important step in the design of new vaccines against meningococci.

Hamrick, T. S., J. R. Horton, et al. (2003). "Influence of Pregnancy on the Pathogenesis of Listeriosis in Mice Inoculated Intragastrically." Infect. Immun. **71**(9): 5202-5209.

http://iai.asm.org/cgi/content/abstract/71/9/5202

Pregnancy increases the risk of listeriosis, a systemic disease caused by Listeria monocytogenes. However, there is incomplete agreement on the reasons for this increased risk. We examined two features of listeriosis in gravid and nongravid female mice following intragastric (gavage) inoculation, namely, (i) disease severity (measured by lethality) and (ii) listerial infectivity (measured by liver and spleen colonization levels up to 120 h postinoculation). Two listerial strains of differing serotype (1/2a and 4nonb) were initially employed. Neither strain produced a lethal infection in nonpregnant female mice (dose range, 106 to 109 CFU/mouse), and only the 4nonb strain produced lethalities in pregnant mice (dose range, 106 to 108 CFU/mouse). The 4nonb strain also produced a higher level of liver and spleen colonization than the 1/2a strain following gavage administration. (The two strains showed similar levels of colonization if parenterally administered.) Both strains were equally capable of binding to and forming plaques upon cultured mouse enterocytes. The ability of the 4nonb strain to produce a lethal infection in pregnant animals did not correlate with an increased incidence or level of liver and spleen colonization over that in nonpregnant females. However, the lethality rate did correlate well with the rate at which embryos and their surrounding decidual covering became infected, suggesting that intrauterine infection could be responsible for the increased disease severity in the gravid females.

Hase, K., L. Eckmann, et al. (2002). "Cell Differentiation Is a Key Determinant of Cathelicidin LL-37/Human Cationic Antimicrobial Protein 18 Expression by Human Colon Epithelium." Infect. Immun. **70**(2): 953-63.

http://iai.asm.org/cgi/content/abstract/70/2/953

Antimicrobial peptides are highly conserved evolutionarily and are thought to play an important role in innate immunity at intestinal mucosal surfaces. To better understand the role of the antimicrobial peptide human cathelicidin LL-37/human cationic antimicrobial protein 18 (hCAP18) in intestinal mucosal defense, we characterized the regulated expression and production of this peptide by human intestinal epithelium. LL-37/hCAP18 is shown to be expressed within epithelial cells located at the surface and upper crypts of normal human colon. Little or no expression was seen within the deeper colon crypts or within epithelial cells of the small intestine. Paralleling its expression in more differentiated epithelial cells in vivo, LL-37/hCAP18 mRNA and protein expression was upregulated in spontaneously differentiating Caco-2 human colon epithelial cells and in HCA-7 human colon epithelial cells treated with the cell differentiation-inducing agent

sodium butyrate. LL-37/hCAP18 expression by colon epithelium does not require commensal bacteria, since LL-37/hCAP18 is produced with a similar expression pattern by epithelial cells in human colon xenografts that lack a luminal microflora. LL-37/hCAP18 mRNA was not upregulated in response to tumor necrosis factor alpha, interleukin 1{alpha} (IL-1{alpha}), gamma interferon, lipopolysaccharide, or IL-6, nor did the expression patterns and levels of LL-37/hCAP18 in the epithelium of the normal and inflamed colon differ. On the other hand, infection of HCA-7 cells with Salmonella enterica serovar Dublin or enteroinvasive Escherichia coli modestly upregulated LL-37/hCAP18 mRNA expression. We conclude that differentiated human colon epithelium expresses LL-37/hCAP18 as part of its repertoire of innate defense molecules and that the distribution and regulated expression of LL-37/hCAP18 in the colon differs markedly from that of other enteric antimicrobial peptides, such as defensins.

Ikebe, T., A. Wada, et al. (2002). "Dissemination of the Phage-Associated Novel Superantigen Gene speL in Recent Invasive and Noninvasive Streptococcus pyogenes M3/T3 Isolates in Japan." Infect. Immun. **70**(6): 3227-3233.

http://iai.asm.org/cgi/content/abstract/70/6/3227

In Japan, more than 10% of streptococcal toxic shock-like syndrome (TSLS) cases have been caused by Streptococcus pyogenes M3/T3 isolates since the first reported TSLS case in 1992. Most M3/T3 isolates from TSLS or severe invasive infection cases during 1992 to 2001 and those from noninvasive cases during this period are indistinguishable in pulsed-field gel electropherograms. The longest fragments of these recent isolates were 300 kb in size, whereas those of isolates recovered during or before 1973 were 260 kb in size. These 260- and 300-kb fragments hybridized to each other, suggesting the acquisition of an about 40-kb fragment by the recent isolates. The whole part of the acquired fragment was cloned from the first Japanese TSLS isolate, NIH1, and its nucleotide sequence was determined. The 41,796-bp fragment is temperate phage {phi}NIH1.1, containing a new superantigen gene speL near its right attachment site. The C-terminal part of the deduced amino acid sequence of speL has 48 and 46% similarity with well-characterized erythrogenic toxin SpeC and the most potent superantigen, SmeZ-2, respectively. None of 10 T3 isolates recovered during or before 1973 has speL, whereas all of 18 M3/T3 isolates recovered during or after 1992 and, surprisingly, Streptococcus equi subsp. equi ATCC 9527 do have this gene. Though plaques could not be obtained from {phi}NIH1.1, its DNA became detectable from the phage particle fraction upon mitomycin C induction, showing that this phage is not defective. A horizontal transfer of the phage carrying speL may explain the observed change in M3/T3 S. pyogenes isolates in Japan.

John, M., I. T. Kudva, et al. (2005). "Use of In Vivo-Induced Antigen Technology for Identification of Escherichia coli O157:H7 Proteins Expressed during Human Infection." Infect. Immun. **73**(5): 2665-2679.

http://iai.asm.org/cgi/content/abstract/73/5/2665

Using in vivo-induced antigen technology (IVIAT), a modified immunoscreening technique that circumvents the need for animal models, we directly identified immunogenic Escherichia coli O157:H7 (O157) proteins expressed either specifically during human infection but not during growth under standard laboratory conditions or at significantly higher levels in vivo than in vitro. IVIAT identified 223 O157 proteins expressed during human infection, several of which were unique to this study. These in vivo-induced (ivi) proteins, encoded by ivi genes, mapped to the backbone, O islands (OIs), and pO157. Lack of in vitro expression of O157-specific ivi proteins was confirmed by proteomic analysis of a mid-exponential-phase culture of E. coli O157 grown in LB broth. Because ivi proteins are expressed in response to specific cues during infection and

might help pathogens adapt to and counter hostile in vivo environments, those identified in this study are potential targets for drug and vaccine development. Also, such proteins may be exploited as markers of O157 infection in stool specimens.

Judge, N. A., H. S. Mason, et al. (2004). "Plant Cell-Based Intimin Vaccine Given Orally to Mice Primed with Intimin Reduces Time of Escherichia coli O157:H7 Shedding in Feces." Infect. Immun. **72**(1): 168-175.

http://iai.asm.org/cgi/content/abstract/72/1/168

Intimin is the primary adhesin of Escherichia coli O157:H7, the most common infectious cause of bloody diarrhea in the United States and the leading cause of acute kidney failure in children who develop hemolytic uremic syndrome. Cattle are the primary reservoir of E. coli O157:H7. Indeed, most cases of E. coli O157:H7 infection in the United States occur after ingestion of contaminated undercooked hamburger or produce that had contact with bovine manure. Because intimin is required for persistent colonization of neonatal calves and adult cattle, we hypothesized that an intimin-based vaccination strategy in calves would reduce colonization of cattle with E. coli O157:H7. To test this concept in a small-animal model, we developed transgenic tobacco plant cells that express the carboxy-terminal host cell-binding domain of E. coli O157:H7 intimin. Mice were either immunized intraperitoneally with intimin expressed from the plant cells, fed transgenic plant cells, or both. Here we show that these mice generated an intimin-specific mucosal immune response when primed parenterally and then boosted orally and also exhibited a reduced duration of E. coli O157:H7 fecal shedding after challenge.

Jung, Y.-J., R. LaCourse, et al. (2002). "Evidence Inconsistent with a Negative Influence of T Helper 2 Cells on Protection Afforded by a Dominant T Helper 1 Response against Mycobacterium tuberculosis Lung Infection in Mice." Infect. Immun. **70**(11): 6436-6443.

http://iai.asm.org/cgi/content/abstract/70/11/6436

Mice incapable of generating an efficient Th2 response because of functional deletion of the genes for signal transducer and activation of transcription 6 (Stat6), interleukin-4 receptor alpha chain (IL-4R{alpha}), or IL-4 plus IL-13 (IL-4/IL-13) were no more resistant than wild-type (WT) mice to airborne infection with virulent Mycobacterium tuberculosis. WT mice were able to control infection and hold it at a stationary level following 20 days of log linear M. tuberculosis growth. Likewise, infection was kept under control and was held at the same stationary level in IL-4/IL-13-/- mice but progressed to a slightly higher level in Stat6-/- and IL-4R{alpha}-/- mice. The onset of stationary-level infection in WT mice was associated with the expression of Th1-mediated immunity, as evidenced by an approximately 100- to 1,000-fold increase in the lungs in the synthesis of mRNA for IL-12, gamma interferon (IFN-{gamma}), and inducible nitric oxide synthase (NOS2) that was sustained for at least 100 days. IL-12 is essential for the induction of Th1 immunity, IFN-{gamma} is a key Th1 cytokine involved in mediation of immunity, and NOS2 is an inducible enzyme of macrophages and is needed by these cells to express immunity. In response to infection, the lungs of Stat6-/- mice showed increases in synthesis of mRNA for IL-12, IFN-{gamma}, and NOS2 similar to that seen in WT mice. In IL-4/IL-13-/- mice, however, synthesis of mRNA for IFN-{gamma} and NOS2 reached higher levels than in WT mice. These results argue against the notion that a Th2 response is partly or wholly responsible for the inability of Th1-mediated immunity to resolve infection with a virulent strain of M. tuberculosis.

Kim, H., K. J. Boor, et al. (2004). "Listeria monocytogenes {sigma}B Contributes to Invasion of Human Intestinal Epithelial Cells." Infect. Immun. **72**(12): 7374-7378.

http://iai.asm.org/cgi/content/abstract/72/12/7374

The role of {sigma}B in Listeria monocytogenes infection of human intestinal epithelial cells was investigated. Invasion defects associated with loss of {sigma}B paralleled those of a {Delta}inlA strain independently of the {sigma}B-dependent P2prfA promoter. Concomitantly, amounts of inlA transcript and InlA protein were significantly decreased in the {Delta}sigB strain.

Koguchi, Y., K. Kawakami, et al. (2002). "Penicillium marneffei Causes Osteopontin-Mediated Production of Interleukin-12 by Peripheral Blood Mononuclear Cells." Infect. Immun. **70**(3): 1042-1048.

http://iai.asm.org/cgi/content/abstract/70/3/1042

We investigated the role of osteopontin (OPN) in interleukin-12 (IL-12) production from peripheral blood mononuclear cells (PBMCs) stimulated with Penicillium marneffei. Kinetic studies showed that OPN synthesis preceded that of IL-12 at both mRNA and protein levels when PBMCs were cocultured with P. marneffei. Treatment with anti-OPN monoclonal antibodies (MAb) significantly suppressed IL-12 secretion. Furthermore, native OPN induced a profound level of synthesis of IL-12 from noninfected PBMCs. The major cellular source of OPN was monocytes, because depletion of CD14+ cells resulted in the abrogation of such production. We also examined the regulatory role of granulocyte-macrophage colony-stimulating factor (GM-CSF) in OPN secretion from P. marneffei-stimulated PBMCs. Neutralizing anti-GM-CSF MAb significantly reduced OPN secretion, and treatment with this cytokine induced OPN production from both infected and noninfected PBMCs. Finally, antagonists against the mannose receptor but not the {beta}-glucan receptor almost completely abrogated the production of OPN. Our results demonstrated that OPN secreted from monocytes is involved in the production of IL-12 from PBMCs after stimulation with P. marneffei and that OPN production is regulated by GM-CSF. Our results also indicated the possible involvement of the mannose receptor as a signal-transducing receptor for triggering the secretion of OPN by P. marneffei-stimulated PBMCs.

Kohler, A. K., D. M. Stone, et al. (2003). "Rhodococcus equi Secreted Antigens Are Immunogenic and Stimulate a Type 1 Recall Response in the Lungs of Horses Immune to R. equi Infection." Infect. Immun. **71**(11): 6329-6337.

http://iai.asm.org/cgi/content/abstract/71/11/6329

Rhodococcus equi is an opportunistic pathogen in immunocompromised humans and an important primary pathogen in young horses. Although R. equi infection can produce life-threatening pyogranulomatous pneumonia, most foals develop a protective immune response that lasts throughout life. The antigen targets of this protective response are currently unknown; however, Mycobacterium tuberculosis is a closely related intracellular pathogen and provides a model system. Based on previous studies of M. tuberculosis protective antigens released into culture filtrate supernatant (CFS), a bacterial growth system was developed for obtaining R. equi CFS antigens. Potential immunogens for prevention of equine rhodococcal pneumonia were identified by using immunoblots. The 48-h CFS contained five virulence-associated protein bands that migrated between 12 and 24 kDa and were recognized by sera from R. equi-infected foals and immune adult horses. Notably, the CFS contained the previously characterized proteins VapC, VapD, and VapE, which are encoded by genes on the R. equi virulence plasmid. R. equi CFS was also examined for the ability to stimulate a type 1-like memory response in immune

horses. Three adult horses were challenged with virulent R. equi, and cells from the bronchoalveolar lavage fluid were recovered before and 1 week after challenge. In vitro stimulation of pulmonary T-lymphocytes with R. equi CFS resulted in significant proliferation and a significant increase in gamma interferon mRNA expression 1 week after challenge. These results were consistent with a memory effector response in immune adult horses and provide evidence that R. equi CFS proteins are antigen targets in the immunoprotective response against R. equi infection.

Lieke, T., S. E. B. Graefe, et al. (2004). "NK Cells Contribute to the Control of Trypanosoma cruzi Infection by Killing Free Parasites by Perforin-Independent Mechanisms." Infect. Immun. **72**(12): 6817-6825.

http://iai.asm.org/cgi/content/abstract/72/12/6817

The protozoan parasite Trypanosoma cruzi circulates in the blood as trypomastigotes and invades a variety of cells to multiply intracellularly as amastigotes. The acute phase leads to an immune response that restricts the proliferation of the parasite. However, parasites are able to persist in different tissues, which causes the pathology of Chagas' disease. Natural killer (NK) cells play an important role in innate resistance to a variety of pathogens. In the present study we analyzed whether NK cells participated in the control of experimental T. cruzi infection. NK cells were depleted from C57BL/6 mice by antiasialo antibodies. This treatment caused an increased parasitemia during the acute phase, but tissue parasite burdens were not significantly altered according to quantitative real-time PCR. Our results demonstrated that NK cells were activated during the initial phase of a T. cruzi infection and exhibited a contact-dependent antiparasitic activity against extracellular parasites that was independent from perforin. Thus, NK cells limit the propagation of the parasite by acting on circulating T. cruzi trypomastigotes.

Lohr, C. V., F. R. Rurangirwa, et al. (2002). "Specific Expression of Anaplasma marginale Major Surface Protein 2 Salivary Gland Variants Occurs in the Midgut and Is an Early Event during Tick Transmission." Infect. Immun. **70**(1): 114-120.

http://iai.asm.org/cgi/content/abstract/70/1/114

Infectivity of Anaplasma spp. develops when infected ticks feed on a mammalian host (transmission feed). Specific Anaplasma marginale major surface protein 2 (MSP2) variants are selected for within the tick and are expressed within the salivary glands. The aims of this study were to determine when and where MSP2 variant selection occurs in the tick, how MSP2 expression is regulated in salivary glands of transmission-feeding ticks, and whether the number of A. marginale organisms per salivary gland is significantly increased during transmission feeding. The South Idaho strain of A. marginale was used, as MSP2 expression is restricted to two variants, SGV1 and SGV2, in Dermacentor andersoni. Using Western blot, real-time PCR, and DNA sequencing analyses it was shown that restriction and expression of MSP2 occurs early in the midgut within the first 48 h of the blood meal, when ticks acquire infection. A. marginale is present in the tick salivary glands before transmission feeding is initiated, but the msp2 mRNA and MSP2 protein levels per A. marginale organism increase only minimally and transiently in salivary glands of transmission-feeding ticks compared to that of unfed ticks. A. marginale numbers per tick increase gradually in salivary glands of both transmission-fed and unfed ticks. It is concluded that MSP2 variant selection is an early event in the tick and that MSP2 variants SGV1 and SGV2 are expressed both in the midgut and salivary glands. While MSP2 may be required for infectivity, there is no strict temporal correlation between MSP2 expression and the development of infectivity.

Long, C. D., D. M. Tobiason, et al. (2003). "Low-Level Pilin Expression Allows for Substantial DNA Transformation Competence in Neisseria gonorrhoeae." Infect. Immun. **71**(11): 6279-6291.

http://iai.asm.org/cgi/content/abstract/71/11/6279

The gonococcal pilus is a major virulence factor that has well-established roles in mediating epithelial cell adherence and DNA transformation. Gonococci expressing four gonococcal pilin variants with distinct piliation properties under control of the lac regulatory system were grown in different levels of the inducer isopropyl-{beta}-D-thiogalactopyranoside (IPTG). These pilin variants expressed various levels of pilin message and pilin protein in response to the level of IPTG in the growth medium. Moreover, posttranslational modifications of the variant pilin proteins were detected, including S-pilin production and glycosylation. The ratio of the modified and unmodified pilin forms did not substantially change with different levels of pilin expression, showing that these modifications are not linked to pilin expression levels. DNA transformation competence was also influenced by IPTG levels in the growth medium. Substantial increases in transformation competence over an isogenic, nonpiliated mutant were observed when limited amounts of three of the pilin variants were expressed. Immunoelectron microscopy showed that when limited amounts of pilin are expressed, pili are rare and do not explain the pilin-dependent transformation competence. This pilin-dependent transformation competence required prepilin processing, the outer membrane secretin PilQ, and the twitching-motility-regulating protein PilT. These requirements show that a fully functional pilus assembly apparatus is required for DNA uptake when limited pilin is produced. We conclude that the pilus assembly apparatus functions to import DNA into the bacterial cell in a pilin-dependent manner but that extended pili are not required for transformation competence.

Lorenz, E., D. C. Chemotti, et al. (2005). "Differential Involvement of Toll-Like Receptors 2 and 4 in the Host Response to Acute Respiratory Infections with Wild-Type and Mutant Haemophilus influenzae Strains." Infect. Immun. **73**(4): 2075-2082.

http://iai.asm.org/cgi/content/abstract/73/4/2075

We used a mouse model of acute respiratory infections to investigate the role of Toll-like receptor 2 (TLR2) and TLR4 in the host response to Haemophilus influenzae. Acute aerosol exposures to wild-type strains of H. influenzae showed that TLR4 function was essential for TNF-{alpha} induction, neutrophil influx, and bacterial clearance. To determine how lipooligosaccharide (LOS) modifications would affect the role of TLR4 in inducing the host response, we used acute infections with an H. influenzae strain expressing a mutation in the htrB gene. This mutant strain expresses an LOS subunit with decreased acylation. In response to H. influenzae htrB infection, tumor necrosis factor alpha (TNF-{alpha}) secretion remained TLR4 dependent. But the decrease in LOS acylation made the neutrophil influx and the bacterial clearance also dependent on TLR2, as shown by the decreased host response elicited in TLR2 knockout mice compared to C57BL/6 mice. A subsequent analysis of TLR2 and TLR4 gene expression by quantitative PCR indicated that TLR4 function induces TLR2 expression and vice versa. These results indicate that some changes in the LOS subunit of H. influenzae can favor signaling through non-TLR4 receptors, such as TLR2. The results also indicate a close interaction between TLR4 and TLR2 that tightly regulates the expression of both receptors.

Maeda, K., H. Nagata, et al. (2004). "Glyceraldehyde-3-Phosphate Dehydrogenase of Streptococcus oralis Functions as a Coadhesin for Porphyromonas gingivalis Major Fimbriae." Infect. Immun.

72(3): 1341-1348.

http://iai.asm.org/cgi/content/abstract/72/3/1341

Cohesive interactions between Porphyromonas gingivalis and plaque-forming bacteria, such as Streptococcus oralis, are considered to play an important role in the colonization of P. gingivalis in periodontal sites. Although P. gingivalis fimbriae have been reported to mediate coaggregation with S. oralis, the S. oralis molecule involved has not been identified. We identified the coadhesin of S. oralis ATCC 9811 and purified it by affinity column chromatography. We found that the molecular mass of the purified protein was approximately 40 kDa. Dot blot and Western blot assays showed binding of the 40-kDa protein to P. gingivalis fimbriae. Further, turbidimetric assays showed that the coadhesin inhibited coaggregation between P. gingivalis and S. oralis in a dose-dependent manner. Analyses of the amino-terminal sequences of the protein and its lysyl endopeptidase-cleaved fragments revealed that the coadhesin was identical to glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Next, we cloned the gene that encodes S. oralis GAPDH and found that the sequence had a high degree of homology with the sequences of GAPDHs of various bacteria, including Streptococcus gordonii and Fusobacterium nucleatum. To confirm the contribution of S. oralis GAPDH to the interaction with P. gingivalis, a recombinant GAPDH protein was generated in Escherichia coli; this protein bound to P. gingivalis fimbriae and had an inhibitory effect on coaggregation. These results suggest that S. oralis GAPDH functions as a coadhesin for P. gingivalis fimbriae. In addition, considering the high degree of homology of the GAPDHs of various bacteria, those of other plaque-forming bacteria also may contribute to the colonization of P. gingivalis.

McCutchan, T. F., K. C. Grim, et al. (2004). "Measuring the Effects of an Ever-Changing Environment on Malaria Control." Infect. Immun. **72**(4): 2248-2253.

http://iai.asm.org/cgi/content/abstract/72/4/2248

The effectiveness of malaria control measures depends not only on the potency of the control measures themselves but also upon the influence of variables associated with the environment. Environmental variables have the capacity either to enhance or to impair the desired outcome. An optimal outcome in the field, which is ultimately the real goal of vaccine research, will result from prior knowledge of both the potency of the control measures and the role of environmental variables. Here we describe both the potential effectiveness of control measures and the problems associated with testing in an area of endemicity. We placed canaries with different immunologic backgrounds (e.g., naive to malaria infection, vaccinated naive, and immune) directly into an area where avian malaria, Plasmodium relictum, is endemic. In our study setting, canaries that are naive to malaria infection routinely suffer approximately 50% mortality during their first period of exposure to the disease. In comparison, birds vaccinated and boosted with a DNA vaccine plasmid encoding the circumsporozoite protein of P. relictum exhibited a moderate degree of protection against natural infection ($P < 0.01$). In the second year we followed the fate of all surviving birds with no further manipulation. The vaccinated birds from the first year were no longer statistically distinguishable for protection against malaria from cages of naive birds. During this period, 36% of vaccinated birds died of malaria. We postulate that the vaccine-induced protective immune responses prevented the acquisition of natural immunity similar to that concurrently acquired by birds in a neighboring cage. These results indicate that dominant environmental parameters associated with malaria deaths can be addressed before their application to a less malleable human system.

Mederle, I., I. Bourguin, et al. (2002). "Plasmidic versus Insertional Cloning of Heterologous Genes in Mycobacterium bovis BCG: Impact on In Vivo Antigen Persistence and Immune Responses."

Infect. Immun. **70**(1): 303-314.

http://iai.asm.org/cgi/content/abstract/70/1/303

Bivalent recombinant strains of Mycobacterium bovis BCG (rBCG) expressing the early regulatory nef and the structural gag(p26) genes from the simian immunodeficiency virus (SIV) SIVmac251 were engineered so that both genes were cotranscribed from a synthetic operon. The expression cassette was cloned into a multicopy-replicating vector, and the expression levels of both nef and gag in the bivalent rBCG(nef-gag) strain were found to be comparable to those of monovalent rBCG(nef) or rBCG(gag) strains. However, extrachromosomal cloning of the nef-gag operon into a replicative plasmid resulted in strains of low genetic stability that rapidly lost the plasmid in vivo. Thus, the nef-gag operon was inserted site specifically into the BCG chromosome by means of mycobacteriophage Ms6-derived vectors. The resulting integrative rBCG(nef-gag) strains showed very high genetic stability both in vitro and in vivo. The in vivo expression of the heterologous genes was much longer lived when the expression cassette was inserted into the BCG chromosome. In one of the strains obtained, integrative cloning did not reduce the expression levels of the genes even though a single copy was present. Accordingly, this strain induced cellular immune responses of the same magnitude as that of the replicative rBCG strain containing several copies of the genes.

Menge, C., M. Blessenohl, et al. (2004). "Bovine Ileal Intraepithelial Lymphocytes Represent Target Cells for Shiga Toxin 1 from Escherichia coli." Infect. Immun. **72**(4): 1896-1905.

http://iai.asm.org/cgi/content/abstract/72/4/1896

The discovery that bovine peripheral lymphocytes are sensitive to Stx1 identified a possible mechanism for the persistence of infections with Shiga toxin (Stx)-producing Escherichia coli (STEC) in the bovine reservoir host. If intraepithelial lymphocytes (IEL) are also sensitive to Stx1, the idea that Stx1 affects inflammation in the bovine intestine is highly attractive. To prove this hypothesis, ileal IEL (iIEL) were prepared from adult cattle, characterized by flow cytometry, and subjected to functional assays in the presence and absence of purified Stx1. We found that 14.9% of all iIEL expressed Gb3/CD77, the Stx1 receptor on bovine lymphocytes, and 7.9% were able to bind the recombinant B subunit of Stx1. The majority of Gb3/CD77+ cells were activated CD3+ CD6+ CD8{alpha}+ T cells, whereas only some CD4+ T cells and B cells expressed Gb3/CD77. However, Stx1 blocked the mitogen-induced transformation to enlarged blast cells within all subpopulations to a similar extent and significantly reduced the percentage of Gb3/CD77+ cells. Although Stx1 did not affect the natural killer cell activity of iIEL, the toxin accelerated the synthesis of interleukin-4 (IL-4) mRNA and reduced the amount of IL-8 mRNA in bovine iIEL cultures. Because the intestinal system comprises a rich network of interactions between different types of cells and any dysfunction may influence the course of intestinal infections, this demonstration that Stx1 can target bovine IEL may be highly relevant for our understanding of the interplay between STEC and its reservoir host.

Munoz-Elias, E. J., J. Timm, et al. (2005). "Replication Dynamics of Mycobacterium tuberculosis in Chronically Infected Mice." Infect. Immun. **73**(1): 546-551.

http://iai.asm.org/cgi/content/abstract/73/1/546

The dynamics of host-pathogen interactions have important implications for the design of new antimicrobial agents to treat chronic infections such as tuberculosis (TB), which is notoriously refractory to conventional drug therapy. In the mouse model of TB, an acute phase of exponential

bacterial growth in the lungs is followed by a chronic phase characterized by relatively stable numbers of bacteria. This equilibrium could be static, with little ongoing replication, or dynamic, with continuous bacterial multiplication balanced by bacterial killing. A static model predicts a close correspondence between "viable counts" (live bacteria) and "total counts" (live plus dead bacteria) in the lungs over time. A dynamic model predicts the divergence of total counts and viable counts over time due to the accumulation of dead bacteria. Here, viable counts are defined as bacterial CFU enumerated by plating lung homogenates; total counts are defined as bacterial chromosome equivalents (CEQ) enumerated by using quantitative real-time PCR. We show that the viable and total bacterial counts in the lungs of chronically infected mice do not diverge over time. Rapid degradation of dead bacteria is unlikely to account for the stability of bacterial CEQ numbers in the lungs over time, because treatment of mice with isoniazid for 8 weeks led to a marked reduction in the number of CFU without reducing the number of CEQ. These observations support the hypothesis that the stable number of bacterial CFU in the lungs during chronic infection represents a static equilibrium between host and pathogen.

Oetjen, J., P. Fives-Taylor, et al. (2002). "The Divergently Transcribed Streptococcus parasanguis Virulence-Associated fimA Operon Encoding an Mn2+-Responsive Metal Transporter and pepO Encoding a Zinc Metallopeptidase Are Not Coordinately Regulated." Infect. Immun. **70**(10): 5706-5714.

http://iai.asm.org/cgi/content/abstract/70/10/5706

The study of how bacteria respond to and obtain divalent metal ions provides insight into the regulation of virulence factors in the host environment. Regulation of metal permease operons in gram-positive bacteria may involve the binding of metal-responsive repressors to palindromic domains in their control regions. The Streptococcus parasanguis fimA operon, which encodes an ATP-binding cassette (ABC) transporter system with sequence homology to the LraI family of metal transporters, possesses a palindromic regulatory region with high homology to that of the Streptococcus gordonii ScaR binding domain. Mapping of the promoter and regulatory regions of fimA and the divergently transcribed pepO gene, which encodes a zinc metalloendopeptidase, indicated that their promoter and regulatory elements overlap. fimA had one transcriptional start site, whereas pepO had three. Analysis of truncated versions of the pepO promoter suggested that all three transcriptional start sites are functional. Analysis of promoter activity under various environmental conditions indicated that the fimA operon promoter and the pepO promoter are not coordinately regulated. The fimA operon is responsive to changes in Mn2+ concentration, but the pepO promoter is not. A S. parasanguis fimA mutant showed a growth deficiency under conditions of limiting Mn2+. This deficiency was not alleviated by compensation with either Mg2+ or Fe3+. Wild-type S. parasanguis could take up Mn2+ and Fe3+, while the fimA mutant showed a marked reduction in this ability. These data suggested that FimA is a component of a metal transporter system capable of transporting both Mn2+ and Fe3+. FimA expression itself was shown to be responsive to Mn2+ concentration, but not to availability of Fe3+ or Mg2+.

Oscarsson, J., M. Westermark, et al. (2002). "Characterization of a Pore-Forming Cytotoxin Expressed by Salmonella enterica Serovars Typhi and Paratyphi A." Infect. Immun. **70**(10): 5759-5769.

http://iai.asm.org/cgi/content/abstract/70/10/5759

Cytolysin A (ClyA) is a pore-forming cytotoxic protein encoded by the clyA gene that has been characterized so far only in Escherichia coli. Using DNA sequence analysis and PCR, we established that clyA is conserved in the human-specific typhoid Salmonella enterica serovars Typhi and Paratyphi A and that the entire clyA gene locus is absent in many other S. enterica serovars, including Typhimurium. The gene products, designated ClyASTy and ClyASPa, show

[>=]90% amino acid identity to E. coli cytolysin A, ClyAEC, and they are immunogenically related. The Salmonella proteins showed a pore-forming activity and are hence functional homologues to ClyAEC. The chromosomal clyASTy gene locus was expressed at detectable levels in the serovar Typhi strains S2369/96 and S1112/97. Furthermore, in the serovar Typhi vaccine strain Ty21a, expression of clyASTy reached phenotypic levels, as detected on blood agar plates. The hemolytic phenotype was abolished by the introduction of an in-frame deletion in the clyASTy chromosomal locus of Ty21a. Transcomplementation of the mutant with a cloned clyASTy gene restored the hemolytic phenotype. To our knowledge, Ty21a is the first reported phenotypically hemolytic Salmonella strain in which the genetic determinant has been identified.

Papazisi, L., S. Frasca, Jr., et al. (2002). "GapA and CrmA Coexpression Is Essential for Mycoplasma gallisepticum Cytadherence and Virulence." Infect. Immun. **70**(12): 6839-6845.

http://iai.asm.org/cgi/content/abstract/70/12/6839

It was previously demonstrated that avirulent Mycoplasma gallisepticum strain Rhigh (passage 164) is lacking three proteins that are expressed in its virulent progenitor, strain Rlow (passage 15). These proteins were identified as the cytadhesin molecule GapA, the putative cytadhesin-related molecule CrmA, and a component of a high-affinity transporter system, HatA. Complementation of Rhigh with wild-type gapA restored expression in the transformant (GT5) but did not restore the cytadherence phenotype and maintained avirulence in chickens. These results suggested that CrmA might play an essential role in the M. gallisepticum cytadherence process. CrmA is encoded by the second gene in the gapA operon and shares significant sequence homology to the ORF6 gene of Mycoplasma pneumoniae, which has been shown to play an accessory role in the cytadherence process. Complementation of Rhigh with wild-type crmA resulted in the transformant (SDCA) that lacked the cytadherence and virulence phenotype comparable to that found in Rhigh and GT5. In contrast, complementation of Rhigh with the entire wild-type gapA operon resulted in the transformant (GCA1) that restored cytadherence to the level found in wild-type Rlow. In vivo pathogenesis trials revealed that GCA1 had regained virulence, causing airsacculitis in chickens. These results demonstrate that both GapA and CrmA are required for M. gallisepticum cytadherence and pathogenesis.

Pernthaner, A., S.-A. Cole, et al. (2005). "Increased Expression of Interleukin-5 (IL-5), IL-13, and Tumor Necrosis Factor Alpha Genes in Intestinal Lymph Cells of Sheep Selected for Enhanced Resistance to Nematodes during Infection with Trichostrongylus colubriformis." Infect. Immun. **73**(4): 2175-2183.

http://iai.asm.org/cgi/content/abstract/73/4/2175

Cytokine gene expression in cells migrating in afferent and efferent intestinal lymph was monitored for extended time periods in individual sheep experimentally infected with the nematode Trichostrongylus colubriformis. Animals from stable selection lines with increased levels of either genetic resistance (R) or susceptibility (S) to nematode infection were used. Genes for interleukin-5 (IL-5), IL-13, and tumor necrosis factor alpha (TNF-{alpha}), but not for IL-4, IL-10, or gamma interferon (IFN-{gamma}), were consistently expressed at higher levels in both afferent and efferent lymph cells of R sheep than in S sheep. However, only minor differences were observed in the surface phenotypes and antigenic and mitogenic responsiveness of cells in intestinal lymph between animals from the two selection lines. The IL-4 and IL-10 genes were expressed at higher levels in afferent lymph cells than in efferent lymph cells throughout the course of the nematode infection in animals of both genotypes, while the proinflammatory TNF-{alpha} gene was relatively highly expressed in both lymph types. These relationships notwithstanding, expression of the IL-10 and TNF-{alpha} genes declined

significantly in afferent lymph cells but not in efferent lymph cells during infection. Collectively, the results showed that R-line sheep developed a strong polarization toward a Th2-type cytokine profile in immune cells migrating in lymph from sites where the immune response to nematodes was initiated, although the IFN-{gamma} gene was also expressed at moderate levels. Genes or alleles that predispose an animal to develop this type of response appear to have segregated with the R selection line and may contribute to the increased resistance of these animals.

Post, D. M. B., M. R. Ketterer, et al. (2003). "The msbB Mutant of Neisseria meningitidis Strain NMB Has a Defect in Lipooligosaccharide Assembly and Transport to the Outer Membrane." Infect. Immun. **71**(2): 647-655.

http://iai.asm.org/cgi/content/abstract/71/2/647

A deletion-insertion mutation in msbB, a gene that encodes a lipid A acyltransferase, was introduced into encapsulated Neisseria meningitidis serogroup B strain NMB and an acapsular mutant of the same strain. These mutants were designated NMBA11K3 and NMBA11K3cap-, respectively. Neither lipooligosaccharide (LOS) nor lipid A could be isolated from NMBA11K3 although a number of techniques were tried, but both were easily extracted from NMBA11K3cap-. Immunoelectron microscopy using monoclonal antibody (MAb) 6B4, which recognizes the terminal Gal{beta}1-4GlcNAc of LOS, demonstrated that NMB, NMBcap-, and NMBA11K3cap- expressed LOS circumferentially, while MAb 6B4 did not bind to the surface of NMBA11K3. However, cytoplasmic staining of NMBA11K3 with MAb 6B4 was a consistent observation. Mass-spectrometric analyses demonstrated that the relative amounts of the lipid A-specific C12:0 3-OH and C14:0 3-OH present in the membrane preparations (MP) from NMBA11K3 were substantially decreased (25- and 23-fold, respectively) compared to the amount in MP from its parent strain, NMB. Western blot analyses of MP from NMBA11K3 demonstrated that the levels of porin in the outer membrane of NMBA11K3 were also substantially decreased. These studies suggest that the lipid A acylation defect in encapsulated NMBA11K3 influences the assembly of the lipid A and consequently the incorporation of porin in the outer membrane.

Post, D. M. B., N. J. Phillips, et al. (2002). "Intracellular Survival of Neisseria gonorrhoeae in Male Urethral Epithelial Cells: Importance of a Hexaacyl Lipid A." Infect. Immun. **70**(2): 909-920.

http://iai.asm.org/cgi/content/abstract/70/2/909

Neisseria gonorrhoeae is a strict human pathogen that invades and colonizes the urogenital tracts of males and females. Lipooligosaccharide (LOS) has been shown to play a role in gonococcal pathogenesis. The acyl transferase MsbB is involved in the biosynthesis of the lipid A portion of the LOS. In order to determine the role of an intact lipid A structure on the pathogenesis of N. gonorrhoeae, the msbB gene was cloned and sequenced, a deletion and insertion mutation was introduced into N. gonorrhoeae, and the mutant strain was designated 1291A11K3. Mass spectrometric analyses of 1291A11K3 LOS determined that this mutation resulted in a pentaacyl rather than a hexaacyl lipid A structure. These analyses also demonstrated an increase in the phosphorylation of lipid A and an increase in length of the oligosaccharide of a minor species of the msbB LOS. The interactions of this mutant with male urethral epithelial cells (uec) were examined. Transmission and scanning electron microscopy studies indicated that the msbB mutants formed close associations with and were internalized by the uec at levels similar to those of the parent strain. Gentamicin survival assays performed with 1291A11K3 and 1291 bacteria demonstrated that there was no difference in the abilities of the two strains to adhere to uec; however, significantly fewer 1291A11K3 bacteria than parent strain bacteria were recovered from gentamicin-treated uec. These studies suggest that the lipid A modification in the N. gonorrhoeae msbB mutant may render it more susceptible to innate intracellular killing mechanisms when

internalized by uec.

Recktenwald, J. and H. Schmidt (2002). "The Nucleotide Sequence of Shiga Toxin (Stx) 2e-Encoding Phage {phi}P27 Is Not Related to Other Stx Phage Genomes, but the Modular Genetic Structure Is Conserved." Infect. Immun. **70**(4): 1896-1908.

http://iai.asm.org/cgi/content/abstract/70/4/1896

In this study we determined the complete nucleotide sequence of Shiga toxin 2e-encoding bacteriophage {phi}P27, isolated from the Shiga toxin-producing Escherichia coli patient isolate 2771/97. {phi}P27 is integrated as a prophage in the chromosomal yecE gene. This integration generates identity segments of attL and attR sites with lengths of 11 nucleotides. The integrated prophage genome has a size of 42,575 bp. We identified 58 open reading frames (ORFs), each with a length of >150 nucleotides. The deduced proteins of 44 ORFs showed significant homologies to other proteins present in sequence databases, whereas 14 putative proteins did not. For 29 proteins, we could deduce a putative function. Most of these are related to the basic phage propagation cycle. The {phi}P27 genome represents a mosaic composed of genetic elements which are obviously derived from related and unrelated phages. We identified five short linker sequences of 22 to 151 bp in the {phi}P27 sequence which have also been detected in a couple of other lambdoid phages. These linkers are located between functional modules in the phage genome and are thought to play a role in genetic recombination. Although the overall DNA sequence of {phi}P27 is not highly related to other known phages, the data obtained demonstrate a typical lambdoid genome structure.

Reglier-Poupet, H., E. Pellegrini, et al. (2003). "Identification of LpeA, a PsaA-Like Membrane Protein That Promotes Cell Entry by Listeria monocytogenes." Infect. Immun. **71**(1): 474-482.

http://iai.asm.org/cgi/content/abstract/71/1/474

The intracellular life of Listeria monocytogenes starts by a complex process of entry involving several bacterial ligands and eukaryotic receptors. In this work, we identified in silico from the sequence of the genome of L. monocytogenes a previously unknown gene designated lpeA (for lipoprotein promoting entry) encoding a 35-kDa protein homologous to PsaA, a lipoprotein belonging to the LraI family and implicated in the cell adherence of Streptococcus pneumoniae and related species. By constructing a mutant of L. monocytogenes in which lpeA is deleted (lpeA mutant), we show that the PsaA-like protein LpeA is not involved in bacterial adherence but is required for entry of L. monocytogenes in eukaryotic cells. In contrast to wild-type bacteria, mutant bacteria failed to invade the epithelial Caco-2 and hepatocyte TIB73 cell lines, as confirmed by confocal microscopy. The mutant bacteria rapidly penetrated in mouse bone marrow-derived macrophages. Surprisingly, lpeA mutant bacteria survive better in macrophages than do wild-type bacteria. This was correlated with a weak exacerbation of virulence of the lpeA mutant in the mouse. LpeA is therefore a novel invasin favoring the entry of L. monocytogenes into nonprofessional phagocytes but not its invasion of macrophages. This is the first report of a lipoprotein promoting cell invasion of an intracellular pathogen.

Satola, S. W., P. L. Schirmer, et al. (2003). "Genetic Analysis of the Capsule Locus of Haemophilus influenzae Serotype f." Infect. Immun. **71**(12): 7202-7207.

http://iai.asm.org/cgi/content/abstract/71/12/7202

A 19-kb DNA region containing genes involved in the biosynthesis of the capsule of Haemophilus influenzae serotype f (Hif) has been cloned and characterized. The Hif cap locus organization is typical of group II capsule biosynthetic loci found in other H. influenzae serotype b bacteria and other gram-negative bacteria. However, the Hif cap locus was not associated with an IS1016 element. Three new open reading frames, Fcs1, Fcs2, and Fcs3, were identified in the Hif capsule-specific region II. The chromosomal location of the Hif cap locus and the organization of the flanking sequences differed significantly from previously described division I H. influenzae serotypes.

Savkovic, S. D., J. Villanueva, et al. (2005). "Mouse Model of Enteropathogenic Escherichia coli Infection." Infect. Immun. **73**(2): 1161-1170.

http://iai.asm.org/cgi/content/abstract/73/2/1161

Enteropathogenic Escherichia coli (EPEC) is an important cause of diarrhea in humans. EPEC infection of cultured intestinal epithelial cells induces attaching and effacing (A/E) lesions, alters intestinal ion transport, increases paracellular permeability, and stimulates inflammation. The lack of a small-animal model has restricted in vivo studies examining EPEC-host interactions. The aim of this study was to characterize the C57BL/6J mouse as a model of EPEC infection. We have shown that EPEC can adhere to and colonize the intestinal epithelium of C57BL/6J mice. Animal weight and water intake were not altered during 10 days of EPEC infection. The proximal colon of infected mice contained semisolid stool, with stool pellets forming only in the distal colon. In contrast, the entire colon of control mice contained formed stool. Microvillous effacement and actin rearrangement, characteristic of A/E lesions, were seen in the intestine of infected mice but not control mice. Histological assessment revealed increased numbers of lamina propria neutrophils with occasional crypt abscesses, intraepithelial lymphocytes, and goblet cells in the intestine of EPEC-infected mice. Altogether, these data suggest that the C57BL/6J mouse is susceptible to infection by EPEC and will provide a suitable in vivo model for studying the consequences of EPEC infection.

Schuppler, M., K. Lotzsch, et al. (2004). "An Abundance of Escherichia coli Is Harbored by the Mucosa-Associated Bacterial Flora of Interleukin-2-Deficient Mice." Infect. Immun. **72**(4): 1983-1990.

http://iai.asm.org/cgi/content/abstract/72/4/1983

Mice deficient in interleukin-2 are well suited for use as an animal model for inflammatory bowel disease. Raised under specific-pathogen-free conditions, interleukin-2-deficient mice develop an inflammatory bowel disease resembling ulcerative colitis in humans. The finding that colitis was attenuated when the mice were kept under germfree conditions implies that the resident intestinal flora is involved in the pathogenesis of colitis. The present study addresses the composition of the mucosa-associated bacterial flora in colon samples from interleukin-2-deficient mice that developed colitis. This was investigated by comparative 16S ribosomal DNA (rDNA) sequence analysis and fluorescence in situ hybridization using rRNA-targeted fluorescent probes to quantify the bacterial populations of the mucosa-associated flora. The investigations revealed distinct differences in the bacterial composition of the mucosa-associated flora between interleukin-2-deficient mice and healthy controls. Fluorescence in situ hybridization identified up to 10% of the mucosa-associated flora in interleukin-2-deficient mice as Escherichia coli, whereas no E. coli was detected in the mucosa from healthy wild-type mice. This finding was consistent with the results from comparative 16S rDNA analysis. About one-third of the clones analyzed from 16S rDNA libraries of interleukin-2-deficient mice represented Enterobacteriaceae, whereas none of the clones analyzed from the healthy controls harbored 16S rDNA from Enterobacteriaceae. The abundance of E. coli in the colonic mucosa of interleukin-2-deficient mice strongly suggests a

participation in the pathogenesis of colitis in the interleukin-2-deficient mouse model for inflammatory bowel disease.

Shea, R. J. and M. H. Mulks (2002). "ohr, Encoding an Organic Hydroperoxide Reductase, Is an In Vivo-Induced Gene in Actinobacillus pleuropneumoniae." Infect. Immun. **70**(2): 794-802.

http://iai.asm.org/cgi/content/abstract/70/2/794

Actinobacillus pleuropneumoniae is the causative agent of porcine pleuropneumonia, a disease characterized by pulmonary necrosis and hemorrhage caused in part by neutrophil degranulation. In an effort to understand the pathogenesis of this disease, we have developed an in vivo expression technology (IVET) system to identify genes that are specifically up-regulated during infection. One of the genes that we have identified as being induced in vivo is ohr, encoding organic hydroperoxide reductase, an enzyme that could play a role in detoxification of organic hydroperoxides generated during infection. Among the 12 serotypes of A. pleuropneumoniae, ohr was found in only serotypes 1, 9, and 11. This distribution correlated with increased resistance to cumene hydroperoxide, an organic hydroperoxide, but not to hydrogen peroxide or to paraquat, a superoxide generator. Functional assays of Ohr activity demonstrated that A. pleuropneumoniae serotype 1 cultures, but not serotype 5 cultures, were able to degrade cumene hydroperoxide. In A. pleuropneumoniae serotype 1, expression of ohr was induced by cumene hydroperoxide, but not by either hydrogen peroxide or paraquat. In contrast, an ohr gene from serotype 1 cloned into A. pleuropneumoniae serotype 5 was not induced by cumene hydroperoxide or by other forms of oxidative stress, suggesting the presence of a serotype-specific positive regulator of ohr in A. pleuropneumoniae serotype 1.

Sheehan, B. J., J. T. Bosse, et al. (2003). "Identification of Actinobacillus pleuropneumoniae Genes Important for Survival during Infection in Its Natural Host." Infect. Immun. **71**(7): 3960-3970.

http://iai.asm.org/cgi/content/abstract/71/7/3960

Actinobacillus pleuropneumoniae is a strict respiratory tract pathogen of swine and is the causative agent of porcine pleuropneumonia. We have used signature-tagged mutagenesis (STM) to identify genes required for survival of the organism within the pig. A total of 2,064 signature-tagged Tn10 transposon mutants were assembled into pools of 48 each, and used to inoculate pigs by the endotracheal route. Out of 105 mutants that were consistently attenuated in vivo, only 11 mutants showed a >2-fold reduction in growth in vitro compared to the wild type, whereas 8 of 14 mutants tested showed significant levels of attenuation in pig as evidenced from competitive index experiments. Inverse PCR was used to generate DNA sequence of the chromosomal domains flanking each transposon insertion. Only one sibling pair of mutants was identified, but three apparent transposon insertion hot spots were found--an anticipated consequence of the use of a Tn10-based system. Transposon insertions were found within 55 different loci, and similarity (BLAST) searching identified possible analogues or homologues for all but four of these. Matches included proteins putatively involved in metabolism and transport of various nutrients or unknown substances, in stress responses, in gene regulation, and in the production of cell surface components. Ten of the sequences have homology with genes involved in lipopolysaccharide and capsule production. The results highlight the importance of genes involved in energy metabolism, nutrient uptake and stress responses for the survival of A. pleuropneumoniae in its natural host: the pig.

Sommer, F., H. Wilken, et al. (2004). "Systemic Th1 Immunization of Mice against Helicobacter pylori Infection with CpG Oligodeoxynucleotides as Adjuvants Does Not Protect from Infection but Enhances Gastritis." Infect. Immun. **72**(2): 1029-1035.

http://iai.asm.org/cgi/content/abstract/72/2/1029

Recent reports have suggested that oral vaccination of mice against Helicobacter pylori is dependent on a Th1-mediated immune response. However, oral vaccination in mice neither induces sterilizing immunity nor leads to complete protection from disease. Therefore, in this study we investigated whether a systemic subcutaneous immunization against H. pylori by using CpG oligodeoxynucleotides as a Th1 adjuvant could achieve protection in a mouse model of H. pylori infection. CpG oligodeoxynucleotides are known for their ability to induce nearly entirely Th1-biased immune responses and may be approved for human use in future. Immunization of mice with H. pylori lysate and CpG induced a strong local and systemic Th1 immune response. Despite this strong Th1 response, mice were not protected from infection with H. pylori yet had a 10-fold reduction in the number of H. pylori in the gastric mucosa compared to nonimmunized mice. Of note, reduction of the bacterial density in immunized mice was accompanied by a significantly enhanced gastritis. Hence, systemic Th1 immunization of mice, even though being able to reduce the bacterial load in the stomach, is associated with aggravated pathology.

Spears, P. A., L. M. Temple, et al. (2003). "Unexpected Similarities between Bordetella avium and Other Pathogenic Bordetellae." Infect. Immun. **71**(5): 2591-2597.

http://iai.asm.org/cgi/content/abstract/71/5/2591

Bordetella avium causes an upper respiratory tract disease (bordetellosis) in avian species. Commercially raised turkeys are particularly susceptible. Like other pathogenic members of the genus Bordetella (B. pertussis and B. bronchiseptica) that infect mammals, B. avium binds preferentially to ciliated tracheal epithelial cells and produces similar signs of disease. These similarities prompted us to study bordetellosis in turkeys as a possible nonmammalian model for whooping cough, the exclusively human childhood disease caused by B. pertussis. One impediment to accepting such a host-pathogen model as relevant to the human situation is evidence suggesting that B. avium does not express a number of the factors known to be associated with virulence in the other two Bordetella species. Nevertheless, with signature-tagged mutagenesis, four avirulent mutants that had lesions in genes orthologous to those associated with virulence in B. pertussis and B. bronchiseptica (bvgS, fhaB, fhaC, and fimC) were identified. None of the four B. avium genes had been previously identified as encoding factors associated with virulence, and three of the insertions (in fhaB, bvgS, and fimC) were in genes or gene clusters inferred as being absent or incomplete in B. avium, based upon the lack of DNA sequence similarities in hybridization studies and/or the lack of immunological cross-reactivity of the putative products. We further found that the genotypic arrangements of most of the B. avium orthologues were very similar in all three Bordetella species. In vitro tests, including hemagglutination, tracheal ring binding, and serum sensitivity, helped further define the phenotypes conferred by the mutations. Our findings strengthen the connection between the causative agents and the pathogenesis of bordetellosis in all hosts and may help explain the striking similarities of the histopathologic characteristics of this upper airway disease in avian and mammalian species.

Straubinger, R. K., A. Greiter, et al. (2003). "Quantitative Evaluation of Inflammatory and Immune Responses in the Early Stages of Chronic Helicobacter pylori Infection." Infect. Immun. **71**(5): 2693-2703.

http://iai.asm.org/cgi/content/abstract/71/5/2693

The early consequences of Helicobacter pylori infection and the role of bacterial virulence determinants in disease outcome remain to be established. The present study sought to measure the development of host inflammatory and immune responses and their relationship to the putative bacterial virulence factors cag pathogenicity island (cagPAI), vacA allele, and oipA in combination with bacterial colonization density in a feline model of the early stages of H. pylori infection. Gastric tissues obtained from infected and uninfected cats were evaluated for H. pylori ureB, cagPAI, vacA allele, and oipA and colonization density (urease, histology, and real-time PCR). Inflammation was assessed by measuring mRNA upregulation of gamma interferon (IFN-{gamma}), interleukin (IL)-1{alpha}, IL-1{beta}, IL-4, IL-6, IL-8, IL-10, and IL-12 p40 and histopathology. The mucosal immune response was characterized by morphometric analysis of lymphoid follicles and by differentiating lymphocyte populations with antibodies against surface markers. Infecting H. pylori strains were positive for vacAs1 but lacked cagPAI and an active oipA gene. Colonization density was uniform throughout the stomach. Upregulation of IFN-{gamma}, IL-1{alpha}, IL-1{beta}, and IL-8 and increased severity of inflammatory infiltrates and fibrosis were observed in infected cats. The median number and total area of lymphoid aggregates were 5 and 10 times greater, respectively, in the stomachs of infected than uninfected cats. Secondary lymphoid follicles in uninfected cats were rare and positive for BLA.36 and B220 but negative for CD3 and CD79{alpha}, whereas in infected cats they were frequent and positive for BLA.36, CD79{alpha}, and CD3 but negative for B220. Upregulation of IFN-{gamma}, IL-1{alpha}, IL-1{beta}, and IL-8 and marked hyperplasia of secondary lymphoid follicles are early consequences of H. pylori infection in cats. The response appears to be similar to that of infected people, particularly children, can develop independently of the pathogenicity factors cagPAI and oipA, and is not correlated with the degree of colonization density or urease activity.

Swords, W. E., D. L. Chance, et al. (2002). "Acylation of the Lipooligosaccharide of Haemophilus influenzae and Colonization: an htrB Mutation Diminishes the Colonization of Human Airway Epithelial Cells." Infect. Immun. **70**(8): 4661-4668.

http://iai.asm.org/cgi/content/abstract/70/8/4661

Haemophilus influenzae is a commensal and opportunistic pathogen of the human airways. A number of surface molecules contribute to colonization of the airways by H. influenzae, such as adhesins, including structures found in the lipooligosaccharide (LOS). A human bronchiolar xenograft model was employed to investigate the host-bacterial interactions involved in the colonization of the airway by H. influenzae. Differential display was used to identify H. influenzae mRNA that reflect genes which were preferentially expressed in the xenograft compared to growth. Eleven mRNA fragments had consistent increased expression when the bacteria grew in xenografts. On sequencing these fragments, eight open reading frames were identified. Three of these had no match in the NCBI or the TIGR database, while an additional three were homologous to genes involved in heme or iron acquisition and utilization: two of the mRNAs encoded proteins homologous to enzymes involved in LOS biosynthesis: a heptosyl transferase (rfaF) involved in the synthesis of the LOS core and a ketodeoxyoctonate phosphate-dependent acyltransferase (htrB) that performs one of the late acylation reactions in lipid A synthesis. Inoculation of human bronchiolar xenografts revealed a significant reduction in colonization capacity by htrB mutants. In vitro, htrB mutants elicited lesser degrees of cytoskeletal rearrangement and less stimulation of host cell signaling with 16HBE14o- cells and decreased intracellular survival. These results implicate acylation of H. influenzae lipid A as playing a key role in the organisms' colonization of the normal airway.

Tanabe, H., T. Ayabe, et al. (2005). "Mouse Paneth Cell Secretory Responses to Cell Surface Glycolipids

of Virulent and Attenuated Pathogenic Bacteria." Infect. Immun. **73**(4): 2312-2320.

http://iai.asm.org/cgi/content/abstract/73/4/2312

Mouse Paneth cells respond to bacteria and bacterial cell surface antigens by discharging secretory granules into the lumen of small intestinal crypts (T. Ayabe et al., Nat. Immunol. 1:113-118, 2000). To investigate mechanisms regulating these responses, purified surface glycolipid molecules with known acyl chain modifications and attenuated properties were tested for the ability to stimulate Paneth cell secretion. The antigens included lipopolysaccharide (LPS) from wild-type and msbB-null Escherichia coli and phoP-null and phoP-constitutive Salmonella enterica serovar Typhimurium strains, as well as LPS, lipid A, and lipoteichoic acid from Pseudomonas aeruginosa and Listeria monocytogenes grown in Mg2+-limited media. Measurements of total secreted protein, secreted lysozyme, and the bactericidal peptide activities of collected secretions showed that the purified antigens elicited similar secretory responses from Paneth cells in mouse crypts ex vivo, regardless of glycolipid acyl chain modification. Despite their impaired Tlr4 pathway, Paneth cells in ex vivo C3H/HeJ mouse crypts released equivalent amounts of bactericidal peptide activity in response to purified bacterial antigens, including lipid A. Thus, mouse Paneth cells respond equivalently to purified bacterial cell envelope glycolipids, regardless of functional Tlr4, the structural properties of glycolipid acyl chains, or their association with virulence in humans.

Tarr, C. L., T. M. Large, et al. (2002). "Molecular Characterization of a Serotype O121:H19 Clone, a Distinct Shiga Toxin-Producing Clone of Pathogenic Escherichia coli." Infect. Immun. **70**(12): 6853-6859.

http://iai.asm.org/cgi/content/abstract/70/12/6853

Most illnesses caused by Shiga toxin-producing Escherichia coli (STEC) have been attributed to E. coli serotype O157:H7, but non-O157 STEC infections are now increasingly recognized as public health problems worldwide. The O121:H19 serotype is being isolated more frequently from clinical specimens and has been implicated in one waterborne outbreak. We used multilocus virulence gene profiling, a PCR-based assay, to characterize the virulence gene content of 24 isolates of serotype O121:H19 and nonmotile variants. We also performed multilocus enzyme electrophoresis and multilocus sequencing to establish the clonal relatedness of O121 isolates and to elucidate the relationship of O121 to common STEC clones. The 24 isolates were found to represent a single bacterial clone, as there was no allelic variation across 18 enzyme loci among the isolates. The complete nucleotide sequence of the intimin gene differed by four substitutions from that of the epsilon (Int-{varepsilon}) allele of O103:H2 strain PMK5. The typical O121 virulence gene profile was similar to the profiles of enterohemorrhagic E. coli (EHEC) clones of E. coli: it included a Shiga toxin 2 gene (stx2), two genes on the EHEC plasmid (toxB and ehxA), and the gene encoding intimin (eae). Despite the similarities, putative virulence genes distributed on O islands--large chromosomal DNA segments present in the O157:H7 genome--were useful for discriminating among STEC serotypes and the O121:H19 clone had a composite profile that was distinct from the profiles of the other major EHEC clones of pathogenic E. coli. On the basis of sequencing analysis with 13 housekeeping genes, the O121:H19 clone did not fall into any of the four classical EHEC and enteropathogenic E. coli groups but instead was closely related to two eae-negative STEC strains.

Teel, L. D., A. R. Melton-Celsa, et al. (2002). "One of Two Copies of the Gene for the Activatable Shiga Toxin Type 2d in Escherichia coli O91:H21 Strain B2F1 Is Associated with an Inducible Bacteriophage." Infect. Immun. **70**(8): 4282-4291.

http://iai.asm.org/cgi/content/abstract/70/8/4282

Shiga toxin (Stx) types 1 and 2 are encoded within intact or defective temperate bacteriophages in Stx-producing Escherichia coli (STEC), and expression of these toxins is linked to bacteriophage induction. Among Stx2 variants, only stx2e from one human STEC isolate has been reported to be carried within a toxin-converting phage. In this study, we examined the O91:H21 STEC isolate B2F1, which carries two functional alleles for the potent activatable Stx2 variant toxin, Stx2d, for the presence of Stx2d-converting bacteriophages. We first constructed mutants of B2F1 that produced one or the other Stx2d toxin and found that the mutant that produced only Stx2d1 made less toxin than the Stx2d2-producing mutant. Consistent with that result, the Stx2d1-producing mutant was attenuated in a streptomycin-treated mouse model of STEC infection. When the mutants were treated with mitomycin C to promote bacteriophage induction, Vero cell cytotoxicity was elevated only in extracts of the Stx2d1-producing mutant. Additionally, when mice were treated with ciprofloxacin, an antibiotic that induces the O157:H7 Stx2-converting phage, the animals were more susceptible to the Stx2d1-producing mutant. Moreover, an stx2d1-containing lysogen was isolated from plaques on strain DH5{alpha} that had been exposed to lysates of the mutant that produced Stx2d1 only, and supernatants from that lysogen transformed with a plasmid encoding RecA were cytotoxic when the lysogen was induced with mitomycin C. Finally, electron-microscopic examination of extracts from the Stx2d1-producing mutant showed hexagonal particles that resemble the prototypic Stx2-converting phage 933W. Together these observations provide strong evidence that expression of Stx2d1 is bacteriophage associated. We conclude that despite the sequence similarity of the stx2d1- and stx2d2-flanking regions in B2F1, Stx2d1 expression is repressed within the context of its toxin-converting phage while Stx2d2 expression is independent of phage induction.

Vallance, B. A., W. Deng, et al. (2002). "Mice Lacking T and B Lymphocytes Develop Transient Colitis and Crypt Hyperplasia yet Suffer Impaired Bacterial Clearance during Citrobacter rodentium Infection." Infect. Immun. **70**(4): 2070-2081.

http://iai.asm.org/cgi/content/abstract/70/4/2070

The bacterial pathogen Citrobacter rodentium belongs to a family of gastrointestinal pathogens that includes enteropathogenic and enterohemorrhagic Escherichia coli and is the causative agent of transmissible colonic hyperplasia in mice. The molecular mechanisms used by these pathogens to colonize host epithelial surfaces and form attaching and effacing (A/E) lesions have undergone intense study. In contrast, little is known about the host's immune response to these infections and its importance in tissue pathology and bacterial clearance. To address these issues, wild-type mice and mice lacking T and B lymphocytes (RAG1 knockout [KO]) were infected with C. rodentium. By day 10 postinfection (p.i.), both wild-type and RAG1 KO mice developed colitis and crypt hyperplasia, and these responses became more exaggerated in wild-type mice over the next 2 weeks, as they cleared the infection. By day 24 p.i., bacterial clearance was complete, and the colitis had subsided; however, crypt heights remained increased. In contrast, inflammatory and crypt hyperplastic responses in the RAG1 KO mice were transient, subsiding after 2 weeks. By day 24 p.i., RAG1 KO mice showed no signs of bacterial clearance and infection was often fatal. Surprisingly, despite remaining heavily infected, tissues from RAG1 KO mice surviving the acute colitis showed few signs of disease. These results thus emphasize the important contribution of the host immune response during infection by A/E bacterial pathogens. While T and/or B lymphocytes are essential for host defense against C. rodentium, they also mediate much of the tissue pathology and disease symptoms that occur during infection.

Weijer, S., M. E. Sewnath, et al. (2003). "Interleukin-18 Facilitates the Early Antimicrobial Host Response

to Escherichia coli Peritonitis." Infect. Immun. **71**(10): 5488-5497.

http://iai.asm.org/cgi/content/abstract/71/10/5488

To determine the role of endogenous interleukin-18 (IL-18) during peritonitis, IL-18 gene-deficient (IL-18 KO) mice and wild-type mice were intraperitoneally (i.p.) infected with Escherichia coli, the most common causative agent found in septic peritonitis. Peritonitis was associated with a bacterial dose-dependent increase in IL-18 concentrations in peritoneal fluid and plasma. After infection, IL-18 KO mice had significantly more bacteria in the peritoneal lavage fluid and were more susceptible for progression to systemic infection at 6 and 20 h postinoculation than wild-type mice. The relative inability of IL-18 KO mice to clear E. coli from the abdominal cavity was not due to an intrinsic defect in the phagocytosing capacity of their peritoneal macrophages or neutrophils. IL-18 KO mice displayed an increased neutrophil influx into the peritoneal cavity, but these migratory neutrophils were less activate, as reflected by a reduced CD11b surface expression. These data suggest that endogenous IL-18 plays an important role in the early antibacterial host response during E. coli-induced peritonitis.

Weiss, D. J., O. A. Evanson, et al. (2002). "Differential Responses of Bovine Macrophages to Mycobacterium avium subsp. paratuberculosis and Mycobacterium avium subsp. avium." Infect. Immun. **70**(10): 5556-5561.

http://iai.asm.org/cgi/content/abstract/70/10/5556

Mycobacterium avium subsp. paratuberculosis and Mycobacterium avium subsp. avium are antigenically and genetically similar organisms; however, they differ in their virulence for cattle. M. avium subsp. paratuberculosis causes a chronic intestinal infection leading to a chronic wasting disease termed paratuberculosis or Johne's disease, whereas M. avium subsp. avium causes only a transient infection. We compared the response of bovine monocyte-derived macrophages to ingestion of M. avium subsp. paratuberculosis and M. avium subsp. avium organisms by determining organism survival, superoxide and nitric oxide production, and expression of the cytokines tumor necrosis factor alpha (TNF-{alpha}), gamma interferon (IFN-{gamma}), interleukin-8 (IL-8), IL-10, IL-12, and granulocyte-monocyte colony-stimulating factor (GM-CSF). Unlike M. avium subsp. paratuberculosis, macrophages were able to kill approximately half of the M. avium subsp. avium organisms after 96 h of incubation. This difference in killing efficiency was not related to differences in nitric oxide or superoxide production. Compared to macrophages activated with IFN-{gamma} and lipopolysaccharide, macrophages incubated with M. avium subsp. paratuberculosis showed greater expression of IL-10 and GM-CSF (all time points) and IL-8 (72 h) and less expression of IL-12 (72 h), IFN-{gamma} (6 h), and TNF-{alpha} (6 h). When cytokine expression by macrophages incubated with M. avium subsp. paratuberculosis was compared to those of macrophages incubated with M. avium subsp. avium, M. avium subsp. paratuberculosis-infected cells showed greater expression of IL-10 (6 and 24 h) and less expression of TNF-{alpha} (6 h). Therefore, the combination of inherent resistance to intracellular degradation and suppression of macrophage activation through oversecretion of IL-10 may contribute to the virulence of M. avium subsp. paratuberculosis in cattle.

Infection, Genetics and Evolution (2)

Mahdi, M., E. M. Elamin, et al. (2005). "Sudanese mucosal leishmaniasis: isolation of a parasite within the Leishmania donovani complex that differs genotypically from L. donovani causing classical visceral leishmaniasis." Infection, Genetics and Evolution **5**(1): 29.

http://www.sciencedirect.com/science/article/B6W8B-4CXMP6N-1/2/f59e67a951becd2a8f4a599a8a660eca

Mucosal leishmaniasis, which is a sporadic disease in the Sudan, was shown by isoenzyme characterization and PCR to be caused by Leishmania donovani. However, it was not clear if the parasite was exactly the same strain as that causing visceral leishmaniasis (VL), or of a different strain. We utilized a new generation of molecular DNA markers, minisatellites and kinetoplast DNA, for rapid characterization of the parasite. The results show that the genotypes of some of the parasites causing VL are different from those causing mucosal leishmaniasis. The L. donovani isolates causing visceral disease, as well as post-kala-azar mucosal leishmaniasis (PKML), have been shown to possess characteristic haplotypes. However, sequencing of a portion of the cytochrome oxidase II (COII) gene indicates that the parasite that invades the oral mucosa is divergent from other parasites causing VL. It appears to possess features of a more ancestral parasite with pronounced sequence homology to L. major. This agrees with earlier studies where isolates of mucosal leishmaniasis have been shown to possess an isoenzyme profile distinct from L. donovani and a different geographical distribution, albeit often overlapping with that of L. donovani.

Prager, R., A. Liesegang, et al. (2002). "Clonal diversity of Shiga toxin-producing Escherichia coli O103:H2/H- in Germany." Infection, Genetics and Evolution **1**(4): 265.

http://www.sciencedirect.com/science/article/B6W8B-45C1JW2-2/2/6e11ca8e750cbf012143d41ba48986ef

Shiga toxin producing Escherichia coli O103:H2/H- belong to the third most frequently isolated EHEC serotypes in Germany following isolates of O157:H7/H- and O26:H11/H-. A total of 145 respective E. coli 103 isolates from single cases of diarrhoea and haemolytic uremic syndrome (HUS) in 1997-2000 were characterised by a range of molecular subtyping methods (PFGE, P-gene profiling, ribotyping, electrotyping) and phage typing in order to analyse their genetic relatedness and the practicability for new epidemiological tracing back. All isolates cluster into a distinct EHEC subgroup and reveal a high clonal diversity together with a considerable stability. Since strains of this serotype rank up to the third most frequently isolated EHEC in Germany a large population of this serotype, and therefore, a great supply of such strains may exist in this country.

Insect Biochemistry and Molecular Biology (34)

Albert, S., J. Klaudiny, et al. (1999). "Molecular characterization of MRJP3, highly polymorphic protein of honeybee (Apis mellifera) royal jelly." Insect Biochemistry and Molecular Biology **29**(5): 427.

http://www.sciencedirect.com/science/article/B6T79-3WJFBBW-4/2/3c4c5a49378e976e150875261c2baf12

Major proteins of honey bee (Apis mellifera) royal jelly are members of the MRJP protein family. One MRJP protein termed MRJP3 exhibits a size polymorphism as detected by SDS-PAGE. In this report we show that polymorphism of the MRJP3 protein is a consequence of the polymorphism of a region with a variable number of tandem repeats (VNTR) located at the C-terminal part of the MRJP3 coding region. We present the characterization of five polymorphic alleles of MRJP3 by DNA sequencing. By PCR analyses, at least 10 alleles of distinct sizes were found in randomly sampled bees. Studies with nurse bees from a single honeybee colony revealed both Mendelian inheritance and very high variability of the MRJP3 genomic locus. The high variability and simple detection of the MRJP3 polymorphism may be useful for genotyping of individuals in studies of the honeybee.

Arnon, T., T. Potikha, et al. (2005). "Bj[alpha]IT: a novel scorpion [alpha]-toxin selective for insects--unique pharmacological tool." Insect Biochemistry and Molecular Biology **35**(3): 187.

http://www.sciencedirect.com/science/article/B6T79-4F4WXNM-1/2/fff14ec0d6df281e2b2a8192a4edc044

Long-chain neurotoxins derived from the venom of the Buthidae scorpions, which affect voltage-gated sodium channels (VGSCs) can be subdivided according to their toxicity to insects into insect-selective excitatory and depressant toxins ([beta]-toxins) and the [alpha]-like toxins which affect both mammals and insects.In the present study by the aid of reverse-phase HPLC column chromatography, RT-PCR, cloning and various toxicity assays, a new insect selective toxin designated as Bj[alpha]IT was isolated from the venom of the Judean Black Scorpion (Buthotus judaicus), and its full primary sequence was determined: MNYLVVICFALLLMTVVESGRDAYIADNLNCAYTCGSNSYCNTECTKNGAVSGYCQWLGKYGN ACWCINLPDKVPIRIPGACR (leader sequence is underlined).Despite its lack of toxicity to mammals and potent toxicity to insects, Bj[alpha]IT reveals an amino acid sequence and an inferred spatial arrangement that is characteristic of the well-known scorpion [alpha]-toxins highly toxic to mammals. Bj[alpha]ITs sharp distinction between insects and mammals was also revealed by its effect on sodium conductance of two cloned neuronal VGSCs heterloguously expressed in Xenopus laevis oocytes and assayed with the two-electrode voltage-clamp technique. Bj[alpha]IT completely inhibits the inactivation process of the insect para/tipE VGSC at a concentration of 100 nM, in contrast to the rat brain Nav1.2/[beta]1 which is resistant to the toxin.The above categorical distinction between mammal and insect VGSCs exhibited by Bj[alpha]IT enables its employment in the clarification of the molecular basis of the animal group specificity of scorpion venom derived neurotoxic polypeptides and voltage-gated sodium channels.

Bischof, L. J. and E. E. Enan (2004). "Cloning, expression and functional analysis of an octopamine receptor from Periplaneta americana." Insect Biochemistry and Molecular Biology **34**(6): 511.

http://www.sciencedirect.com/science/article/B6T79-4BVNV0B-2/2/4d549717d457fce61d070519710e113a

Octopamine regulates multiple physiological functions in invertebrates. The biological effects of octopamine and the pharmacology of octopamine receptors have been extensively studied in the American cockroach, Periplaneta americana. This paper reports the cloning of the first octopamine receptor from Periplaneta americana. A cDNA encoding a putative 7 transmembrane receptor was isolated from the head of Periplaneta americana. The encoded protein contains 628 amino acids and has sequence similarity to other biogenic amine receptors. This protein was expressed in COS-7 cells for radioligand binding studies using the antagonist 3H-yohimbine. Competitive binding comparing biogenic amines that could potentially function as endogenous

ligands demonstrated this receptor had the highest affinity for octopamine (Ki=13.3 [mu]M) followed by tyramine, dopamine, serotonin and histamine. Octopamine increased both cAMP levels (EC50=1.62 [mu]M) and intracellular concentrations of calcium through the receptor expressed in HEK-293 cells. Tyramine increased levels of both of these second messengers but only at significantly higher concentrations than octopamine. The cAMP increase by octopamine was independent of the increase in calcium. Competitive binding with antagonists revealed this receptor is similar to Lym oa1 from Lymnaea stagnalis. The data indicate that this cDNA is the first octopamine receptor cloned from Periplaneta americana and therefore has been named Pa oa1.

Chiou, S.-J., J. Vanden Broeck, et al. (1998). "Cloning of the cDNA encoding Scg-SPRP, an unusual Ser-protease-related protein from vitellogenic female desert locusts (Schistocerca gregaria)." Insect Biochemistry and Molecular Biology **28**(10): 801.

http://www.sciencedirect.com/science/article/B6T79-3TWYNNS-B/2/aeeed34b8dad71f5485c723dbb60b28c

The cDNA coding for a Ser-protease-related protein (Scg-SPRP)1 was cloned from desert locust (Schistocerca gregaria) midgut. The derived amino acid sequence consists of 260 residues and shows strong sequence similarity to insect trypsin-like molecules. It is, however, likely that Scg-SPRP is not a proteolytically active enzyme and that it plays another physiologically relevant role, since two out of three residues which are indispensable for catalytic activity of Ser-proteases are replaced. Northern analysis revealed that the Scg-SPRP gene is expressed in midgut tissue and that this expression is strongly induced in adult female locusts. Moreover, the occurrence of the transcript (1.2 kb) fluctuates during the molting cycle and during the female reproductive cycle. Juvenile hormone (JH III) dependence of transcription was investigated by chemical allatectomy (precocene I) of adult females. This resulted in inhibition of vitellogenesis and in disappearance of the Scg-SPRP transcript. Expression of Scg-SPRP in precocene-treated locusts could be reinduced by additional treatment with JH III or with 20-OH-ecdysone.

Donly, B. C., M. Fuse, et al. (1996). "Characterization of the gene for leucomyosuppressin and its expression in the brain of the cockroach Diploptera punctata." Insect Biochemistry and Molecular Biology **26**(6): 627.

http://www.sciencedirect.com/science/article/B6T79-3W2T43D-G/2/fb33e2c28ebd12288029fee4b9a417bb

Using HPLC separation, radioimmunoassay, and subsequent bioassay, we have detected the presence of an active peptide, which co-elutes with the insect myoinhibitory peptide leucomyosuppressin, in the brain of the cockroach Diploptera punctata. We have isolated a cDNA encoding the precursor for this peptide from cDNA libraries representing D. punctata brain RNA. The cDNA sequence contains an open reading frame that upon translation would result in a prepropolypeptide of 96 amino acids. Proteolytic cleavage of the predicted precursor could result in several peptides, including a 10 amino acid C-terminal peptide that would, upon modification of the NH2 and COOH-terminal amino acids, be identical to the insect FLRFamide, leucomyosuppressin. No other RFamide products are predicted to be processed from the precursor. Southern blot analysis indicates that the gene is present in the D. punctata genome in a single copy. Northern blot analysis shows that the gene is predominantly expressed as a 3.8 kb mRNA in cockroach brain. Study of the expression of the leucomyosuppressin gene in D. punctata brain, using in situ hybridization, indicates that expression occurs primarily in the pars intercerebralis of the protocerebrum, a region showing abundant FMRFamide-like immunoreactive neurosecretory cells. Immunohistochemistry and HPLC coupled to

radioimmunoassay indicates that leucomyosuppressin represents a significant proportion of FMRFamide-related peptide production in the brain. However, HPLC analysis also indicates the presence of significant levels of other related peptides, demonstrating the presence of more than one FMRFamide-related gene in this insect.

Douglas, L. J., P. M. Untalan, et al. (2004). "Molecular sexing in the Mediterranean fruit fly, Ceratitis capitata." Insect Biochemistry and Molecular Biology **34**(2): 159.

http://www.sciencedirect.com/science/article/B6T79-4B4XM8C-4/2/ef01fa0ac1fa4db0a0ebed9bca8c24ef

Molecular methods have been devised for sexing Mediterranean fruit fly (medfly) individuals using minimal amounts of material from any stage of the life cycle. Molecular sexing methods are particularly valuable when material is obtained from pre-adult stages and sex identification based on morphological characters is not possible. These methods may also be useful for adult stage material in situations where only limited amounts or poorly preserved specimens are available. The sexing methods described here use the polymerase chain reaction (PCR) to amplify sequences known to originate from the sex chromosomes of this species. One method co-amplifies homologous regions of the ITS1 ribosomal DNA from both the X and Y chromosomes. Males and females are distinguished based on the restriction fragment pattern produced after digestion of the PCR products with the restriction enzyme ApoI. A second method identifies males based on the positive amplification of a repetitive DNA sequence originating from the Y chromosome. Both methods are shown to be capable of establishing the sex identity of individuals using only minimal amounts of material from any stage of the life cycle.

Fatima Grossi De Sa, M. and M. J. Chrispeels (1997). "Molecular cloning of bruchid (Zabrotes subfasciatus) [alpha]-amylase cDNA and interactions of the expressed enzyme with bean amylase inhibitors." Insect Biochemistry and Molecular Biology **27**(4): 271.

http://www.sciencedirect.com/science/article/B6T79-3RGSWMN-D/2/a24463b492251e2e4adbac5194ce60b1

[alpha]-Amylases are important digestive enzymes in weevils that infest starchy seeds, and plants have evolved proteinaceous [alpha]-amylase inhibitors ([alpha]AI) for protection. To gain a better understanding of the interaction between weevil [alpha]-amylases and [alpha]AIs, we cloned the [alpha]-amylase cDNA of Zabrotes subfasciatus larvae. Larvae of this bruchid infest seeds of cultivated varieties of the common bean, Phaseolus vulgaris, although the seeds contain high levels of an [alpha]AI. The [alpha]-amylase cDNA, called ZsAmy, encodes a mature protein of 466 amino acids with a signal peptide of 17 amino acids. This protein has 50-60% amino acid identity with the other five known insect [alpha]-amylases. Three amino acid residues known to be important for catalysis and three histidine residues involved in substrate binding are conserved in the derived amino acid sequence of ZsAmy. Expression of ZsAmy with a baculovirus vector in cultured insect cells resulted in the production of active [alpha]-amylase. [alpha]AI-1, the form of the inhibitor found in cultivated beans, does not inhibit larval or expressed bruchid [alpha]-amylase, but [alpha]AI-2, a form of the inhibitor found in certain wild bean accessions, does inhibit the larval, as well as the expressed bruchid [alpha]-amylase. These and other observations lead to the conclusion that ZsAmy encodes the major larval amylase of this bruchid species.

Gorman, M. J., O. V. Andreeva, et al. (2000). "Molecular characterization of five serine protease genes

cloned from Anopheles gambiae hemolymph." Insect Biochemistry and Molecular Biology **30**(1): 35.

http://www.sciencedirect.com/science/article/B6T79-3XYG4HF-5/2/a4f9e5193c88d125cdad994ea261b30a

We identified five new serine protease cDNAs from the hemolymph of the malaria vector, Anopheles gambiae. All five show sequence similarity to genes thought to be involved in vertebrate or invertebrate defense responses. Sp14A, Sp14D2 and Sp22D demonstrate changes in transcript abundance in response to bacteria injections. Sp14A and Sp14D2, as well as the previously characterized Sp14D1, are induced by infection with the malaria parasite, Plasmodium berghei. These three proteases, along with Sp18D, are related to a group of secreted proteases that have amino-terminal clip domains and trypsin-like substrate specificity. BLAST results and phylogenetic analyses group Sp14A, Sp14D1 and Sp14D2 with the Drosophila protease EASTER, and three prophenoloxidase activating enzymes from other insects. EASTER's substrate is SPAETZLE, a ligand involved in embryogenesis but also in activating anti-microbial peptide synthesis. Their similarity to EASTER and immune inducibility suggest that one of these proteases may activate a SPAETZLE-like ligand during anti-parasite responses in mosquitoes. Alternatively, as potential prophenoloxidase activators, Sp14A, Sp14D1 or Sp14D2 may play a role in melanotic encapsulation of Plasmodium.

Gu, S. and J. H. Willis (2003). "Distribution of cuticular protein mRNAs in silk moth integument and imaginal discs." Insect Biochemistry and Molecular Biology **33**(12): 1177.

http://www.sciencedirect.com/science/article/B6T79-49KS3H1-1/2/ff24497ebb6e9c120450d693250ec9e2

The distributions of mRNAs for two cuticular proteins of Hyalophora cecropia were examined with RT-PCR and in situ hybridization. For major regions of larval and pupal cuticle, there was a strong correspondence between the type of cuticle and the predominant cuticular protein message found. Epidermal cells underlying soft cuticle had mRNA for HCCP12, with a RR-1 consensus attributed to soft cuticle, while the epidermal cells associated with hard cuticle had predominantly mRNA for HCCP66, a protein with the RR-2 consensus attributed to hard cuticle. Both messages were found in all areas of the pupal fore- and hind-wings, with modest area-specific difference in concentration being much less than differences in the relative abundance of these cuticular proteins.mRNA for HCCP12 was present in imaginal discs of feeding larvae of H cecropia. Data from Bombyx mori available at SilkBase (http://www.ab.a.u-tokyo.ac.jp/silkbase/) revealed that imaginal discs from feeding larvae had abundant mRNA for RR-1 cuticular proteins, representing six distinct gene products. Only discs from spinning larvae had mRNAs that coded for RR-2 proteins arising from 10 distinct genes. Thus, lepidopteran wing imaginal discs can no longer be regarded as inactive in larval cuticle production.

Guerrero, F. D. (2000). "Cloning of a horn fly cDNA, Hi[alpha]E7, encoding an esterase whose transcript concentration is elevated in diazinon-resistant flies." Insect Biochemistry and Molecular Biology **30**(11): 1107.

http://www.sciencedirect.com/science/article/B6T79-416BXBB-F/2/f9576e4587290da188db31a1f3a0984a

Reverse transcriptase-polymerase chain reaction (PCR) was used to clone two esterase cDNAs from a diazinon-resistant field population of horn flies that expresses qualitative and quantitative

differences in esterases compared with a susceptible population. The open reading frame from one of the esterase cDNAs, Hi[alpha]E7, exhibits substantial amino-acid identity to an esterase associated with diazinon resistance in Lucilia cuprina. RNA Northern blots showed that Hi[alpha]E7 mRNA was more abundant in the diazinon-resistant population than the susceptible population. DNA copy number analysis did not reveal major differences in Hi[alpha]E7 gene copy number between the two populations. The full-length cDNA to Hi[alpha]E7 was cloned and sequenced, and found to contain all of the highly conserved sequence elements associated with carboxyl/cholinesterases. The Hi[alpha]E7 homologs in diazinon-resistant strains of L. cuprina and Musca domestica have been shown to possess an amino-acid substitution conferring diazinon hydrolytic activity to the esterase enzyme. This amino-acid substitution was not found in diazinon-resistant horn flies examined by allele-specific PCR. Individual flies from the resistant field population were phenotyped as diazinon-resistant or diazinon-susceptible by topical diazinon application bioassays and total RNA isolated and hybridized to Hi[alpha]E7 probe in ribonuclease protection assays. Hi[alpha]E7 transcript was expressed at a five-fold higher level in resistant female individual flies than in susceptible female individuals.

He, M. and D. S. Haymer (1994). "The actin gene family in the oriental fruit fly Bactrocera dorsalis. Muscles specific actins." Insect Biochemistry and Molecular Biology **24**(9): 891.

http://www.sciencedirect.com/science/article/B6T79-47PR7BM-2C/2/6dc92f0ab396977f3fb316c9b2c9354a

The actin protein is a critical protein in eukaryotic cells. Four actin genes, constituting what appear to be a set of muscle specific actin genes, have been isolated from the genome of the oriental fruit fly Bactrocera dorsalis. DNA sequences have been determined for the coding as well as 3' and 5' flanking regions for each of these genes. These genes have also been characterized in terms of RNA expression patterns, and comparisons have been made to actin genes from other species. Consistent with other actins, there is a high degree of amino acid sequence conservation in the coding regions of these genes. However, even within the coding regions codon usage patterns in the oriental fruit fly are quite different from some other well characterized species. In addition, the DNA sequences in the intermediate 3' and 5' flanking regions exhibit virtually no detectable sequence homology both within and between species. In terms of nitrons, three of the four actin genes from the oriental fruit fly described here have a single intervening sequence. Two of these genes share the same intron position with the two muscle specific actin genes act79B and act88F from Drosophila melanogaster and with one muscle specific actin gene CcA1 from the Mediterranean fruit fly, Ceratitis capitata. Another gene from the oriental fruit fly shares the same intron position as the muscle specific actin gene act57B from D. melanogaster. Such conservation of intron positioning between species is highly unusual among previously characterized actin genes. Using unique sequences found in the 3' untraslated regions, gene specific probes have also been constructed. These have been used to detect the expression patterns of individual genes in a temporal and spatial manner. Each of the four genes examined here show differential patterns of expression. The patterns indicate that all four genes are most likely to encode muscle specific actins.

Huang, T.-s., O. Melefors, et al. (1999). "An atypical Iron-Responsive Element (IRE) within crayfish ferritin mRNA and an Iron Regulatory Protein 1 (IRP1)-like protein from crayfish hepatopancreas." Insect Biochemistry and Molecular Biology **29**(1): 1.

http://www.sciencedirect.com/science/article/B6T79-3VNPHG5-1/2/1846ac7523f763944fb85efcfc48c36d

A putative crayfish iron-responsive element (IRE) is present in the 5'-untranslated region of the

crayfish ferritin mRNA. The putative crayfish IRE is in a cap-proximal position and shares most of the structural features of the consensus IRE, but the RNA stem-loop structure contains a bulge of a guanine instead of a cytosine at the expected position, so far thought to be a hallmark of IREs. By using an electromobility shift assay this IRE was shown to specifically bind purified recombinant human iron regulatory protein 1 (IRP1) as well as a factor(s) present in a homogenate of crayfish hepatopancreas, likely to be a crayfish IRP1 homologue. With mutations in the crayfish IRE, the affinity of IRP to IRE was drastically decreased. A cDNA2 encoding an IRP1-like protein was cloned from the hepatopancreas of crayfish. This protein has sequence similarities to IRP1, and contains all the active-site residues of aconitase, two putative RNA-binding regions and a putative contact site between RNA and IRP. These results show that a crayfish IRE, lacking the bulged C, can bind IRP1 in vitro and that an IRP1-like protein present in crayfish hepatopancreas may have both aconitase and RNA-binding activities.

Iwami, M., I. Furuya, et al. (1996). "Bombyxin-related peptides: cDNA structure and expression in the brain of the hornworm Agrius convoluvuli." Insect Biochemistry and Molecular Biology **26**(1): 25.

http://www.sciencedirect.com/science/article/B6T79-3Y2N09C-K/2/9975c340000ec4498f281e56ee869d2e

We have cloned three cDNAs from the sweet potato hornworm Agrius convolvuli that encode precursor molecules for peptides structurally related to bombyxin, an insulin-related brain secretory peptide in Bombyx mori. The Agrius bombyxin-related peptide (ABRP) cDNAs are classified into type A and B according to their sequence similarity. The prepro-ABRPs deduced from the cDNA sequences have the insulin-like domain organization of signal peptide/B chain/C peptide/A chain. The ABRP transcripts in Agrius brain were shown to locate in four pairs of medial neurosecretory cells, the homologous group of neurosecretory cells that produce bombyxins in Bombyx brain. Genomic Southern analysis indicated the presence of multiple copies of ABRP gene in the Agrius genome. Results showed that the ABRP genes are remarkably different from the vertebrate insulin genes in the number of copy and spatial localization of the transcripts.

Jamroz, R. C., F. D. Guerrero, et al. (1998). "Role of the kdr and super-kdr sodium channel mutations in pyrethroid resistance: correlation of allelic frequency to resistance level in wild and laboratory populations of horn flies (Haematobia irritans)." Insect Biochemistry and Molecular Biology **28**(12): 1031.

http://www.sciencedirect.com/science/article/B6T79-3VBSBR0-F/2/6afef567ad04ee1f0e4193ba6208b38f

The kdr and super-kdr point mutations found in the insect sodium channel gene are postulated to confer knockdown resistance (kdr) to pyrethroids. Using an allele-specific PCR assay to detect these mutations in individual horn flies, Haematobia irritans (L.), we determined the allelic frequency of the kdr and super-kdr mutations in several wild and laboratory populations. Wild populations with very similar allelic frequencies had resistance levels that ranged widely from 3- to 18-fold relative to a susceptible population. Conversely, the kdr allele frequency in a lab population with 17-fold resistance was nearly double that found in a heavily pressured wild population with 18-fold resistance. We conclude that, although the kdr mutation confers significant levels of pyrethroid resistance, a substantial component of resistance in insecticidally pressured populations is conferred by mechanisms that are PBO-suppressible. High super-kdr allele frequencies were detected in two resistant lab populations, but in wild populations with equivalent resistance the super-kdr allele frequency was very low. Interestingly, in over 1200 individuals assayed, the super-kdr mutation was never detected in the absence of the kdr

mutation.

Jindra, M., F. Sehnal, et al. (1994). "Isolation and developmental expression of the ecdysteroid-induced GHR3 gene of the wax moth Galleria mellonella." Insect Biochemistry and Molecular Biology **24**(8): 763.

http://www.sciencedirect.com/science/article/B6T79-47PR7TS-10/2/c3fdaddd2f05550c72cd9182b1eae7dd

Three degenerate primers were designed to match the most conserved regions within the DNA-binding domains of several selected members of the steroid hormone receptor family. Use of these primers in the polymerase chain reaction with cDNA from Galleria mellonella prepupae detected a 177 bp fragment that had 87% identity to the Manduca sexta gene MHR3 and 75% to the Drosophila melanogaster DHR3 gene, and therefore was named "GHR3". Screening of a Galleria penultimate instar cDNA library with this fragment yielded a cDNA clone that contained a 557 condon open reading frame, predicting a 62.3 kDa protein. The deduced amino acid sequence of GHR3 showed 92% overall identity with the MHR3 protein and 97 and 70% identity with DHR3 in the putative DNA- and ligand-binding domains, respectively. Hybridization of whole body RNA revealed high GHR3 mRNA levels during both the larval and pupal molts, coincident with the molt-inducing ecdysteroid pulses, and low or undetectable levels during the first half of the last instar. During the larval-pupal transformation, no GHR3 mRNA was found at the beginning of the stemmatal pigment retraction at the onset of the ecdysteroid rise; maximal levels were observed 4 h later, coincident with the peak ecdysteroid titer (over 2.3 [mu]g 20E equivalents/ml hemolymph). Two mRNAs (4.6 and 3.6 kb) were detected when the ecdysteroid titer was high. Injection of 2 [mu]g/gm 20E into isolated final instar larval abdomens induced the appearance of the 4.6 kb mRNA within 1.5 h; the mRNA level then reached maximum by 3 h and declined by 6 h. No 3.6 kb mRNA was detectable during that time. A 10-fold lower 20E dose caused only trace induction by 3 h.

Jones, A. M. E., P. Winge, et al. (2002). "Characterization and evolution of a myrosinase from the cabbage aphid Brevicoryne brassicae." Insect Biochemistry and Molecular Biology **32**(3): 275.

http://www.sciencedirect.com/science/article/B6T79-44XM1S4-3/2/53d8b15bbe12e1c60f50005445c9daba

The aphid myrosinase gene has been elucidated using Rapid Amplification of cDNA Ends--PCR. Sequencing has shown that aphid myrosinase has significant sequence similarity (35%) to plant myrosinases and other members of glycosyl hydrolase family 1 (GHF1). The residues acting as proton donor and nucleophile, in the hydrolysis of glucosinolates by aphid myrosinase, are identified as Glu 167 and Glu 374 respectively. The equivalent residues in plant myrosinase are Gln 187 and Glu 409 and for the cyanogenic [beta]-glucosidase Glu 183 and Glu 397. Thus it would appear that the absence of a proton donor is not necessary for the hydrolysis of glucosinolates as was thought to be the case for the plant myrosinases. Aphid myrosinase appears to be more similar to animal [beta]-O-glucosidases than to plant myrosinases, as assessed by sequence similarity and phylogenetic techniques. These results strongly suggest that myrosinase activity has twice arisen from [beta]-O-glucosidases in plants and animals. Comparison of aphid myrosinase with plant myrosinase has highlighted Lys 173 and Arg 312 as possibly playing a crucial role in the hydrolysis of glucosinolates by aphid myrosinase.

Kang, D., G. Liu, et al. (1996). "PCR differential display of immune gene expression in Trichoplusia ni." Insect Biochemistry and Molecular Biology **26**(2): 177.

http://www.sciencedirect.com/science/article/B6T79-3Y2N08T-8/2/c9a723cd78a4eab783c44022ad68e68a

The immune state of insects is defined by a set of proteins that is absent in the naive state. To explore the immune system of Trichoplusia ni in more detail we have employed a PCR differential display technique to compare the mRNA population of untreated last instar larvae to that of immunized animals. In the primary display, more than one hundred bands seemed induced upon bacterial challenge. When they were used as probes in Northern blots, 35% of these probes detected inducible mRNA species. Such probes were used to screen a cDNA library from immunized larvae. We isolated clones for T. ni homologs of cecropin A, lysozyme and attacin. One differentially expressed band hybridized to clones for BJHSP1, a hemacyanin-related protein which is hormonally up-regulated in last instar larvae; this induction is probably not related to the bacterial infection. Still other probes recognized inducible mRNAs of 1.6 and 1.0 kb. The corresponding cDNA clones did not show strong sequence homology to any known proteins. We have demonstrated the potential of this PCR technique to display both known and unknown genes specific for the immune state of whole insects against a background of genes involved in larval development.

Klaudiny, J., S. Albert, et al. (2005). "Two structurally different defensin genes, one of them encoding a novel defensin isoform, are expressed in honeybee Apis mellifera." Insect Biochemistry and Molecular Biology **35**(1): 11.

http://www.sciencedirect.com/science/article/B6T79-4DR1FRH-1/2/ff7b6bdfd7c205d104aa5cf0d10efe65

Two defensins showing high mutual similarity have previously been characterized in honeybee Apis mellifera: royalisin, a peptide isolated from the royal jelly, and defensin, found in the hemolymph of bacterially infected bees. Here we show that both these peptides are encoded by the same polymorphic gene, which we termed defensin1. Besides this gene, we identified an additional defensin gene coding for a novel honeybee defensin designated defensin2. The pre-pro-peptide sequence of defensin 2 was inferred from its cDNA. Mature defensin 2 peptide shows 55.8% identity with defensin 1. Sequences of genomic loci of the two defensin genes revealed their different structure. Defensin1 possesses an exon-intron structure unique among arthropoda defensin genes. Its second intron splits exactly the common structural module of defensins from a short amidated C-terminal extension found only in hymenopteran defensins. Transcription of defensin genes in some nurse honeybees tissues was studied by RT-PCR. Both defensins are expressed in heads and thoraces. Defensin1 but not defensin2 mRNA was detected in hyphopharyngeal, mandibular and thoracic salivary glands. Immune response elements were identified by computer analysis of the promoter regions of defensin genes. Their different representation in these genes reflects presumably observed tissue-specific expression of defensins.

Krapcho, K. J., J. R. M. Kral, et al. (1995). "Characterization and cloning of insecticidal peptides from the primitive weaving spider Diguetia canities." Insect Biochemistry and Molecular Biology **25**(9): 991.

http://www.sciencedirect.com/science/article/B6T79-3YF4B49-G/2/0b081bb0d59599924f90b9c35c3ed518

Three potent insecticidal peptide toxins were purified from the venom of the primitive weaving spider, Diguetia cavities. The toxins share significant homology (> 40%) in their amino acid sequences and are of related size (masses of 6371-7080 Da). In lepidopteran larvae, the toxins cause a progressive spastic paralysis, with 50% paralytic doses (PD50s) ranging from 0.38 to 3.18 nmol/g, suggesting them to be among the most potent insecticidal compounds yet described from arthropod venoms. The most potent of these toxins, DTX9.2, was cloned using a reverse transcription-polymerase chain reaction (RT-PCR). The cDNA encodes a 94 amino acid precursor which is processed to the active 56 amino acid peptide by removal of a signal and propeptide sequence. The gene encoding DTX9.2 was isolated and characterized. The transcriptional unit spans 5.5 kilobases and is segregated into five exons. DNA sequences upstream from the first exon contain a TATA box and two palindromic sequences (one with homology to a CAAT consensus) which together may constitute a functional promoter. The highly segmented gene structure observed for this small peptide suggests that a mechanism such as exon shuffling may have played a role in the evolution of this toxin family.

Lung, O. and M. F. Wolfner (2001). "Identification and characterization of the major Drosophila melanogaster mating plug protein." Insect Biochemistry and Molecular Biology **31**(6-7): 543.

http://www.sciencedirect.com/science/article/B6T79-42M1D7K-4/2/e3bfd4722bd5b805d2152a90e30fea3f

In many insects, semen coagulates into a mating plug at the distal part of the female's genital tract. Mating plugs have been proposed to facilitate sperm movement or to prevent subsequent matings or sperm loss. The molecular constituents of insect mating plugs have not previously been characterized. Here we report that an abundant autofluorescent protein made by the Drosophila melanogaster male's ejaculatory bulb is a major constituent of the posterior region of the mating plug. Identities in size, chromosomal location and expression pattern indicate that the autofluorescent protein is PEB-me, an abundant ejaculatory bulb protein reported by Ludwig et al. [Biochem. Genet. 29 (1991) 215]. We cloned and sequenced the RNA encoding this protein. The transcript, which is male-specific and expressed only in the ejaculatory bulb, encodes a 377 a.a. predicted secreted protein with PGG repeats similar to those in homopolymer-forming proteins found in spider silk.

Martin, J., R. T. Hoffman, et al. (1996). "Identification of divergent homologs of Chironomus tentans sp185 and its Balbiani ring 3 gene in Australasian species of Chironomus and Kiefferulus." Insect Biochemistry and Molecular Biology **26**(5): 465.

http://www.sciencedirect.com/science/article/B6T79-3W49339-7/2/d7b46be065a5c84815fd86690ecc2bbc

A 185-kDa silk protein (sp185) from Chironomus tentans, present in both larval and prepupal silks, contains a striking amino acid sequence motif, Cys-X-Cys-X-Cys, which occurs about every 22-26 residues. Homologous proteins have been found in Chironomus pallidivittatus (sp185) and Chironomus thummi (sp220), which apparently differ in size but are very similar in overall composition and sequence. While surveying Australasian species of Chironomus and Kefferulus we obtained evidence for immunologically related silk proteins having similar size and amino acid composition, but noticeably less Cys. Interspecies in situ hybridization to polytene chromosomes with C. tentans and C. pallidivittatus cDNA probes indicated that each species had a related gene. One pair of C. tentans cDNA-derived primers enabled polymerase chain reaction amplification of a discrete fragment of this gene from Kiefferulus 'cornishi'. Preliminary sequence information for this fragment confirmed the presence of an encoded Cys-X-Cys-X-Cys motif in what appeared to be a similar protein region containing less Cys. We conclude that homologs of

C. tentans sp185 and its gene have been identified which may contain significant deviations in structure. Once suitable libraries are available, probes described here will be useful for selecting cDNA and genomic clones for detailed study.

Martinez, L., J. C. Almagro, et al. (2004). "Sequence variability in the fibroin-H intron of domesticated and wild silk moths." Insect Biochemistry and Molecular Biology **34**(4): 343.

http://www.sciencedirect.com/science/article/B6T79-4BJK5JM-1/2/411cf77405d69774fe60cb144671b562

The single intron of the heavy-chain fibroin gene in domesticated (Bombyx mori) and wild (B. mandarina) silk moths has a length of approximately 1000 nucleotides. It is located only 57 bp from the gene's core promoter and harbors multiple AT-rich regulatory elements that have been found to enhance the basal level of transcription in vitro. In this work, the intronic nucleotide variability among members of both Bombyx species is analyzed. The intron sequences of B. mori strains k120 and Nistari, as well as B. mandarina specimens from Japan and Korea, were obtained. This information was compared with the previously reported sequences of B. mori strains p50 and C-108, as well as an additional B. mandarina specimen collected in Japan. We found a total of 26 variant positions, including variants shared by members of both species and species-specific changes. The potential functional role of these variants was investigated by using the program MatInspector to search for putative binding sites of transcription factors within the intron. We detected a multitude of putative binding elements distributed along the entire intronic sequence. Among them, 22 correspond to protein binding domains that are known to regulate fibroin transcription. The mapping of multiple variant positions within these putative binding sequences as well as in known regulatory elements of the intron argue for functional significance on the regulation of transcription.

Matsunaga, T. M. and H. Fujiwara (2002). "Identification and characterization of genes abnormally expressed in wing-deficient mutant (flugellos) of the silkworm, Bombyx mori." Insect Biochemistry and Molecular Biology **32**(6): 691.

http://www.sciencedirect.com/science/article/B6T79-44GHTVH-2/2/93f3c55640c9b31ee9aaf77b8602d760

The wing-deficient mutant, flugellos (fl), of the silkworm lacks four wings in the pupa and the adult, due to aberrant wing morphogenesis during metamorphosis. To elucidate the mechanisms of wing-specific deficiencies in the fl mutant, we used mRNA differential display and identified five genes abnormally expressed in the fl wing discs. Northern blot and RT-PCR analyses revealed that four genes were overexpressed, but the fifth one was not transcribed in the fl wing discs. The expression level of ribosome-associated protein p40 in the fl wing discs was elevated approximately 10 times compared to the wild-type (WT) discs. Another overexpressed gene CB10 encodes a novel wing-specific protein with a putative zinc-finger motif. Overexpression of two components of extracellular matrix, cuticle protein 18 (BMCP18) and a fibrillin-like protein AD10, may result in the abnormal wing morphogenesis in the fl mutant. In contrast, a novel member of multifunctional Ca2+-binding protein annexins, designated as annexin b13 (Anx b13), was expressed dominantly in the wing discs of WT but completely repressed in the fl tissues. Strong expression of Anx b13 in wing discs during the fourth and fifth instar indicates that ANX B13 plays an important role in wing morphogenesis.

Mukabayire, O., A. J. Cornel, et al. (1996). "The Tryptophan oxygenase gene of Anopheles gambiae." Insect Biochemistry and Molecular Biology **26**(6): 525.

http://www.sciencedirect.com/science/article/B6T79-3W2T43D-2/2/210616228cf7a5ae2bed03ea05ef2b46

The Anopheles gambiae gene encoding tryptophan oxygenase, a homolog of the Drosophila melanogaster vermilion gene, has been molecularly cloned and characterized. Unlike Drosophila, where it is X-linked, the A. gambiae gene maps to chromosome 2R, subdivision 12E, by in situ hybridization to the polytene chromosomes. Of the six introns present, four are positioned identically to those of the Drosophila homolog, one is similarly positioned, and one is novel. A 1955 nt cDNA potentially encodes a 392 amino acid protein of an estimated 45 kDa. Amino acid comparisons between the deduced protein and previously known tryptophan oxygenases revealed 74% identity between Anopheles and Drosophila, and 53% identity between Anopheles and nematode or mammalian proteins. Northern analysis detected a developmentally regulated transcript about 2 kb in length. Since this gene is known to control adult eye color in other flies, its cloning from A. gambiae provides the basis for a dominant phenotypic marker for germline transformation, one whose expression, unlike that of white, is not cell autonomous.

Rasmussen, C. and G. F. Rohrmann (1994). "Characterization of the Spodoptera frugiperda TATA-binding protein: Nucleotide sequence and response to baculovirus infection." Insect Biochemistry and Molecular Biology **24**(7): 699.

http://www.sciencedirect.com/science/article/B6T79-47PR7VD-6M/2/8fc0591c2fdea95bde8a942c21ccf96e

A cDNA clone containing a 921 bp open-reading frame (307 amino acids; 34 kDa) homologous to the TATA-binding protein (TBP) was isolated and sequenced from a Spodoptera frugiperda cell line that is commonly used in the baculovvrus expression system. Analysis of the S. frugiperda TBP (SfTBP) sequence showed that the amino-terminal portion of SfTBP diverged significantly from that of other TBP sequences including Drosophila melanogaster whereas the carboxy-terminal sequence was highly conserved. Southern blot analysis indicated that SfTBP was encoded by a single gene in the S. frugiperda genome. Northern blot analysis indicated that steady-state levels of the 1.3 kb SfTBP transcript declined by 24 h post-infection corresponding to the time of virus-induced inhibition of host-cell transcription. Corresponding western blot analysis showed that TBP protein levels remain constant up to 72 h post-infection.

Schorderet, S., R. D. Pearson, et al. (1998). "cDNA and deduced amino acid sequences of a peritrophic membrane glycoprotein, 'Peritrophin-48', from the larvae of Lucilia cuprina." Insect Biochemistry and Molecular Biology **28**(2): 99.

http://www.sciencedirect.com/science/article/B6T79-3T6YH4M-4/2/8124b8bd4a212f7b8a04fe66995098ff

The gut of most insects is lined with a semi-permeable peritrophic membrane (or peritrophic matrix) composed of chitin, proteoglycans and proteins. Despite the probable importance of the peritrophic membrane in facilitating the digestive process and protecting insects from invasion by micro-organisms and parasites, there has been little characterization of the specific components and their interactions within this acellular structure. Here we report the characterization of an integral peritrophic membrane glycoprotein, peritrophin-48, from the larvae of the fly Lucilia cuprina, a primary agent of cutaneous myiasis in sheep. Peritrophin-48 was purified from

peritrophic membrane obtained by larval culture and its location within the peritrophic membrane determined by immuno-fluorescence and immuno-gold localizations. The cDNA coding for peritrophin-48 was cloned and sequenced. The deduced amino acid sequence codes for a protein of 375 amino acids containing an amino-terminal signal sequence followed by five similar, but non-identical domains, each approximately 65-70 amino acids in length and characterised by a specific register of six cysteines. The deduced amino acid sequence shows significant similarity to two other peritrophic membrane proteins, peritrophin-95 and peritrophin-44, from the same species. A reverse transcriptase-PCR approach indicated that there are several highly related peritrophin-48 genes expressed in each individual. Reverse transcriptase-PCR also demonstrated the expression of peritrophin-48 in all three larval instars and adults but not pupae or eggs. Peritrophin-48 was expressed only by the cardia and by the larval midgut. A simple structural model of a basic unit of a type 2 peritrophic membrane is presented.

Sun, J., M. Yuda, et al. (1998). "Characterization and cDNA cloning of a hemoprotein in the salivary glands of the blood-sucking insect, Rhodnius prolixus." Insect Biochemistry and Molecular Biology **28**(3): 191.

http://www.sciencedirect.com/science/article/B6T79-3VGRRV2-K/2/e83efc2d7a503583930c0379e07bb3f0

Three major red hemoproteins, named RpSG I, II (identical with prolixin-S) and III, in the salivary glands of the blood-sucking insect, Rhodnius prolixus, show homology in N-terminal amino acid (AA) sequences, and are immunologically related. We focussed on one of these proteins, RpSG-I, in this paper. RpSG-I in fresh salivary gland extract was separated into two components (Ia and Ib) by isoelectric focussing gel electrophoresis. Absorption spectra of RpSG-Ia and Ib showed Soret peaks at 400 nm and 420 nm, respectively, suggesting that they are nitric oxide (NO)-unbound and -bound hemoproteins and function as NO-carriers. RpSG-I is stage-specific in appearance, being absent in 3rd and 4th instar nymphs, appearing and increasing gradually in 5th (last) instar nymphs after engorgement, and present in the adult stage. We purified RpSG-I from salivary gland extract by size exclusion and ion exchange HPLCs. It is a single electrophoretic band with an absorption peak at 400 nm, representing the NO-unbound molecule. Full-size cDNA of RpSG-I was cloned by screening with a specific polyclonal antibody from a salivary gland cDNA library. Sequence analysis of RpSG-I cDNA showed an open reading frame encoding a signal peptide (23 AA) and mature protein (179 AA) of 19778 daltons. The deduced N-terminal AA sequence of the RpSG-I was identical with that of the hemoprotein reported as nitrophorin-3 (Champagne et al., 1995).

Suzuki, Y., T. Matsuoka, et al. (2002). "Ecdysteroid-dependent expression of a novel cuticle protein gene BMCPG1 in the silkworm, Bombyx mori." Insect Biochemistry and Molecular Biology **32**(6): 599.

http://www.sciencedirect.com/science/article/B6T79-44B29F1-3/2/e2f9c0414d8f86b85b1f7cc1ce574458

When insects molt, the exoskeleton is renewed under the controls of insect hormones via the biosynthesis and degradation of cuticle proteins. To understand the hormonal control of cuticle formation, we used the differential display method to look for stage-specific cuticle genes, and identified a novel cDNA named Bombyx mori Cuticle Protein GlyGlyTyr-repeat 1 (BMCPG1). Expression of BMCPG1 mRNA peaked sharply immediately after a pulse of ecdysteroid during the fourth molt and pre-pupal stages, concurrent with the expression of genes for FTZF1 and dopa decarboxylase. BMCPG1 was expressed only in the epidermis, but not in any other tissue. We cultured the larval epidermis and found that BMCPG1 expression is not induced by the continuous presence of ecdysteroid. Removal of ecdysteroid from the medium, which constitutes

a pulse treatment, is required for the induction of BMCPG1 transcription. These results explain well the stage-specific expression of BMCPG1 by ecdysteroid in vivo. Based on its expression patterns and unique structure, we propose that BMCPG1 may be a novel component of epicuticle of B. mori, and is probably involved in cross-linking of proteins via its GGY repeats.

Tellam, R. L., C. Eisemann, et al. (2000). "The intrinsic peritrophic matrix protein peritrophin-95 from larvae of Lucilia cuprina is synthesised in the cardia and regurgitated or excreted as a highly immunogenic protein." Insect Biochemistry and Molecular Biology **30**(1): 9.

http://www.sciencedirect.com/science/article/B6T79-3XYG4HF-2/2/dc82f53343f01eee2ce5b4f07af2f3f9

The intrinsic peritrophic matrix glycoprotein, peritrophin-95, from the midgut of larvae of Lucilia cuprina can only be solubilized from the matrix using strong denaturants. This suggests that the protein has a structural role in the matrix. Consistent with this is the finding that immuno-gold and immuno-fluorescence localizations of the protein showed a uniform distribution within the peritrophic matrix. RT-PCR demonstrated that expression of peritrophin-95 mRNA was restricted to the larval cardia, a small organ located in the anterior midgut from which the type 2 peritrophic matrix originates. Immuno-blots and ELISAs demonstrated that the sera from sheep infested naturally or artificially with these larvae recognised peritrophin-95. This indicates that peritrophin-95 stimulates the ovine immune system during larval infestation even though the protein is firmly attached to the peritrophic matrix in the larval midgut and seemingly "concealed" from the ovine immune surveillance system. Analyses of larval regurgitated or excreted material by immuno-blots, immuno-affinity purification and amino-terminal sequencing demonstrated the presence of soluble monomeric peritrophin-95. These results indicate that peritrophin-95, a candidate vaccine antigen for use in sheep is not a "concealed" antigen as previously thought. The presence of soluble peritrophin-95 in the regurgitated/excreted material from larvae suggests that this protein may be involved in a maturation phase of peritrophic matrix production, a by-product of which is the excretion or regurgitation of soluble peritrophin-95.

Vuocolo, T., C. H. Eisemann, et al. (2001). "Identification and molecular characterisation of a peritrophin gene, peritrophin-48, from the myiasis fly Chrysomya bezziana." Insect Biochemistry and Molecular Biology **31**(9): 919.

http://www.sciencedirect.com/science/article/B6T79-43CH6GC-9/2/9c6ecd862d0a662a5f2ba5921b6cceb0

The peritrophic matrix lines the midgut of most insects and has important roles in digestion, protection of the midgut from mechanical damage and invasion by micro-organisms. Although a few intrinsic peritrophic matrix proteins have been characterised, no direct homologues of any of these proteins have been found in other insect species, even closely related species, suggesting that the peritrophic matrix proteins show considerable sequence divergence. We now report the identification of the cDNA and genomic DNA sequences of a Chrysomya bezziana homologue of the Lucilia cuprina intrinsic peritrophic matrix protein, peritrophin-48. The gene for C. bezziana peritrophin-48 spans 1315 bp and consists of three exons (65, 560 and 690 bp, respectively) separated by introns of 566 and 72 bp. The transcriptional start site, identified by a consensus of cDNA clones and primer extension analysis, is probably located 58 bp upstream from the start codon. However, there may be multiple start sites for transcription. Two potential TATA boxes and a consensus arthropod transcription initiator are located within 134 bp of sequence upstream of the putative transcriptional start site suggesting that this region contains the gene promoter. Immuno-fluorescence localization demonstrated that C. bezziana peritrophin-48 was localised to the larval peritrophic matrix. Protein fold recognition analysis indicated structural similarities

between peritrophin-48 and wheatgerm lectin. As wheatgerm lectin binds chitin, this result suggested that C. bezziana peritrophin-48 may also bind chitin, a constituent of the peritrophic matrix. Chitin binding studies with a recombinant peritrophin-48 protein confirmed that it binds chitin. A Drosophila melanogaster homologue of peritrophin-48 encoded in an EST and a genomic sequence was also identified. The pairwise percentage identities of the deduced amino acid sequences for the peritrophin-48 homologues from the three higher Dipteran species were relatively low, ranging between 32 and 42%. Despite this sequence variability, the predicted structure of these proteins, dictated by five domains, each containing a characteristic distribution of six cysteines, was strictly conserved. It is concluded that considerable sequence variation can be tolerated in this protein because of the constraints imposed on the structure of the protein by an extensive disulphide bonded framework.

Wilson, R., J. Orsetti, et al. (2003). "Post-integration behavior of a Mos1 mariner gene vector in Aedes aegypti." Insect Biochemistry and Molecular Biology **33**(9): 853.

http://www.sciencedirect.com/science/article/B6T79-4967CHH-1/2/6b1c6d10c779a20de62c765d3cda7f1a

The post-integration behavior of insect gene vectors will determine the types of applications for which they can be used. Transposon mutagenesis, enhancer trapping, and the use of transposable elements as genetic drive systems in insects requires transposable elements with high rates of remobilization in the presence of transposase. We investigated the post-integration behavior of the Mos1 mariner element in transgenic Aedes aegypti by examining both germ-line and somatic transpositions of a non-autonomous element in the presence of Mos1 transposase. Somatic transpositions were occasionally detected while germ-line transposition was only rarely observed. Only a single germ-line transposition event was recovered after screening 14,000 progeny. The observed patterns of transposition suggest that Mos1 movement takes place between the S phase and anaphase. The data reported here indicate that Mos1 will be a useful vector in Ae. aegypti for applications requiring a very high degree of vector stability but will have limited use in the construction of genetic drive, enhancer trap, or transposon tagging systems in this species.

Wu, Q., P. Andolfatto, et al. (2001). "Cloning and sequence of the gene encoding the muscle fatty acid binding protein from the desert locust, Schistocerca gregaria." Insect Biochemistry and Molecular Biology **31**(6-7): 553.

http://www.sciencedirect.com/science/article/B6T79-42M1D7K-5/2/53b67de498c43f389992b54ae3d46389

Muscle fatty acid binding protein (FABP) is a major cytosolic protein in flight muscle of the desert locust, Schistocerca gregaria. FABP expression varies greatly during development and periods of increased fatty acid utilization, but the molecular mechanisms that regulate its expression are not known. In this study, the gene coding for locust muscle FABP was amplified by PCR and cloned, together with 1.2 kb of upstream sequence. The sequence coding for the 607 bp cDNA is interrupted by two introns of 12.7 and 2.9 kb, inserted in analogous positions as the first and third intron of the mammalian homologues. Both introns contain repetitive sequences also found in other locust genes, and the second intron contains a GT-microsatellite. The promoter sequence includes a canonical TATA box 24 bp upstream of the transcription start site. The upstream sequence contains various potential myocyte enhancer sequences and a 160 bp segment that is repeated three times. In database searches in the genome database of Drosophila melanogaster, a gene with the same gene organization and promoter structure was identified, likely the dipteran homologue of muscle FABP. Upstream of both insect genes, a conserved 19 bp inverted repeat

sequence was detected. A similar but reverse palindrome is also present upstream of all mammalian heart FABP genes, possibly representing a novel element involved in muscle FABP expression.

Zhang, J. and N. H. Haunerland (1998). "Transcriptional regulation of FABP expression in flight muscle of the desert locust, Schistocerca gregaria." Insect Biochemistry and Molecular Biology **28**(9): 683.

http://www.sciencedirect.com/science/article/B6T79-3TXSR1F-7/2/ce39bc5978279c7d2f2a1b7b61f06d7c

FABP is the most abundant cytosolic protein in developed flight muscle of adult locusts, but it is completely absent in nymphs. During the two weeks following adult ecdysis, FABP rises to 18% of the total soluble proteins. Its mRNA increases steadily up to day 8, before it gradually declines until reaching a low concentration at day 15, which remains constant for the rest of the animal's life. Using a PCR primer combination specific for a 597 bp sequence of intron 1, we have developed a reverse transcription PCR assay to quantify the amount of primary transcript present in muscle tissue at various ages. The FABP gene is not transcribed prior to metamorphosis; its primary transcript rises rapidly during the first two days of adult life, and remains close to maximal until day 5. Subsequently its level rapidly declines, ultimately reaching values of less than 0.02% of the maximal level. The correlation between primary transcript, mRNA and FABP content were analyzed by modeling transcription, translation and degradation with computer modeling software. The computer simulation is in good agreement with the experimentally obtained data, suggesting that the control of FABP expression in locust flight muscle occurs predominantly at the level of transcription initiation.

Zhu, K. Y. and J. M. Clark (1995). "Cloning and sequencing of a cDNA encoding acetylcholinesterase in colorado potato beetle, Leptinotarsa decemlineata (say)." Insect Biochemistry and Molecular Biology **25**(10): 1129.

http://www.sciencedirect.com/science/article/B6T79-3YF4B3R-8/2/b5c0907dc4e249f0298ef46357e3ec52

A cDNA encoding acetylcholinesterase (AChE, EC 1.1.1.7) was cloned from a cDNA library constructed from an insecticide-susceptible strain of Colorado potato beetle, Leptinotarsa decemlineata (Say). The complete amino acid sequence of AChE deduced from the cDNA consisted of 29 residues for the putative signal peptide and 600 residues for the mature protein with a predicted molecular weight of 67,994. Northern blot analysis of poly(A) RNA showed an approx 13.1-kb transcript. The mature protein sequence had 57 and 61% of amino acid residues identical to those of Drosophila melanogaster and Anopheles stephensi, respectively, and produced a remarkably similar hydropathy profile when compared to those of the two dipterous species. The three residues (Ser, Glu and His) that putatively form the catalytic triad and the six Cys that form intra-subunit disulfide bonds were completely conserved when compared to the other seven AChEs from a broad range of animal species reported to date. Other properties of the deduced protein of AChE, including molecular weight and amino acid composition, agreed well with those of a previously reported study on the purified AChE from the same insect species. All these features firmly established that the cloned cDNA encodes AChE in Colorado potato beetle.

Bjorkroth, K. J., U. Schillinger, et al. (2002). "Taxonomic study of Weissella confusa and description of Weissella cibaria sp. nov., detected in food and clinical samples." Int J Syst Evol Microbiol **52**(1): 141-148.

http://ijs.sgmjournals.org/cgi/content/abstract/52/1/141

Bonnet, R., A. Suau, et al. (2002). "Differences in rDNA libraries of faecal bacteria derived from 10- and 25-cycle PCRs." Int J Syst Evol Microbiol **52**(3): 757-763.

http://ijs.sgmjournals.org/cgi/content/abstract/52/3/757

Bosshard, P. P., R. Zbinden, et al. (2002). "Paenibacillus turicensis sp. nov., a novel bacterium harbouring heterogeneities between 16S rRNA genes." Int J Syst Evol Microbiol **52**(6): 2241-2249.

http://ijs.sgmjournals.org/cgi/content/abstract/52/6/2241

Bozal, N., M. J. Montes, et al. (2002). "Shewanella frigidimarina and Shewanella livingstonensis sp. nov. isolated from Antarctic coastal areas." Int J Syst Evol Microbiol **52**(1): 195-205.

http://ijs.sgmjournals.org/cgi/content/abstract/52/1/195

Distel, D. L., W. Morrill, et al. (2002). "Teredinibacter turnerae gen. nov., sp. nov., a dinitrogen-fixing, cellulolytic, endosymbiotic gamma-proteobacterium isolated from the gills of wood-boring molluscs (Bivalvia: Teredinidae)." Int J Syst Evol Microbiol **52**(6): 2261-2269.

http://ijs.sgmjournals.org/cgi/content/abstract/52/6/2261

Eribe, E. R. K., B. J. Paster, et al. (2004). "Genetic diversity of Leptotrichia and description of Leptotrichia goodfellowii sp. nov., Leptotrichia hofstadii sp. nov., Leptotrichia shahii sp. nov. and Leptotrichia wadei sp. nov." Int J Syst Evol Microbiol **54**(2): 583-592.

http://ijs.sgmjournals.org/cgi/content/abstract/54/2/583

Sixty strains of Gram-negative, anaerobic, rod-shaped bacteria from human sources initially assigned to Leptotrichia buccalis (n=58) and Leptotrichia pseudobuccalis' (n=2) have been subjected to polyphasic taxonomy. Full-length 16S rDNA sequencing, DNA-DNA hybridization, RAPD, SDS-PAGE of whole-cell proteins, cellular fatty acid analysis and enzymic/biochemical tests supported the establishment of four novel Leptotrichia species from this collection, Leptotrichia goodfellowii sp. nov. (type strain LB 57T=CCUG 32286T=CIP 107915T), Leptotrichia hofstadii sp. nov. (type strain LB 23T=CCUG 47504T=CIP 107917T), Leptotrichia shahii sp. nov.

(type strain LB 37T=CCUG 47503T=CIP 107916T) and Leptotrichia wadei sp. nov. (type strain LB 16T=CCUG 47505T=CIP 107918T). Light and electron microscopy showed that the four novel species were Gram-negative, non-spore-forming and non-motile rods. L. goodfellowii produced arginine dihydrolase, {beta}-galactosidase, N-acetyl-{beta}-glucosaminidase, arginine arylamidase, leucine arylamidase and histidine arylamidase. L. shahii produced {alpha}-arabinosidase. L. buccalis and L. goodfellowii fermented mannose and were{beta} -galactosidase-6-phosphate positive. L. goodfellowii, L. hofstadii and L. wadei were{beta} -haemolytic. L. buccalis fermented raffinose. With L. buccalis, L. goodfellowii showed 3{middle dot}8-5{middle dot}5 % DNA-DNA relatedness, L. shahii showed 24{middle dot}5-34{middle dot}1 % relatedness, L. hofstadii showed 27{middle dot}3-36{middle dot}3 % relatedness and L. wadei showed 24{middle dot}1-35{middle dot}9 % relatedness. 16S rDNA sequencing demonstrated that L. hofstadii, L. shahii, L. wadei and L. goodfellowii each formed individual clusters with 97, 96, 94 and 92 % similarity, respectively, to L. buccalis.

Finneran, K. T., H. M. Forbush, et al. (2002). "Desulfitobacterium metallireducens sp. nov., an anaerobic bacterium that couples growth to the reduction of metals and humic acids as well as chlorinated compounds." Int J Syst Evol Microbiol **52**(6): 1929-1935.

http://ijs.sgmjournals.org/cgi/content/abstract/52/6/1929

Finneran, K. T., C. V. Johnsen, et al. (2003). "Rhodoferax ferrireducens sp. nov., a psychrotolerant, facultatively anaerobic bacterium that oxidizes acetate with the reduction of Fe(III)." Int J Syst Evol Microbiol **53**(3): 669-673.

http://ijs.sgmjournals.org/cgi/content/abstract/53/3/669

To further investigate the diversity of micro-organisms capable of conserving energy to support growth from dissimilatory Fe(III) reduction, Fe(III)-reducing micro-organisms were enriched and isolated from subsurface sediments collected in Oyster Bay, VA, USA. A novel isolate, designated T118T, was recovered in a medium with lactate as the sole electron donor and Fe(III) as the sole electron acceptor. Cells of T118T were Gram-negative, motile, short rods with a single polar flagellum. Strain T118T grew between pH 6{middle dot}7 and 7{middle dot}1, with a temperature range of 4-30 {degrees}C. The optimal growth temperature was 25 {degrees}C. Electron donors utilized by strain T118T with Fe(III) as the sole electron acceptor included acetate, lactate, malate, propionate, pyruvate, succinate and benzoate. None of the compounds tested was fermented. Electron acceptors utilized with either acetate or lactate as the electron donor included Fe(III)-NTA (nitrilotriacetic acid), Mn(IV) oxide, nitrate, fumarate and oxygen. Phylogenetic analysis demonstrated that strain T118T is most closely related to the genus Rhodoferax. Unlike other species in this genus, strain T118T is not a phototroph and does not ferment fructose. However, phototrophic genes may be present but not expressed under the experimental conditions tested. No Rhodoferax species have been reported to grow via dissimilatory Fe(III) reduction. Based on these physiological and phylogenetic differences, strain T118T (=ATCC BAA-621T=DSM 15236T) is proposed as a novel species, Rhodoferax ferrireducens sp. nov.

Gorshkova, N. M., E. P. Ivanova, et al. (2003). "Marinobacter excellens sp. nov., isolated from sediments of the Sea of Japan." Int J Syst Evol Microbiol **53**(6): 2073-2078.

http://ijs.sgmjournals.org/cgi/content/abstract/53/6/2073

Five strains of halophilic, Gram-negative marine bacteria (KMM 3809T, KMM 3814, KMM 3815, KMM 3817 and KMM 3818) were isolated from sediments collected from Chazhma Bay, Sea of Japan. Phylogenetic 16S rRNA gene sequence-based analysis placed these bacteria in a clade within the genus Marinobacter in the{gamma} -Proteobacteria. KMM 3809T showed highest 16S rRNA gene sequence similarity of 97{middle dot}3 % to Marinobacter litoralis and 96{middle dot}9 % to Marinobacter hydrocarbonoclasticus and Marinobacter aquaeolei. DNA-DNA hybridization between the five isolates was at the conspecific level (94-96 %) and that among the closest phylogenetic neighbours ranged from 45{middle dot}0 to 62{middle dot}5 %. The new organisms were susceptible to polymyxin. Predominant fatty acids were C16: 0, C16: 1{omega}9c, C16: 1{omega}7c and C18: 1{omega}9c. Phylogenetic evidence, along with phenotypic and genotypic characteristics, showed that the bacteria constituted a novel species of the genus Marinobacter. The name Marinobacter excellens sp. nov. is proposed for this species, with the type strain KMM 3809T (=CIP 107686T).

Hanninen, M.-L., M. Utriainen, et al. (2003). "Helicobacter sp. flexispira 16S rDNA taxa 1, 4 and 5 and Finnish porcine Helicobacter isolates are members of the species Helicobacter trogontum (taxon 6)." Int J Syst Evol Microbiol **53**(2): 425-433.

http://ijs.sgmjournals.org/cgi/content/abstract/53/2/425

The term flexispira' refers to micro-organisms with a particular morphology: fusiform-shaped with helical periplasmic fibrils and bipolar tufts of sheathed flagella. Two flexispira taxa have been formally named, Helicobacter bilis and Helicobacter trogontum, a third named species is Helicobacter aurati and eight additional 16S rRNA sequence-based flexispira taxa have been described by Dewhirst et al. (Int J Syst Evol Microbiol 50, 1781-1787, 2000) and given the provisional designation Helicobacter sp. flexispira taxa 1-5, 7, 8 and 10. In the present study, seven gastric or intestinal flexispira isolates from seven Finnish pigs originating from different farms were characterized. Morphologically, all these porcine isolates had typical flexispira morphology. Analysis of the 16S rDNA sequences of five isolates showed that they were most closely related to the sequences of flexispira taxa 4 and 5 and H. trogontum (taxon 6), but less closely related to taxa 1-3 and 8, H. bilis and H. aurati. Phenotypic characterization, analysis of RFLPs of 16S and 23S rDNAs and SDS-PAGE profiles revealed that all of the porcine isolates, reference strains of flexispira taxa 1, 4 and 5 and the type strain of H. trogontum (ATCC 700114T) had highly related characteristics that differed from those of the reference strains of taxa 2, 3 and 8 and H. bilis. Furthermore, a high DNA-DNA binding rate was found, in dot-blot hybridization studies, between the Finnish porcine strains, taxa 1, 4 and 5 reference strains and H. trogontum ATCC 700114T. In conclusion, polyphasic characterization of novel porcine flexispira isolates and previously described taxa 1, 4 and 5 reference strains showed that they all belong to a validly described species, H. trogontum, and that the taxonomy of known flexispiras is less complicated than proposed on the basis of 16S rDNA sequence analysis.

Hatano, K., T. Nishii, et al. (2003). "Taxonomic re-evaluation of whorl-forming Streptomyces (formerly Streptoverticillium) species by using phenotypes, DNA-DNA hybridization and sequences of gyrB, and proposal of Streptomyces luteireticuli (ex Katoh and Arai 1957) corrig., sp. nov., nom. rev." Int J Syst Evol Microbiol **53**(5): 1519-1529.

http://ijs.sgmjournals.org/cgi/content/abstract/53/5/1519

The taxonomic status of 64 strains of whorl-forming Streptomyces (formerly Streptoverticillium) species was re-evaluated and strains were reclassified on the basis of their phenotypes, DNA-DNA hybridization data and partial sequences of gyrB, the structural gene of the B subunit of DNA gyrase. These strains, which consisted of 46 species and eight subspecies with validly

published names and 13 species whose names have not been validly published [including 10 strains examined by the International Streptomyces Project (ISP)], were divided into two groups, namely typical and atypical whorl-forming Streptomyces species, based on their phenotypes and gyrB gene sequences. The typical whorl-forming species (59 strains) were divided into six major clusters of three or more species, seven minor clusters of two species and five single-member clusters, based on the threshold value of 97 % gyrB sequence similarity. Major clusters were typified by Streptomyces abikoensis, Streptomyces cinnamoneus, Streptomyces distallicus, Streptomyces griseocarneus, Streptomyces hiroshimensis and Streptomyces netropsis. Phenotypically, members of each cluster resembled each other closely except for the S. distallicus cluster, which was divided phenotypically into the S. distallicus and Streptomyces stramineus subclusters, and the S. netropsis cluster, which was divided into the S. netropsis and Streptomyces eurocidicus subclusters. Strains in each minor cluster closely resembled each other phenotypically. DNA-DNA relatedness between the representative species and others in each major cluster and/or subcluster, and between strains in the minor clusters, was >70 %, indicating that the major clusters and/or subclusters and the minor clusters each comprise a single species. It was concluded that 59 strains of typical whorl-forming Streptomyces species consisted of the following 18 species, including subjective synonym(s): S. abikoensis, Streptomyces ardus, Streptomyces blastmyceticus, S. cinnamoneus, S. eurocidicus, S. griseocarneus, S. hiroshimensis, Streptomyces lilacinus, Streptomyces luteoreticuli', Streptomyces luteosporeus, Streptomyces mashuensis, Streptomyces mobaraensis, Streptomyces morookaense, S. netropsis, Streptomyces orinoci, S. stramineus, Streptomyces thioluteus and Streptomyces viridiflavus.

Hugo, C. J., P. Segers, et al. (2003). "Chryseobacterium joostei sp. nov., isolated from the dairy environment." Int J Syst Evol Microbiol **53**(3): 771-777.

http://ijs.sgmjournals.org/cgi/content/abstract/53/3/771

Among a large collection of South African dairy isolates, a novel Chryseobacterium taxon (DNA group 3) was previously delineated by a polyphasic taxonomic study (Hugo et al., Syst Appl Microbiol 22, 586-595, 1999). In the present paper, this taxon is further characterized using 16S rDNA sequencing, fatty acid methyl ester analysis and a comparative phenotypic analysis, resulting in the proposal of a novel species, Chryseobacterium joostei sp. nov. (type strain Ix 5aT=LMG 18212T=CCUG 46665T).

Ivanova, E. P., Y. V. Alexeeva, et al. (2004). "Formosa algae gen. nov., sp. nov., a novel member of the family Flavobacteriaceae." Int J Syst Evol Microbiol **54**(3): 705-711.

http://ijs.sgmjournals.org/cgi/content/abstract/54/3/705

Four light-yellow-pigmented, Gram-negative, short-rod-shaped, non-motile isolates were obtained from enrichment culture during degradation of the thallus of the brown alga Fucus evanescens. The isolates studied were chemo-organotrophic, alkalitolerant and mesophilic. Polar lipids were analysed and phosphatidylethanolamine was the only phospholipid identified. The predominant cellular fatty acids were 15: 0, i15: 0, ai15: 0, i15: 1 and 15: 1(n-6). The DNA G+C contents of the four strains were 34{middle dot}0-34{middle dot}4 mol%. The level of DNA relatedness of the four isolates was conspecific (88-98 %), indicating that they belong to the same species. The 16S rDNA sequence of strain KMM 3553T was determined. Phylogenetic analysis revealed that KMM 3553T formed a distinct phyletic line in the phylum Bacteroidetes, class Flavobacteria in the family Flavobacteriaceae and that, phylogenetically, this strain could be placed almost equidistant from the genera Gelidibacter and Psychroserpens (16S rRNA gene sequence similarities of 94 %). On the basis of significant differences in phenotypic and chemotaxonomic characteristics, it is

suggested that the isolates represent a novel species in a new genus; the name Formosa algae gen. nov., sp. nov. is proposed. The type strain is KMM 3553T (=CIP 107684T).

Ivanova, E. P., R. Christen, et al. (2004). "Brevibacterium celere sp. nov., isolated from degraded thallus of a brown alga." Int J Syst Evol Microbiol **54**(6): 2107-2111.

http://ijs.sgmjournals.org/cgi/content/abstract/54/6/2107

Two whitish yellow, Gram-positive, non-motile, aerobic bacteria were isolated from enrichment culture during degradation of the thallus of the brown alga Fucus evanescens. The bacteria studied were chemo-organotrophic, mesophilic and grew well on nutrient media containing up to 15 % (w/v) NaCl. The DNA G+C content was 61 mol%. The two isolates exhibited a conspecific DNA-DNA relatedness value of 98 %, indicating that they belong to the same species. A comparative analysis of 16S rRNA gene sequences revealed that strain KMM 3637T formed a distinct phyletic lineage in the genus Brevibacterium (family Brevibacteriaceae, class Actinobacteria) and showed the highest sequence similarity (about 97 %) to Brevibacterium casei. DNA-DNA hybridization experiments demonstrated 45 % binding with the DNA of B. casei DSM 20657T. Physiological and chemotaxonomic characteristics (meso-diaminopimelic acid in the peptidoglycan, major cellular fatty acids 15: 0ai and 17: 0ai) of the bacteria studied were consistent with the genomic and phylogenetic data. On the basis of the results of this study, a novel species, Brevibacterium celere sp. nov., is proposed. The type strain is KMM 3637T (=DSM 15453T=ATCC BAA-809T).

Ivanova, E. P., O. I. Nedashkovskaya, et al. (2003). "Shewanella waksmanii sp. nov., isolated from a sipuncula (Phascolosoma japonicum)." Int J Syst Evol Microbiol **53**(5): 1471-1477.

http://ijs.sgmjournals.org/cgi/content/abstract/53/5/1471

Two marine bacterial strains, KMM 3823T and KMM 3836, isolated from a sipuncula (Phascolosoma japonicum), a common inhabitant of Troitsa Bay in the Gulf of Peter the Great (Sea of Japan), were studied. Comparative 16S rRNA gene sequence-based phylogenetic analysis placed these bacteria into a separate branch of the Gammaproteobacteria' within members of the genus Shewanella. KMM 3823T showed the highest similarity (96{middle dot}6 %) with Shewanella fidelis. The DNA G+C contents of the two strains studied were 43{middle dot}0 mol%. The level of DNA homology between these two strains was conspecific (93 %), indicating that they represent a single genospecies. These organisms were greenish-brown, Gram-negative, polarly flagellated, facultatively anaerobic, mesophilic (temperature range 4-30 {degrees}C), neutrophilic, haemolytic and were able to degrade elastin, gelatin and DNA. They were susceptible to ampicillin, carbenicillin, gentamicin and kanamycin. The predominant fatty acids were characteristic for shewanellas: 13: 0-i, 15: 0-i and 16: 1(n-7); up to 6{middle dot}7 % of eicosapentaenoic fatty acid, 20: 5(n-3), was produced during growth at 28 {degrees}C. Phylogenetic evidence, confirmed by DNA hybridization and phenotypic characteristics revealed that the two bacteria studied constitute a new species, Shewanella waksmanii sp. nov., the type strain of which is KMM 3823T (=CIP 107701T=ATCC BAA-643T).

Kashefi, K., J. M. Tor, et al. (2002). "Geoglobus ahangari gen. nov., sp. nov., a novel hyperthermophilic archaeon capable of oxidizing organic acids and growing autotrophically on hydrogen with Fe(III) serving as the sole electron acceptor." Int J Syst Evol Microbiol **52**(3): 719-728.

http://ijs.sgmjournals.org/cgi/content/abstract/52/3/719

Kukolya, J., I. Nagy, et al. (2002). "Thermobifida cellulolytica sp. nov., a novel lignocellulose-decomposing actinomycete." Int J Syst Evol Microbiol **52**(4): 1193-1199.

http://ijs.sgmjournals.org/cgi/content/abstract/52/4/1193

Lee, I. M., D. E. Gundersen-Rindal, et al. (2004). "'Candidatus Phytoplasma asteris', a novel phytoplasma taxon associated with aster yellows and related diseases." Int J Syst Evol Microbiol **54**(4): 1037-1048.

http://ijs.sgmjournals.org/cgi/content/abstract/54/4/1037

Aster yellows (AY) group (16SrI) phytoplasmas are associated with over 100 economically important diseases worldwide and represent the most diverse and widespread phytoplasma group. Strains that belong to the AY group form a phylogenetically discrete subclade within the phytoplasma clade and are related most closely to the stolbur phytoplasma subclade, based on analysis of 16S rRNA gene sequences. AY subclade strains are related more closely to their culturable relatives, Acholeplasma spp., than any other phytoplasmas known. Within the AY subclade, six distinct phylogenetic lineages were revealed. Congruent phylogenies obtained by analyses of tuf gene and ribosomal protein (rp) operon gene sequences further resolved the diversity among AY group phytoplasmas. Distinct phylogenetic lineages were identified by RFLP analysis of 16S rRNA, tuf or rp gene sequences. Ten subgroups were differentiated, based on analysis of rp gene sequences. It is proposed that AY group phytoplasmas represent at least one novel taxon. Strain OAY, which is a member of subgroups 16SrI-B, rpI-B and tufI-B and is associated with evening primrose (Oenothera hookeri) virescence in Michigan, USA, was selected as the reference strain for the novel taxon Candidatus Phytoplasma asteris'. A comprehensive database of diverse AY phytoplasma strains and their geographical distribution is presented.

Lizama, C., M. Monteoliva-Sanchez, et al. (2002). "Halorubrum tebenquichense sp. nov., a novel halophilic archaeon isolated from the Atacama Saltern, Chile." Int J Syst Evol Microbiol **52**(1): 149-155.

http://ijs.sgmjournals.org/cgi/content/abstract/52/1/149

Minana-Galbis, D., M. Farfan, et al. (2004). "Aeromonas molluscorum sp. nov., isolated from bivalve molluscs." Int J Syst Evol Microbiol **54**(6): 2073-2078.

http://ijs.sgmjournals.org/cgi/content/abstract/54/6/2073

Five Aeromonas strains (848TT, 93M, 431E, 849T and 869N), which were isolated from bivalve molluscs and were recognized previously by numerical taxonomy as members of an unknown Aeromonas taxon, were subjected to a polyphasic taxonomic study. DNA-DNA hybridization experiments showed that DNA of strain 848TT was <70 % similar (27-45 %) to that of the type/reference strains of the current Aeromonas hybridization groups (HGs), but 93 % similar to that of strain 93M. The DNA G+C content of the five strains ranged from 59{middle dot}0 to

59{middle dot}4 mol%. 16S rRNA gene sequence analysis confirmed that the strains belonged to the genus Aeromonas and showed high similarity to Aeromonas encheleia. Amplified fragment length polymorphism fingerprinting clustered the novel strains in a homogeneous group with low genotypic relatedness to other Aeromonas species. Useful phenotypic features for differentiating the five isolates from other Aeromonas species include their negative reactions in tests for indole production, lysine decarboxylase, gas from glucose and starch hydrolysis. From the results of this study, the name Aeromonas molluscorum sp. nov. is proposed for these strains, with the type strain 848TT (=CECT 5864T=LMG 22214T).

Moore, R. B., K. M. Ferguson, et al. (2003). "Highly organized structure in the non-coding region of the psbA minicircle from clade C Symbiodinium." Int J Syst Evol Microbiol **53**(6): 1725-1734.

http://ijs.sgmjournals.org/cgi/content/abstract/53/6/1725

The chloroplast genes of dinoflagellates are distributed among small, circular dsDNA molecules termed minicircles. In this paper, we describe the structure of the non-coding region of the psbA minicircle from Symbiodinium. DNA sequence was obtained from five Symbiodinium strains obtained from four different coral host species (Goniopora tenuidens, Heliofungia actiniformis, Leptastrea purpurea and Pocillopora damicornis), which had previously been determined to be closely related using LSU rDNA region D1/D2 sequence analysis. Eight distinct sequence blocks, consisting of four conserved cores interspersed with two metastable regions and flanked by two variable regions, occurred at similar positions in all strains. Inverted repeats (IRs) occurred in tandem or twin' formation within two of the four cores. The metastable regions also consisted of twin IRs and had modular behaviour, being either fully present or completely absent in the different strains. These twin IRs are similar in sequence to double-hairpin elements (DHEs) found in the mitochondrial genomes of some fungi, and may be mobile elements or may serve a functional role in recombination or replication. Within the central unit (consisting of the cores plus the metastable regions), all IRs contained perfect sequence inverses, implying they are highly evolved. IRs were also present outside the central unit but these were imperfect and possessed by individual strains only. A central adenine-rich sequence most closely resembled one in the centre of the non-coding part of Amphidinium operculatum minicircles, and is a potential origin of replication. Sequence polymorphism was extremely high in the variable regions, suggesting that these regions may be useful for distinguishing strains that cannot be differentiated using molecular markers currently available for Symbiodinium.

Nakamura, K., S. Haruta, et al. (2004). "Cerasibacillus quisquiliarum gen. nov., sp. nov., isolated from a semi-continuous decomposing system of kitchen refuse." Int J Syst Evol Microbiol **54**(4): 1063-1069.

http://ijs.sgmjournals.org/cgi/content/abstract/54/4/1063

A moderately thermophilic and alkaliphilic bacillus, which had been reported and designated BLx (Haruta et al., 2002), was isolated from a semi-continuous decomposing system of kitchen refuse. Cells of strain BLxT were strictly aerobic, rod-shaped, motile and spore forming. The optimum temperature and pH for growth were approximately 50 {degrees}C and pH 8-9. Strain BLxT was able to grow at NaCl concentrations from 0{middle dot}5 to 7{middle dot}5 %, with optimum growth at 0{middle dot}5 % NaCl. The predominant menaquinone was MK-7, and the major fatty acid was iso-C15: 0. Phylogenetic analysis showed that strain BLxT was positioned in an independent lineage within the cluster that includes the genera Virgibacillus and Lentibacillus in Bacillus rRNA group 1. Strain BLxT exhibited 16S rDNA similarity of 92{middle dot}8-94{middle dot}8 % to Virgibacillus species and 92{middle dot}3 % to Lentibacillus salicampi. Phenotypic, chemotaxonomic and phylogenetic analyses supported the classification of strain BLxT in a novel

genus and species. Cerasibacillus quisquiliarum gen. nov., sp. nov. is proposed on the basis of phenotypic, chemotaxonomic and phylogenetic data. The type strain is BLxT (DSM 15825T=IAM15044T=KCTC 3815T).

Nishiguchi, M. K. and V. S. Nair (2003). "Evolution of symbiosis in the Vibrionaceae: a combined approach using molecules and physiology." Int J Syst Evol Microbiol **53**(6): 2019-2026.

http://ijs.sgmjournals.org/cgi/content/abstract/53/6/2019

The family Vibrionaceae is considered to be one of the most diverse and well-studied groups of bacteria. Here, evolution is assessed within the Vibrionaceae to determine whether multiple origins of eukaryotic associations have occurred within this diverse group of bacteria. Analyses were based on a large molecular dataset, along with a matrix that consisted of 100 biochemical and restriction digest characters. By using direct optimization methods to analyse both datasets individually and in combination, a total-evidence cladogram has been produced, which supports the hypothesis that several important symbionts (both mutualistic and pathogenic) within the Vibrionaceae are not monophyletic. This leads us to consider that symbiosis (and subsequently, associations with Eukarya) has evolved multiple times within the Vibrionaceae lineage.

Shlimon, A. G., M. W. Friedrich, et al. (2004). "Methanobacterium aarhusense sp. nov., a novel methanogen isolated from a marine sediment (Aarhus Bay, Denmark)." Int J Syst Evol Microbiol **54**(3): 759-763.

http://ijs.sgmjournals.org/cgi/content/abstract/54/3/759

Strain H2-LRT, a 5-18 {micro}m long and 0{middle dot}7 {micro}m wide filamentous, mesophilic, moderately halophilic, non-motile hydrogenotrophic methanogen, was isolated from marine sediment of Aarhus Bay, Denmark, 1{middle dot}7 m below the sediment surface. On the basis of 16S rRNA gene comparison with sequences of known methanogens, strain H2-LRT could be affiliated to the genus Methanobacterium. The strain forms a distinct line of descent within this genus, with Methanobacterium oryzae (95{middle dot}9 % sequence identity) and Methanobacterium bryantii (95{middle dot}7 % sequence identity) as its closest relatives. The 16S rRNA-based affiliation was supported by comparison of the mcrA gene, which encodes the {alpha}-subunit of methyl-coenzyme M reductase. Strain H2-LRT grew only on H2/CO2. The DNA G+C content is 34{middle dot}9 mol%. Optimum growth temperature was 45 {degrees}C. The strain grew equally well at pH 7{middle dot}5 and 8. No growth or methane production was observed below pH 5 or above pH 9. Strain H2-LRT grew well within an NaCl concentration range of 100 and 900 mM. No growth or methane production was observed at 1 M NaCl. At 50 mM NaCl, growth and methane production were reduced. Based on 16S rRNA gene sequence analysis, the isolate is proposed to represent a novel taxon within the genus Methanobacterium, namely Methanobacterium aarhusense sp. nov. The type strain is H2-LRT (=DSM 15219T=ATCC BAA-828T).

Taillardat-Bisch, A.-V., D. Raoult, et al. (2003). "RNA polymerase {beta}-subunit-based phylogeny of Ehrlichia spp., Anaplasma spp., Neorickettsia spp. and Wolbachia pipientis." Int J Syst Evol Microbiol **53**(2): 455-458.

http://ijs.sgmjournals.org/cgi/content/abstract/53/2/455

Sequence analysis of rpoB, the gene encoding the{beta} -subunit of RNA polymerase, was used in a phylogenetic investigation of nine species from the genera Ehrlichia, Neorickettsia, Wolbachia and Anaplasma. The complete nucleotide sequences obtained for Anaplasma phagocytophilum (HGE agent), Ehrlichia chaffeensis, Neorickettsia sennetsu, Neorickettsia risticii, Anaplasma marginale and Wolbachia pipientis were amongst the longest rpoB sequences in GenBank and ranged from 4074 bp for N. sennetsu to 4311 bp for W. pipientis. Additional partial rpoB sequences were obtained for Ehrlichia canis, Ehrlichia ruminantium and Ehrlichia muris. Identical phylogenetic trees were inferred from multiple sequence alignments of the nucleotide sequences and the derived amino acid sequences using either distance, maximum-likelihood or parsimony methods. This study confirms the phylogeny previously inferred from sequence analyses of the 16S rRNA gene, groESL and gltA and allows the confirmation of four monophyletic clades. The rpoB nucleotide sequences were more variable than the 16S rRNA gene and groESL sequences at the species level.

Thompson, C. C., F. L. Thompson, et al. (2004). "Use of recA as an alternative phylogenetic marker in the family Vibrionaceae." Int J Syst Evol Microbiol **54**(3): 919-924.

http://ijs.sgmjournals.org/cgi/content/abstract/54/3/919

This study analysed the usefulness of recA gene sequences as an alternative phylogenetic and/or identification marker for vibrios. The recA sequences suggest that the genus Vibrio is polyphyletic. The high heterogeneity observed within vibrios was congruent with former polyphasic taxonomic studies on this group. Photobacterium species clustered together and apparently nested within vibrios, while Grimontia hollisae was apart from other vibrios. Within the vibrios, Vibrio cholerae and Vibrio mimicus clustered apart from the other genus members. Vibrio harveyi- and Vibrio splendidus-related species formed compact separated groups. On the other hand, species related to Vibrio tubiashii appeared scattered in the phylogenetic tree. The pairs Vibrio coralliilyticus and Vibrio neptunius, Vibrio nereis and Vibrio xuii and V. tubiashii and Vibrio brasiliensis clustered completely apart from each other. There was a correlation of 0{middle dot}58 between recA and 16S rDNA pairwise similarities. Strains of the same species have at least 94 % recA sequence similarity. recA gene sequences are much more discriminatory than 16S rDNA. For 16S rDNA similarity values above 98 % there was a wide range of recA similarities, from 83 to 99 %.

International Biodeterioration & Biodegradation (2)

Held, B. W., J. A. Jurgens, et al. (2005). "Environmental factors influencing microbial growth inside the historic expedition huts of Ross Island, Antarctica." International Biodeterioration & Biodegradation **55**(1): 45.

http://www.sciencedirect.com/science/article/B6VG6-4D4D2PX-2/2/50410d2debbee8476d49d6a8e9b88358

Explorers to Antarctica during the Heroic Era of exploration built three wooden huts on Ross Island, Antarctica in 1902, 1908 and 1911. The structures were used as bases of operation while their occupants participated in scientific endeavors and strived to reach the South Pole. The huts, and the thousands of artifacts in and around them, have survived in the Antarctic environment for

9-10 decades, but deterioration has taken place. The successful preservation of these important historic structures and materials requires information on the agents causing deterioration and factors that influence microbial growth. Temperature and relative humidity (RH) were monitored in the expedition huts for several years. During the austral summer months of December and January it was common for temperatures to rise above and RH to exceed 80%. Extensive fungal growth was observed on wood and artifacts within the Cape Evans hut, and fungi isolated were identified as species of Cladosporium, Penicillium, Cadophora, Geomyces and Hormonema. The factors that influence RH within the huts and methods to control moisture and arrest microbial growth are discussed.

Pitzurra, L., B. Moroni, et al. (2003). "Microbial growth and air pollution in carbonate rock weathering." International Biodeterioration & Biodegradation **52**(2): 63.

http://www.sciencedirect.com/science/article/B6VG6-47X1X1M-F/2/3b076c9ecee4d61e0ed31647068ad617

Preliminary results on limestone weathering caused by air pollution and microbial colonization are presented in this study. Outdoor exposure experimental assays were performed on Scaglia limestone samples. Samples were exposed in two areas in Perugia (Italy) that differ in degree of urban air pollution. At different times of exposure, ranging from 1 to 12 months, microbial contamination of sampled surfaces was evaluated by microbiological techniques, genotyping and scanning electron microscopy. After 1 year of exposure, a significant fungal colonization and the presence of weathering products (i.e., gypsum) were detected on sampled surfaces.

International Congress Series (27)

Albarran, C., P. Martin, et al. (2003). "Analysis of mitochondrial 12S rRNA gene sequence variation in four ethnically defined populations." International Congress Series **1239**: 75.

http://www.sciencedirect.com/science/article/B7581-47W664D-1F2/2/c4ae867ec43632d053501787d71bdaee

In this study, we have analysed the nucleotide sequence variation of the 12S rRNA mitochondrial gene (648-1601 bp) from five different populations (Spanish Caucasian, Autochthonous from the Basque Country, Chinese, New Guinea Highlander and Africans of Benin) by full sequencing of two overlapping PCR fragments using d-rhodamine cycle sequencing coupled with an ABI377 sequencer. Preliminary data indicate different patterns of sequence variation between Africans and non-Africans. Africans are much more polymorphic than non-African populations, which have only a very restricted subset of haplotypes. Furthermore, the greater part of Africans analysed showed two specific nucleotide substitutions (769G->A and 1018G->A) that were not observed in non-African individuals. In conclusion, the mtDNA 12SRNA gene in combination with other systems could be an interesting ethnic marker that could help to differentiate between African and non-African maternal lineages.

Anjos, M. J., M. Carvalho, et al. (2004). "Sequencing of FES, vWA and SE33 STRs in mother-child

incompatibilities." International Congress Series **1261**: 529.

http://www.sciencedirect.com/science/article/B7581-4C4WDDP-69/2/1c58c88fd2946d45d6d864ffd045cdfc

Our casuistic in paternity testing showed several isolated incompatibilities present in different STRs like FES, SE33, vWA, among others, include homozygote and heterozygote situations, and appear both in father-child and mother-child pairs. As we assume, in paternity testing, that the mother is the real one, most of incompatibility situations are assigned to a mutation in putative father. In fact, only sequencing allows the real explanation for the event.

Balogh, M. K., J. Burger, et al. (2003). "Fingerprints from fingerprints." International Congress Series **1239**: 953.

http://www.sciencedirect.com/science/article/B7581-47W664D-1P4/2/110019c43b4de1249779d61355218f69

Berger, B., H. Niederstatter, et al. (2003). "Male/female DNA mixtures: a challenge for Y-STR analysis." International Congress Series **1239**: 295.

http://www.sciencedirect.com/science/article/B7581-47W664D-1GX/2/acc037f6b4a365d98af3d3cc4c9aa958

We present the optimisation of two Y-STR multiplexes for forensic casework applications especially for the analysis of mixtures of male and female DNA. The procedure involved: (1) a new design of the PCR primers for the loci DYS389, DYS390 and DYS391 in order to improve the PCR efficiency and to reduce the length of the amplification products, and (2) the addition of PCR Enhancer to the reaction mix, increasing the specificity of the method.

Bini, C., S. Ceccardi, et al. (2004). "Development of a heptaplex PCR system to analyse X-chromosome STR loci from five Italian population samples. A collaborative study." International Congress Series **1261**: 272.

http://www.sciencedirect.com/science/article/B7581-4C4WDDP-36/2/20ebea36f2419f9f0310f07ba94c667e

A heptaplex PCR has been developed to amplify DXS6789, HUMARA, DXS10011, DXS7423, HPRTB, DXS6807 and DXS101 on Italian samples from Bologna, Modena, Padova, Ancona and Pisa. Statistical analyses were performed for all the loci.

Carvalho, M., C. Mendes, et al. (2003). "mtDNA analysis in Portuguese populations (Central Portugal and Azores Islands): polymorphic sites in control region sequences." International Congress Series **1239**: 535.

http://www.sciencedirect.com/science/article/B7581-47W664D-1JS/2/71d47bf00e013b4f6916c90985b7a262

Background: The polymorphism of the two hypervariable segments (HVI and HVII) of the control region of mtDNA was analyzed in a population of 81 unrelated individuals from Central Portugal and 48 from the Azores Islands, using a fluorescent-based electrophoresis sequencing method. Methods: Sequences have been obtained with ABI PRISM0 Big Dye Terminator and dRhodamine Terminator Cycle Sequencing Ready Reaction Kits, with Amplitaq DNA Polymerase FS, and have been detected with ABI PRISM 377 DNA sequencer. Results: In the Central Portugal population (n=81), we observed 69 polymorphic sites of sequence in HVI region and 44 in HVII region. In the Azores population (n=48), we observed 48 polymorphic sites of sequence in HVI region and 24 in HVII region. Conclusions: Nucleotide substitution rather than insertion/deletion (1 or 2 bp) was the majority of variation. The distribution showed a large bias towards transitional changes than transversional changes. Our sequencing results are similar to other Caucasian population data.

Cathala, P., E. Baccino, et al. (2003). "Forensic applications of denaturing high-performance liquid chromatography: determination of age at death, human identification and gender determination." International Congress Series **1239**(Supplement 1): S1.

http://www.sciencedirect.com/science/article/B7581-49M5YX9-3/2/6a2d66cb5183bcf8b707479803f5658c

Denaturing high-performance liquid chromatography (dHPLC) is a new automated sizing method when used in non-denaturing conditions and is a powerful mutation detection tool in denaturing conditions. We introduce three possible forensic applications of this new technology.(1)Determination of age at death. Various mutations accumulate in mtDNA during ageing. According to this, we are developing a new method to determine age at death based on dHPLC ability to detect mtDNA mutations. mtDNA is extracted from autopsy tissues (iliopsoas, liver, kidney, putamen and heart) of numerous individuals representing a wide age spectrum. After amplification and digestion of the entire mtDNA in 90-600 bp fragments, separation is performed by dHPLC at different temperatures. We are studying the qualitative and quantitative differential accumulation of mutations with age among the various tissues. Our methodology is presented.(2)Human identification. In non-denaturing conditions, dHPLC gives size-based separation of DNA fragments. This allows separation of short tandem repeats (STRs) fragments. The different alleles of the HUMTH01, F13A01, vWa31 and FES/FPS loci were separated and sized in 5 min.(3)Gender determination. Used in non-denaturing conditions, dHPLC can separate the two sex-specific alleles of the amelogenin locus in 4 min without any preparation of the PCR product.

Coene, A., N. Vanderheyden, et al. (2004). "Analysis of SNP-variation in the coding region of mitochondrial DNA." International Congress Series **1261**: 97.

http://www.sciencedirect.com/science/article/B7581-4C4WDDP-15/2/a0118765706b57bdc0c0193d9e9f88fc

A multiplex-PCR procedure was developed for the analysis of single nucleotide polymorphism (SNP) variation defining the European mtDNA-haplotypes in the coding part of the mitochondrial genome. The obtained PCR-products were genotyped for 26 SNPs in four mini-sequencing (SNaPshot(TM)) reactions and analyzed on the ABI PRISM(R) 3100 Genetic Analyzer. A population study of 157 individuals of Belgian descent revealed 25 different haplotypes belonging to 5 major haplogroups.

Dewa, K., N. Quang Tuyen, et al. (2003). "13 Y-chromosomal STRs in a Vietnamese population." International Congress Series **1239**: 315.

http://www.sciencedirect.com/science/article/B7581-47W664D-1H2/2/718a3750b0a508a4a07961059417b621

We present the frequency distributions of 13 Y-specific STR polymorphisms (DYS19, DXYS156, DYS385, DYS389 I and II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, DYS439 and YCAII) and the frequency of the combination of these haplotypes in Vietnamese males.

Hoffmann, E., N. Zhou, et al. (2001). "Eight-plasmid rescue system for influenza A virus." International Congress Series **1219**: 1007.

http://www.sciencedirect.com/science/article/B7581-47W60JD-4X/2/d71c9a20c7930e85b9294a30fdef8264

Plasmid-driven synthesis of viral RNA and protein allows the recovery of infectious influenza virus without the need for helper virus infection. Because no selection system is required for this approach, genetic manipulation of all eight viral gene segments without technical limitations is possible. We have developed a system which requires the construction and transfection of only eight plasmids for the recovery of influenza A viruses. In this DNA transfection system, viral cDNA is inserted between the human RNA polymerase I (pol I) promoter and murine terminator sequences. The entire pol I transcription unit is flanked by an RNA polymerase II (pol II) promoter and a poly(A) site. As a first step to evaluate the utility of this plasmid-based system for the production of vaccines, we generated the master strain A/PR/8/34 (H1N1) currently used for the production of inactivated vaccines entirely from cloned cDNAs. The virus yield as determined by HA-assay after passage of the recombinant virus in eggs was as high as the virus yield of the parental wild-type virus. These results prove that the generated recombinant virus has the same growth properties as the parental egg grown virus and indicate that the eight-plasmid transfection method has the potential to improve currently used methods for the production of vaccine viruses.

Iacovacci, G., M. Serafini, et al. (2003). "STR typing from human faeces: a modified DNA extraction method." International Congress Series **1239**: 917.

http://www.sciencedirect.com/science/article/B7581-47W664D-1NV/2/4f44ac088f3d7ccc65fa233e158fa59b

Kloosterman, A. D. and P. Kersbergen (2003). "Efficacy and limits of genotyping low copy number DNA samples by multiplex PCR of STR loci." International Congress Series **1239**: 795.

http://www.sciencedirect.com/science/article/B7581-47W664D-1MV/2/a76d7c70f0f10866e899768adfcd9ad8

In this validation study, we have evaluated the efficacy and the validity of the SGM Plus test using an amplification regime of 34 cycles. We obtained valid DNA typing results from pristine extracts with an extremely low DNA content. In this context, the aspects of single cell PCR typing were also evaluated. In these experiments, the allele dropout phenomenon was clearly demonstrated. From actual casework samples, we obtained conclusive DNA profiles from highly purified extracts of bone and teeth that failed to demonstrate typing results using the standard PCR protocol of 28

cycles. Moreover, low copy number (LCN) DNA typing offered us the possibility to reanalyse crime samples that failed to produce a conclusive profile after 28 cycles. Unfortunately, several complications accompany ultrasensitive PCR amplification. During our validation studies, we have observed increased risk of contamination, allelic dropout, locus dropout and heightened stutters. Analyses of heterozygote balance, between-loci balance and stutter heights, show that the 34-cycle PCR has its own characteristic features. We finally show that reamplification of SGM Plus PCR products by an extra 6 PCR cycles offers a promising new alternative if too little of the original sample extract is left for a complete reanalysis.

Matsuki, T., K. Sawazaki, et al. (2003). "DXS10011: a hypervariable TTTC/GAAA repeat marker on human chromosome Xq27-q28." International Congress Series **1239**: 363.

http://www.sciencedirect.com/science/article/B7581-47W664D-1HF/2/b6aa2eb52e67691460aa5d43ce62481d

We mapped human STS UT413 on human chromosome Xq27-q28, renamed it DXS10011 and developed an easy method of analysis using capillary electrophoresis. The probability of discrimination was 0.954 from 1198 chromosomes in the Japanese population. DXS10011 is a hypervariable and stable marker on the human X chromosome.

Medeiros, R., N. Escriou, et al. (2001). "Hemagglutinin residues of recent human A(H3N2) viruses that affect agglutination of chicken erythrocytes." International Congress Series **1219**: 369.

http://www.sciencedirect.com/science/article/B7581-47W60JD-1T/2/023af6806577d635a2b76ed4e6ad50a4

Background: The hemagglutination remains crucial in diagnosis of influenza virus and for the antigenic characterization of the hemagglutinin (HA) and neuraminidase. However, the human influenza viruses A(H3N2) isolated recently appear to have lost the ability to agglutinate chicken erythrocytes (ER) (RBC). The molecular determinants of this phenomenon are not known. Methods: Two viruses isolated in Paris were studied, since their ability to agglutinate chicken RBC was observed after serial passages either in Madin Darby canine kidney (MDCK) cells or embryonated hen's eggs. Sequencing analyses and hemadsorption assays were performed to demonstrate the role of amino acid substitutions in the HA gene. Results: Sequencing of the HA gene revealed the presence of either the Leu194Ile or the Val226Ile mutation following the phenotypic change. Hemadsorption assays performed following transfection of COS-1 cells, with plasmids expressing wild-type or mutated HA molecules, showed that the Leu194Ile and Val226Ile mutations were responsible for the ability of the HA to bind chicken RBCs. Conclusion: These findings suggest that a valine at position 226 in the HA molecule, found in recent clinical isolates of human A(H3N2) viruses, could be responsible for their inability to agglutinate chicken erythrocytes.

Nata, M. and M. Hashiyada (2003). "Rapid detection of GYPA, LDLR, HBGG, D7S8 and GC alleles by real-time fluorescence PCR." International Congress Series **1239**: 27.

http://www.sciencedirect.com/science/article/B7581-47W664D-1DN/2/708647627c0adb225adac1051e3fd3f8

We developed the allele-specific TaqMan polymerase chain reaction (AS-TaqMan PCR) and

SYBR Green PCR assays for detecting glycophorin A (GYPA), low-density lipoprotein receptor (LDLR), hemoglobin G (HBGG), D7S8 and group-specific component (GC) alleles. We improved the specificity of detecting a nucleotide substitution by introducing the additional mismatches at position 2 (3 in GYPA). The differences between threshold cycle (Ct) values of different genotypes on each of the loci were statistically significantly different. All the genotypes agreed with the results using the AmpliType PM+DQA1 PCR Amplification and Typing kit. The AS-TaqMan PCR and SYBR Green PCR assays are simple, rapid, and accurate, as well as suitable for high-throughput applications in a forensic investigation.

Rapone, C., G. D'Errico, et al. (2003). "DNA typing after [alpha]-amylase test." International Congress Series **1239**: 911.

http://www.sciencedirect.com/science/article/B7581-47W664D-1NT/2/c4b0cafebdadc36c6a16f7ea26b082a1

Reisacher, R. B. K., B. Glock, et al. (2004). "Short tandem repeat polymorphisms across the HLA-complex: sequence and population data of D6S389 and D6S1051." International Congress Series **1261**: 236.

http://www.sciencedirect.com/science/article/B7581-4C4WDDP-2S/2/8e9f10c4a62a8e7d5a2250f5fab6d93a

The tetranucleotide repeat loci D6S389 and D6S1051 situated nearby the HLA class II region (6p) were investigated in an Austrian Caucasoid population sample. Typing of the amplification products and cycle sequencing were carried out using denaturing capillary electrophoresis. For D6S389 and D6S1051, 19 and 7 different alleles were observed. Sequencing of D6S389 revealed a (GAAA)n repeat pattern in the common alleles. Additionally, infrequent ".1" alleles were seen. Alleles of D6S1051 showed a simple (GATA)n repeat structure and an A/G-SNP next to the repeat region as well as a T/C-SNP in the 3'-flanking region. D6S389 proved to be a highly polymorphic marker. D6S1051 is less polymorphic, but interesting because of the existing SNPs.

Sobrino, B., M. Lareu, et al. (2004). "SNP genotyping with single base extension-tag microarrays." International Congress Series **1261**: 331.

http://www.sciencedirect.com/science/article/B7581-4C4WDDP-3X/2/c9eb763ad68ae2e78e89a43e7778e793

During the last few years, there is an increasing interest in the use of Single Nucleotide Polymorphisms (SNPs) for forensic purposes as an alternative to STR analysis. At this moment, development of SNP genotyping technologies to analyse several markers in the same reaction with high accuracy, simplicity and reasonable cost is the key to progress in SNP typing for forensic genetics. A promising approach for this purpose is DNA microarrays. We have developed a microarray for typing a set of 29 Y-chromosome SNPs for European populations. Single Base Extension and Tags (SBE-Tag) has been the strategy selected.

Stadlbacher, S., E. M. Dauber, et al. (2003). "The tetranucleotide repeat polymorphism C 2_4_4: sequence and population data." International Congress Series **1239**: 91.

http://www.sciencedirect.com/science/article/B7581-47W664D-1F7/2/d1d36c9b398ba26117182304282821dc

The STR locus C 2_4_4 situated in the HLA class I region (6p21.3) was investigated in an Austrian Causasoid population sample of 247 unrelated individuals. Beside length polymorphism, the alleles at this locus also revealed sequence polymorphism.

Tsukada, K., K. Takayanagi, et al. (2004). "Simultaneous PCR of eight loci for very short Y-STR fragment size." International Congress Series **1261**: 322.

http://www.sciencedirect.com/science/article/B7581-4C4WDDP-3T/2/3d09c41cf2ee671e0dc3997b317fa45c

We selected the DYS19, DYS385, DYS389, DYS390, DYS392, DYS393, DYS483, and amelogenin loci, and designed a new pair of primers to minimize the fragment sizes of these loci as much as possible. As a consequence, these loci were able to detect in the range of 79-259 bp using multiplex PCR amplification. The optimum DNA amount was 100 pg to 10 ng. The haplotype diversity was 0.9979.

Tsukada, K., K. Takayanagi, et al. (2003). "Multiplex PCR using newly designed primers for very short fragments of TH01, TPOX, CSF1PO, and vWA loci." International Congress Series **1239**: 755.

http://www.sciencedirect.com/science/article/B7581-47W664D-1MH/2/4d767eb9a4eac74d0a6dee1a5999bf67

We performed multiplex PCR for the TH01, TPOX, CSF1PO, and vWA loci using newly designed pairs of primers that yield smaller fragments than previously reported [Int. J. Leg. Med. 114 (2001) 285; Am. J. Hum. Genet. 55 (1994) 175; Int. J. Leg. Med. 106 (1994) 183.] [[1, 2 and 3]]. This system required genomic DNA in a range of 50 pg-2 ng, and proved to be sensitive as a typing method. Furthermore, it was possible to determine the allele types even from 18-year-old bloodstains.

Turrina, S. and D. De Leo (2004). "Population genetic comparisons of three X-chromosomal STRs (DXS7132, DXS7133 and GATA172D05) in North and South Italy." International Congress Series **1261**: 302.

http://www.sciencedirect.com/science/article/B7581-4C4WDDP-3J/2/edc69691f3af8fe8b7778ca595db8066

Population genetic data of three tetranucleotide X-chromosomal STRs, DXS7132, DXS7133 and GATA172D05, were obtained by analyzing 295 unrelated healthy individuals living in North and South Italy (160 females and 135 males), and 40 family trios with female children. PCR primers for the loci DXS7132 and DXS7133 were redesigned in order to reduce the length of the amplification products compared with conventional design so that improved typing success rate when highly degraded DNA is used as a template. The comparison of the allele frequencies of these three ChrX markers gave similar distributions for North and South Italy although minor variations were found for some alleles. Additionally, some differences were found when comparing the allele frequencies of the male and female samples independently. Based on the investigated meiotic events, mutations were not found.

Verrecas, M., K. Knaepen, et al. (2004). "Forensic toxicology: development of an SNP-assay for genotyping CYP2D6 and CYP2C19 variants." International Congress Series **1261**: 583.

http://www.sciencedirect.com/science/article/B7581-4C4WDDP-6Y/2/48b75002cc2e00bcde61e0aef652e3c9

A procedure was developed for genotyping one CYP2C19 and eight CYP2D6 polymorphisms. After multiplex PCR of the exons containing the SNPs, a mini-sequencing reaction was performed with the SNapShot(TM) Multiplex Kit and the resultant primer-extension products were analyzed on an ABI PRISM(R) 3100 Genetic Analyzer. The developed procedure was validated in a population of 199 Caucasians.

Vieira Silva, C., C. Cruz, et al. (2004). "16 Y-specific STR analysis in human remains identification." International Congress Series **1261**: 251.

http://www.sciencedirect.com/science/article/B7581-4C4WDDP-2Y/2/65cc3db2aaec5dad408c97c545bc9709

Degraded samples were studied in order to obtain Y-STR haplotype to provide additional data in paternal lineage identification caseworks. Multiplex reactions were used comprising the minimal Y-STR haplotype DYS19, DYS390, DYS391, DYS392, DYS393, DYS19, DYS389I/II and DYS385 belonging to the Y-STR database. The other Y-STR loci--GATA A 7.1, GATA A 7.2, GATA C4, GATA H4, DYS437, DYS438 and DYS439--were included in the Y-Chromosome Quality Control Group of the Spanish and Portuguese Group (GEPY) of the ISFG. Y-STRs results were successful in almost all the samples when applied some modifications in amplification methods.

Wenda, S., E. M. Dauber, et al. (2004). "Multiplex PCR investigation of the STR loci C1_4_4, C2_4_4 and C3_3_6 in the HLA class I region." International Congress Series **1261**: 188.

http://www.sciencedirect.com/science/article/B7581-4C4WDDP-27/2/b0378627912cd1e63214b270f5ec393e

In this study, we investigated the short tandem repeat loci C1_4_4, C2_4_4 and C3_3_6 situated in the HLA class I region in a sample of 153 unrelated Austrian Caucasoid individuals by multiplex PCR. The sequence structure of C1_4_4 and C3_3_6 alleles is described, as well as the sequence of a new C2_4_4 allele, allele 23.

Yen, H. L., M. C. Cheng, et al. (2001). "Influenza surveillance in poultry market and its inter-species transmission in Taiwan." International Congress Series **1219**: 201.

http://www.sciencedirect.com/science/article/B7581-47W60JD-13/2/3c6ae1fb0f18c608626b592c549cf834

Background: Since Taiwan has high population density and a similar ecological environment to Mainland China, the epi-center of influenza viruses, it is very important to establish influenza virologic surveillance systems in both animal and human populations. The H5N1 Hong Kong Flu

in 1997 and H9N2 in 1999 have showed that avian influenza viruses can cross the receptor of host species boundary and transmit to human. Therefore, the specific aim of this study is to understand the frequency of inter-species transmission in Taiwan. Methods: We have established an avian influenza virologic surveillance system in one of the largest live poultry markets in Taipei City from October 1999 to March 2000. Serum samples were collected from 341 blood donors, including veterinarians, poultry farm workers, and market employees. HI and microneutralization were used to detect specific antibody against H6, an endemic virus in chicken farms in southern Taiwan, and antibodies against H3, H4, and H9 viruses. Results: Among about 1300 fecal specimens of chickens and ducks collected, we isolated 12 H3 viruses, 14 H4 viruses, and 2 H6 viruses (i.e. 9 serotypes of HA and NA) from ducks. The isolation rates were 0% (0/580) and 7% (28/400) in chickens and ducks, respectively. Phylogenetic analysis of HA from 7 of our 12 H3 isolates showed the highest (93%) homology with A/equine/Jilin/89 (H3N8). Both phylogenetic relationship of HA and NP genes from selected representative strains (7 H3, 7 H4, and 2 H6) found they all fell into Eurasian lineage of avian influenza viruses. Their NP genes were away from the G1 lineage that was found in H5N1 strain isolated in Hong Kong in 1997. In addition, the results of HI and microneutralization tests found that they were all seronegative against two avian influenza virus strains [A/Duck/Czechoslovakia/56 (H4N6) and A/Shearwater/Australia/1/72 (H6N5)]. Conclusion: Continuous efforts by integrating animal, market and human influenza surveillance systems have provided the best early warning signals to detect new influenza virus activities for preventing potential pandemics and providing effective controls.

Zoledziewska, M. and T. Dobosz (2003). "Gender determination in highly degraded DNA samples." International Congress Series **1239**: 593.

http://www.sciencedirect.com/science/article/B7581-47W664D-1K6/2/bd3ca57048b84af02c2186bc984260ad

Based on the method described in Stone et al.'s [Am. J. Phys. Anthropol. 99 (1999) 231] publication, a fragment of the amelogenin gene (exon 6) was shortened. In a new method, a flanked and amplified 78-bp fragment from X and Y copies of the amelogenin gene, followed by hybridization and ligation with fluorescent-labeled oligonucleotides, resulted in two gender-specific products analyzed by capillary electrophoresis. The method was tested using fresh DNA samples and DNA isolated from bones of different ages.

International Dairy Journal (4)

Bottero, M. T., T. Civera, et al. (2003). "A multiplex polymerase chain reaction for the identification of cows', goats' and sheep's milk in dairy products." International Dairy Journal **13**(4): 277.

http://www.sciencedirect.com/science/article/B6T7C-47VS61M-1/2/346576973b671ab137397d3a96e4b516

A multiplex PCR able to identify cows', goats' and sheep's milk in dairy products was developed. Specific primers were designed in the mitochondrial 12s and 16s rRNA genes so as to generate fragments of different length.The assay was applied to 19 cheeses from the retail trade in order to verify the label statements. The multiplex PCR results were confirmed by PCR-restriction fragment length polymorphism.The proposed multiplex PCR represents a rapid and sensitive

method applicable on a routine basis. In fact it enables to detect, in a single step, goats', sheep's and cows' milk in dairy products with a good sensitivity threshold (0.5%).

Lombardi, A., M. Gatti, et al. (2004). "Characterization of Streptococcus macedonicus strains isolated from artisanal Italian raw milk cheeses." International Dairy Journal **14**(11): 967.

http://www.sciencedirect.com/science/article/B6T7C-4D2XFXX-1/2/611c7b6d43f638bf46e1e1ad94db9c95

The aim of this research was to elucidate the potential technological role of Streptococcus macedonicus in cheese. For this purpose, phenotypic and randomly amplified polymorphic DNA (RAPD)-PCR subtyping as well as biochemical characterization were carried out on 37 S. macedonicus isolates collected from several artisanal, Italian raw milk cheeses. Identification was achieved by a species-specific PCR assay developed in this study. A wide phenotypic and genotypic heterogeneity between the isolates was observed. A certain variability of the acidifying and peptidase activities was observed and the comparison between some technological characteristics displayed by S. macedonicus and Streptococcus thermophilus indicated a clear distinction of these species. The presence of dairy grade characteristics, such as peptidase activities, ability to produce inhibitory compounds, absence of antibiotic resistance and haemolytic activity, may suggest the rational design of a novel starter culture for dairy application composed of strains of both S. macedonicus and S. thermophilus species, with the former used as a starter adjunct.

Marilley, L., S. Ampuero, et al. (2004). "Screening of aroma-producing lactic acid bacteria with an electronic nose." International Dairy Journal **14**(10): 849.

http://www.sciencedirect.com/science/article/B6T7C-4CDJHKR-1/2/9463937d67a2442ab2e6fe99e6f999f0

The potential of a mass spectrometry (MS) based electronic nose to screen lactic acid bacteria producing volatile compounds was assessed. Thirty four reference strains and 62 Lactobacillus casei strains isolated from 5 dairies producing Gruyere cheeses were analysed. These isolates were classified into 7 different genotypes by Repetitive extragenic palindromic Polymerase chain reaction (REP-PCR). The strains were incubated in Ultra High Temperature milk supplemented with casamino acids. After 10 days of incubation, the volatile fractions were analysed with the electronic nose. The strains with the same genotype were grouped together. The classification of strains based on the production of volatile compounds was in conformity with the classification obtained with the molecular method. This MS-based electronic nose can be used to differentiate bacterial populations in cheese samples and to screen for new aroma-producing strains.

Rijpens, N., G. Jannes, et al. (2002). "Messenger RNA-based RT-PCR detection of viable Salmonella." International Dairy Journal **12**(2-3): 233.

http://www.sciencedirect.com/science/article/B6T7C-44GF0GD-2/2/0f9c3e16c35eed454323d6876018863e

The objective of our study was to investigate whether certain regions in the rpoD gene of Salmonella were suited for the RT-PCR detection of viable Salmonella cells. We performed RT-PCR on RNA extracted from viable, and heat- or ethanol-killed Salmonella cells. Using RT-PCR,

RNA was easily detected in viable cells. For the heat-killed cells mRNA was undetectable after 1 h when stored at room temperature or 4[deg]C. For the ethanol-killed cells mRNA was detectable till 1 and 48 h after treatment, when the dead Salmonella cells were stored at room temperature and 4[deg]C, respectively. When the ethanol-killed cells that were stored at 4[deg]C, were incubated at 37[deg]C for 1 h prior to RNA extraction, no mRNA was detected. The primerpair used (salmrpod2/5) was directed to the housekeeping gene rpoD and using RT-PCR, signals from Salmonella were obtained and no cross-reaction was observed with other members of the Enterobacteriaceae.

International Journal for Parasitology (36)

Arkush, K. D., M. A. Miller, et al. (2003). "Molecular and bioassay-based detection of Toxoplasma gondii oocyst uptake by mussels (Mytilus galloprovincialis)." International Journal for Parasitology **33**(10): 1087.

http://www.sciencedirect.com/science/article/B6T7F-4961NGV-1/2/4bc2cd1918c679086a7fe5dbf9d58f71

Toxoplasma gondii is associated with morbidity and mortality in a variety of marine mammals, including fatal meningoencephalitis in the southern sea otter (Enhydra lutris nereis). The source(s) of T. gondii infection and routes of transmission in the marine environment are unknown. We hypothesise that filter-feeding marine bivalve shellfish serve as paratenic hosts by assimilation and concentration of infective T. gondii oocysts and their subsequent predation by southern sea otters is a source of infection for these animals. We developed a TaqMan PCR assay for detection of T. gondii ssrRNA and evaluated its usefulness for the detection of T. gondii in experimentally exposed mussels (Mytilus galloprovincialis) under laboratory conditions. Toxoplasma gondii-specific ssrRNA was detected in mussels as long as 21 days post-exposure to T. gondii oocysts. Parasite ssrRNA was most often detected in digestive gland homogenate (31 of 35, i.e. 89%) compared with haemolymph or gill homogenates. Parasite infectivity was confirmed using a mouse bioassay. Infections were detected in mice inoculated with any one of the mussel sample preparations (haemolymph, gill, or digestive gland), but only digestive gland samples remained bioassay-positive for at least 3 days post-exposure. For each time point, the total proportion of mice inoculated with each of the different tissues from T. gondii-exposed mussels was similar to the proportion of exposed mussels from the same treatment groups that were positive via TaqMan PCR. The TaqMan PCR assay described here is now being tested in field sampling of free-living invertebrate prey species from high-risk coastal locations where T. gondii infections are prevalent in southern sea otters.

Aspinall, T. V., D. Marlee, et al. (2002). "Prevalence of Toxoplasma gondii in commercial meat products as monitored by polymerase chain reaction - food for thought?" International Journal for Parasitology **32**(9): 1193.

http://www.sciencedirect.com/science/article/B6T7F-45RFM7B-5/2/04158c2ab3ac5dac750db5c8531b9c44

DNA was extracted from 71 meat samples obtained from UK retail outlets. All of these DNA preparations gave the expected polymerase chain reaction products when amplified with primers

specific for the species from which the meat originated. A second polymerase chain reaction analysis, using primers specific for the Toxoplasma gondii SAG2 locus, revealed the presence of this parasite in 27 of the meat samples. Restriction analysis and DNA sequencing showed that 21 of the contaminated meats contained parasites genotyped as type I at the SAG2 locus, whilst six of the samples contained parasites of both types I and II. Toxoplasma- positive samples were subjected to further polymerase chain reaction analysis to determine whether any carried an allele of the dihydropteroate synthase gene that has recently been shown to be causally associated with sulfonamide resistance in T. gondii. In all cases, this analysis confirmed that parasites were present in the samples and, additionally, revealed that none of them carried the drug-resistant form of dihydropteroate synthase. These results suggest that a significant proportion of meats commercially available in the UK are contaminated with T. gondii. Although none of the parasites detected in this study carried the sulfonamide-resistance mutation, a simplified procedure for monitoring this situation merits development.

Bruna-Romero, O., J. C. R. Hafalla, et al. (2001). "Detection of malaria liver-stages in mice infected through the bite of a single Anopheles mosquito using a highly sensitive real-time PCR." International Journal for Parasitology **31**(13): 1499.

http://www.sciencedirect.com/science/article/B6T7F-4447KS1-C/2/769a78f65756b6318627c7be539ed869

We describe a highly sensitive real-time PCR to detect and measure the development of the liver-stages of malaria parasites in mice infected with sporozoites ranging in number from 25 to more than 164,000, using the same reaction conditions. Furthermore, this assay detects and measures parasite loads in the livers of mice exposed to the bite of a single malaria-infected Anopheles mosquito. This unique method should greatly facilitate studies aimed at evaluating very precisely the efficacy of anti-malarial experimental drug treatments and vaccination regimens in conditions of infection resembling those found in the field.

Casu, R. E., C. H. Eisemann, et al. (1996). "The major excretory/secretory protease from Lucilia cuprina larvae is also a gut digestive protease." International Journal for Parasitology **26**(6): 623.

http://www.sciencedirect.com/science/article/B6T7F-3WF7DY2-4/2/e199dfd576315ec9f38d3b00d1c31081

The larvae of the fly Lucilia cuprina excrete or secrete a chymotrypsin (LCTb) onto the skin of sheep to facilitate the establishment of the larval infestation. A combination of immunoblotting and RT-PCR approaches has established that this protease is also a gut digestive protease. LCTb is synthesized primarily in the cardia, a small highly specialized organ located at the anterior end of the midgut and by midgut cells. There is also some expression by the hindgut but no expression by salivary glands. Excretion of LCTb with waste products or regurgitation of the gut contents of the larvae may explain how this protease is transferred from the larval gut onto ovine skin. LCTb is first expressed in eggs and constitutively expressed throughout each larval instar, but is not expressed in pupae or adult flies. It is concluded that LCTb could be involved in the establishment of larvae on sheep skin as well as acting as a general gut digestive enzyme.

Chalmers, R. M., C. Ferguson, et al. (2005). "Direct comparison of selected methods for genetic categorisation of Cryptosporidium parvum and Cryptosporidium hominis species." International Journal for Parasitology **35**(4): 397.

http://www.sciencedirect.com/science/article/B6T7F-4FH5GV9-2/2/12e8d0f05ff570d5204b0bcb2989e6f1

A study was undertaken to compare the performance of five different molecular methods (available in four different laboratories) for the identification of Cryptosporidium parvum and Cryptosporidium hominis and the detection of genetic variation within each of these species. The same panel of oocyst DNA samples derived from faeces (n=54; coded blindly) was sent for analysis by: (i) DNA sequence analysis of a fragment of the HSP70 gene; (ii) DNA sequence analysis and the ssrRNA gene in laboratory 1; (iii) single-strand conformation polymorphism analysis of part of the ssrRNA; (iv) SSCP analysis of the second internal transcribed spacer (ITS-2) of nuclear ribosomal DNA region in laboratory 2; (v) 60 kDa glycoprotein (gp60) gene sequencing with prior species determination using PCR with restriction fragment length polymorphism analysis of the ssrRNA gene in laboratory 3; and (vi) multilocus genotyping at three microsatellite markers in laboratory 4. For detecting variation within C. parvum and C. hominis, SSCP analysis of ITS-2 was considered to have superior utility and determined 'subgenotypes' in samples containing DNA from both species. SSCP was also most cost effective in terms of time, cost and consumables. Sequence analysis of gp60 and microsatellite markers ML1, ML2 and 'gp15' provided good comparators for the SSCP of ITS-2. However, applicability of these methods to other Cryptosporidium species or genotypes and to environmental samples needs to be evaluated. This trial provided, for the first time, a direct comparison of multiple methods for the genetic characterisation of C. parvum and C. hominis samples. A protocol has been established for the international distribution of samples for the characterisation of Cryptosporidium. This can be applied in further evaluation of molecular methods by investigation of a larger number of unrelated samples to establish sensitivity, typability, reproducibility and discriminatory power based on internationally accepted methods for evaluation of microbial typing schemes.

Cornelissen, J. B. W. J., C. P. H. Gaasenbeek, et al. (2001). "Early immunodiagnosis of fasciolosis in ruminants using recombinant Fasciola hepatica cathepsin L-like protease." International Journal for Parasitology **31**(7): 728.

http://www.sciencedirect.com/science/article/B6T7F-42XB4F7-C/2/bc45a0d3915e84b1e2cdfd41e53cea7c

A diagnostic ELISA with recombinant Fasciola hepatica cathepsin L-like protease as antigen was developed to detect antibodies against F. hepatica in sheep and cattle. The recombinant cathepsin L-like protease was generated by functional expression of the cDNA from adult stage F. hepatica flukes in Saccharomyces cerevisiae. Specificity and sensitivity of the cathepsin L enzyme-linked immunosorbent assay (ELISA) was assessed using sera from sheep and calves experimentally or naturally mono-infected with F. hepatica and six-seven other parasites. The sensitivity of the cathepsin L ELISA for sheep and cattle sera was 99.1 and 100%, respectively. In the experimental setting with established mono-infections, the specificity of the cathepsin L ELISA was 98.5% for cattle sera and 96.5% for sheep sera. In experimentally infected cattle and sheep, the first detection of F. hepatica-specific antibodies appeared first between 5 and 7 weeks post-infection, but depended on the infectious dose of F. hepatica. In ELISA the detection preceded first detection of the infection based on egg counts and remained detectable till at least 23 weeks after a primary F. hepatica infection. Detection of Fasciola gigantica infections was similar to detection of F. hepatica. The first detection occurred at week 5 and signals persisted for at least 20 weeks. All sera from naturally F. hepatica infected sheep were seropositive in the cathepsin L-like ELISA. The relevance of this ELISA format was also evaluated using sera from naturally infected cattle in the Netherlands, Ecuador and Vietnam and compared with results from egg-counts. For the latter two endemic areas with mixed parasitic infections the 'apparent' sensitivity of the cathepsin L ELISA was calculated for all serum samples together to be 90.2%. The 'apparent' specificity under these conditions was calculated to be 75.3%. In cattle, the cathepsin L ELISA was superior to the concurrently evaluated peptide ELISA format using a single epitope as

the antigen both in controlled natural infections as well as in infections in endemic areas. The present ELISA-format contributes a relatively sensitive and reliable tool for the early serodiagnosis of bovine and ovine fasciolosis.

Ey, P. L., J. M. Darby, et al. (1993). "Giardia intestinalis: Detection of major genotypes by restriction analysis of gene amplification products." International Journal for Parasitology **23**(5): 591.

http://www.sciencedirect.com/science/article/B6T7F-476HJH1-1TC/2/2b6f661142794f5c3f5c5c601f3bcfb1, and 1993. Giardia intestinalis: Detection of major genotypes by restriction analysis of gene amplification products. International Journal for Parasitology 23: 591-600. The polymerase chain reaction (PCR) has been used to amplify a 0.52 kb segment of Giardia intestinalis DNA, using primers specific for nucleotide sequences conserved within two genes (tspl 1 and tsa417) that encode homologous, cysteine-rich trophozoite surface proteins. Using products amplified from axenic isolates belonging to genetic groups I and II (defined on the basis of allozyme electrophoresis data), restriction endonuclease analysis revealed both tsp11-like and tsa417-like fragments within all samples. The study also identified among the amplification products of group II organisms an additional fragment, containing a novel PstI site, that is not detected in the reaction products of group I isolates. The recovery of three distinct PCR products from each group II isolate was verified by cloning the fragments into the plasmid vector pGEM-7. Fragments containing the new PstI site possess the ClaI site common to both tsp11 and tsa417-like fragments, but they lack the HindIII site which characterizes tsp11-like fragments and also lack the PstI and KpnI sites which characterize tsa417-like fragments. Spot-blot analyses using cloned fragments of all three types as probes showed strong homologous hybridization but weak heterologous hybridization, indicating that each type diners substantially in nucleotide sequence from the others. Because the samples of Giardia DNA used in the PCR were purified from cultures that had been established from single trophozoites, the data indicate that individual trophozoites belonging to genetic group II possess three homologous genes defined by these related fragments. The presence of a PstI site in the amplified segment of the newly-discovered third gene of group II organisms provides a simple diagnostic means of differentiating group I and II isolates.

Foster, J. M., S. Kumar, et al. (2004). "Construction of bacterial artificial chromosome libraries from the parasitic nematode Brugia malayi and physical mapping of the genome of its Wolbachia endosymbiont." International Journal for Parasitology **34**(6): 733.

http://www.sciencedirect.com/science/article/B6T7F-4BYF7VX-1/2/52b9c6efb7ed6ec0ca47acca0c1feacb

The parasitic nematode, Brugia malayi, causes lymphatic filariasis in humans, which in severe cases leads to the condition known as elephantiasis. The parasite contains an endosymbiotic [alpha]-proteobacterium of the genus Wolbachia that is required for normal worm development and fecundity and is also implicated in the pathology associated with infections by these filarial nematodes. Bacterial artificial chromosome libraries were constructed from B. malayi DNA and provide over 11-fold coverage of the nematode genome. Wolbachia genomic fragments were simultaneously cloned into the libraries giving over 5-fold coverage of the 1.1 Mb bacterial genome. A physical framework for the Wolbachia genome was developed by construction of a plasmid library enriched for Wolbachia DNA as a source of sequences to hybridise to high-density bacterial artificial chromosome colony filters. Bacterial artificial chromosome end sequencing provided additional Wolbachia probe sequences to facilitate assembly of a contig that spanned the entire genome. The Wolbachia sequences provided a marker approximately every 10 kb. Four rare-cutting restriction endonucleases were used to restriction map the genome to a resolution of approximately 60 kb and demonstrate concordance between the bacterial artificial

chromosome clones and native Wolbachia genomic DNA. Comparison of Wolbachia sequences to public databases using BLAST algorithms under stringent conditions allowed confident prediction of 69 Wolbachia peptide functions and two rRNA genes. Comparison to closely related complete genomes revealed that while most sequences had orthologs in the genome of the Wolbachia endosymbiont from Drosophila melanogaster, there was no evidence for long-range synteny. Rather, there were a few cases of short-range conservation of gene order extending over regions of less than 10 kb. The molecular scaffold produced for the genome of the Wolbachia from B. malayi forms the basis of a genomic sequencing effort for this bacterium, circumventing the difficult challenge of purifying sufficient endosymbiont DNA from a tropical parasite for a whole genome shotgun sequencing strategy.

Gale, K. R., C. M. Dimmock, et al. (1996). "Anaplasma marginale: Detection of carrier cattle by PCR-ELISA." International Journal for Parasitology **26**(10): 1103.

http://www.sciencedirect.com/science/article/B6T7F-478R723-9/2/fd8afa819419187724437340e711e311

Gale K. R., Dimmock C. M., Gartside M. & Leatch G. 1996. Anaplasma marginale: detection of carrier cattle by PCR-ELISA. International Journal for Parasitology 26:1103-1109. A highly sensitive and specific polymerase chain reaction (PCR) based assay for the detection of the minute levels of Anaplasma marginale present in the blood of long-term carrier cattle was developed. A simple lysis method was used to remove most of the haemoglobin from the blood to facilitate direct input of samples into the PCR reactions without prior purification of the DNA. PCR product was detected by enzyme-linked immunosorbent assay (ELISA) to simplify the processing of large numbers of samples. The sensitivity limit of the PCR-ELISA was 0.00015% parasitaemia (24 infected erythrocytes per microlitre of blood). No cross-reactivity of the assay was observed when A. marginale-negative blood infected with Babesia bovis or Theileria orientalis was tested. The PCR-ELISA was shown to be 92% efficient in the detection of long-term A. marginale carrier cattle. No false-positive results were obtained. These results compared favourably with 2 serological assays for detection of A. marginale carrier cattle (card agglutination test and ELISA) which were applied to the same experimental animals.

Harmsen, M. M., J. B. W. J. Cornelissen, et al. (2004). "Identification of a novel Fasciola hepatica cathepsin L protease containing protective epitopes within the propeptide." International Journal for Parasitology **34**(6): 675.

http://www.sciencedirect.com/science/article/B6T7F-4BMJ9GJ-2/2/3793794b640a6be2cb9b5a2e64e427a9

Cathepsin L (CL)-like proteases are important candidate vaccine antigens for protection against helminth infections. We previously identified an immunogenic 32 kDa protein specifically present in newly excysted juveniles (NEJs) of Fasciola hepatica. Here we show by N-terminal protein sequencing that this protein represents a CL-like protease still containing the propeptide. Two cDNAs encoding this CL were subsequently isolated from NEJs by RT-PCR. The predicted amino acid sequences of these cDNAs showed approximately 70% sequence homology to both CL1 and CL2 sequences isolated from adult stage F. hepatica and are, therefore, referred to as CL3. The CL3 clones encoded asparagine at position P1 of the propeptide cleavage site, suggesting a dependence on asparaginyl endopeptidases for maturation. Recombinant expression of a CL3 cDNA in Saccharomyces cerevisiae resulted in secretion of the proenzyme form. The propeptide of CL-like proteins was predicted to contain important B-cell epitopes. To determine the contribution of the propeptide to protective immunity, rats were vaccinated with Keyhole Limpet Haemocyanin-conjugated synthetic peptides encoding these putative B-cell epitopes derived from

the CL1 or CL3 sequence. A subsequent challenge infection resulted in a significant (PF. hepatica infection.

Hodgkinson, J. E., S. Love, et al. (2001). "Evaluation of the specificity of five oligoprobes for identification of cyathostomin species from horses." International Journal for Parasitology **31**(2): 197.

http://www.sciencedirect.com/science/article/B6T7F-42G0KDC-9/2/47e74a0aa21a7297eef73f73f57d4ae7

Here, we report evaluation of five oligoprobes designed from intergenic spacer (IGS) region sequences for identification of cyathostomin species. Oligoprobes were designed for identification of Cylicocyclus ashworthi, Cylicocyclus nassatus, Cylicostephanus longibursatus, Cylicostephanus goldi and a fifth probe designed to identify all members of this tribe. PCR amplification of IGS DNA from 16 cyathostomin species allowed sequence comparison and identification of four putative species-specific probes. Southern blotting of amplified products from 16 species showed that all probes were species-specific. The fifth probe recognised all 16 cyathostomin species but did not bind to members of the genus Strongylus. Furthermore, these probes were used to identify individual infective L3, eggs and L4 indicating that they will be invaluable to furthering the study of the epidemiology and pathogenesis of these important equine nematodes.

Hoglund, J., E. Wilhelmsson, et al. (1999). "ITS2 sequences of Dictyocaulus species from cattle, roe deer and moose in Sweden: molecular evidence for a new species." International Journal for Parasitology **29**(4): 607.

http://www.sciencedirect.com/science/article/B6T7F-3WH63GP-B/2/642542f0cede7d05f15647a27fc9587d

Total DNA was isolated from adult lungworms of the genus Dictyocaulus, collected from cattle, moose (Alces alces) and roe deer (Capreolus capreolus) in Sweden. The second ribosomal internal transcribed spacer was amplified with PCR, and DNA sequences were determined from nine individual worms that all came from different hosts in order to avoid analysis of siblings. The sequence data obtained were aligned and compared with similar data derived from German lungworm isolates from cattle and fallow deer (Cervus dama). These analyses clearly showed that specimens of the cattle lungworm, Dictyocaulus viviparus, were almost identical irrespective of their geographical origin. However, when the second internal transcribed spacer sequence of D. viviparus was compared with that of lungworms from moose and roe deer, major differences were noticed. Although lungworms collected from these cervids had identical second internal transcribed spacer sequences, they proved to be genetically different from Dictyocaulus eckerti of German fallow deer, displaying a 66.5% similarity. In an evolutionary tree, inferred by maximum likelihood analysis, the Dictyocaulus species from cattle and wild cervids clustered as compared with Dictyocaulus filaria from sheep. The study has thus demonstrated that A. alces and C. capreolus in Sweden are parasitised with a Dictyocaulus species that is different from D. viviparus and D. eckerti, indicating that we are dealing with a new species in moose and roe deer.

Kinnaird, J. H., J. M. Bumstead, et al. (2004). "EtCRK2, a cyclin-dependent kinase gene expressed during the sexual and asexual phases of the Eimeria tenella life cycle." International Journal for Parasitology **34**(6): 683.

http://www.sciencedirect.com/science/article/B6T7F-4BRKXM3-1/2/4c4ca753312071a31cf0583f992f7ee1

EtCRK2, a cyclin-dependent kinase from the coccidian parasite, Eimeria tenella is closely related to eukaryotic cyclin-dependent kinases that regulate progression of the cell cycle and to several cyclin-dependent kinases identified in the Apicomplexa. Northern blot analyses revealed that EtCRK2 is transcribed during both asexual (first-generation schizogony) and sexual (oocyst sporulation) replicative phases of the parasite life cycle. In addition, it appears to be transcriptionally regulated during meiosis. Recombinant EtCRK2 produced in Escherichia coli has kinase activity which is significantly stimulated by the addition of vertebrate cyclin A. This cyclin-dependent kinase may play a significant role in regulating critical cell cycle events during both asexual proliferation and sexual development of the parasite.

Kirisits, M. J., E. Mui, et al. (2000). "Measurement of the efficacy of vaccines and antimicrobial therapy against infection with Toxoplasma gondii." International Journal for Parasitology **30**(2): 149.

http://www.sciencedirect.com/science/article/B6T7F-3YRVKCC-7/2/65f1d53d0276d04351610b1c7ca1b9b8

To facilitate studies of vaccines and antimicrobial agents effective against Toxoplasma gondii infection, an assay system was developed to semi-quantitate parasitaemia using PCR amplification of T. gondii DNA obtained from the blood of mice infected with the parasite. A competitive internal standard DNA fragment of the B1 gene of T. gondii was generated and used in PCR so that the amplified product could be semi-quantitated and false negative results could be avoided. The PCR assay system was used to analyse the levels of parasitaemia in immunised and antimicrobial agent treated mice at various times after infection with T. gondii. The results of these studies indicate that this highly sensitive detection method is a rapid and reliable procedure that can be employed to assess the abilities of vaccines or antimicrobial agents to provide protection early following T. gondii infection.

Lalle, M., E. Pozio, et al. (2005). "Genetic heterogeneity at the [beta]-giardin locus among human and animal isolates of Giardia duodenalis and identification of potentially zoonotic subgenotypes." International Journal for Parasitology **35**(2): 207.

http://www.sciencedirect.com/science/article/B6T7F-4DXTJJT-1/2/ac446912b091bf6f7b59af1d2d730ff5

Human giardiasis, caused by the intestinal flagellate Giardia duodenalis, is considered a zoonotic infection, although the role of animals in the transmission to humans is still unclear. Molecular characterisation of cysts of human and animal origin represents an objective means to validate or reject this hypothesis. In the present work, cysts were collected in Italy from humans (n=37) and animals (dogs, one cat and calves, n=46), and were characterised by PCR amplification and sequencing of the [beta]-giardin gene. As expected, only Assemblages A and B were identified among human isolates. The host-specific Assemblages C and D were found in the majority of dog isolates; however, 6 dog isolates were typed as Assemblage A. The cat-specific Assemblage F has been identified in the single feline isolate available. Among calf isolates, most were typed as Assemblages A (n=12) and B (n=5), whereas the host-specific Assemblage E was rarely found (n=3). Sequence heterogeneity in the [beta]-giardin gene allowed a number of subgenotypes to be identified within Assemblage A (8 subgenotypes), B (6 subgenotypes), D (2 subgenotypes), and E (3 subgenotypes). Five of these subgenotypes, namely A1, A2, A3, A4 and B3, were found to be associated with infections of humans, of dogs and of calves; these data, therefore,

supported the role of these animals as a source of infection for humans.

Leon, M. P. D., T. Yanagi, et al. (1998). "Characterisation of Trypanosoma cruzi populations by dna polymorphism of the cruzipain gene detected by single-stranded dna conformation polymorphism (SSCP) and direct sequencing." International Journal for Parasitology **28**(12): 1867.

http://www.sciencedirect.com/science/article/B6T7F-3W1HH98-6/2/6253dde1f82aec047d1e48cc30800bb1

Fifty fresh isolates of Trypanosoma cruzi from Triatoma dimidiata vectors and 31 from patients with Chagas disease were analysed for DNA polymorphisms within the 432-bp core region of the cruzipain gene which encodes the active site of cathepsin L-like cystein proteinase. The cruzipain gene showed signs of polymorphism consisting of four different DNA sequences in Central and South American isolates of T. cruzi. The PCR fragments of Guatemalan isolates could be divided into three groups, Groups 1, 2 and 3, based on different patterns of single-stranded DNA conformation polymorphism. All of the strains isolated from Brazil, Chile, and Paraguay, except for the CL strain, showed a Group 4 pattern. Two to four isolates from each group were analysed by cloning and sequencing. A silent mutation occurred between Groups 1 and 2, and five nucleotides and two aa substitutions were detected between Groups 1 and 3. The DNA sequence of Group 4 contained five nucleotides and one aa substitution from Group 1. All of the DNA sequences corresponded well with the single-stranded DNA conformation polymorphism. The Group 1 isolates, the majority in the Guatemalan population (70/81, 86.4%), were isolated from both triatomines and humans, but Group 3 were isolated only from humans. Moreover, the Group 2 isolates were detected only in triatomine vectors (9/50; 18%), but never in humans (0/32, $P<0.05$) suggesting that this group has an independent life-cycle in sylvatic animals and is maintained by reservoir hosts other than humans.

Littlewood, D. T. J., K. Rohde, et al. (1997). "Parasite speciation within or between host species?--Phylogenetic evidence from site-specific polystome monogeneans." International Journal for Parasitology **27**(11): 1289.

http://www.sciencedirect.com/science/article/B6T7F-3RSP2P5-3/2/55758718387abbf2ff5f110138205ef2

Partial nuclear 28S ribosomal RNA and mitochondrial cytochrome c oxidase subunit I (COI) gene sequences (953 and 385 nucleotides, respectively) of one fish monogenean (outgroup) and six polystome monogeneans (four Polystomoides spp. from the oral cavities and urinary bladders of freshwater turtles in Australia and Malaya, two Neopolystoma spp. from the urinary bladder and conjunctival sac of a freshwater turtle in Australia) were used to examine the question of whether congeneric species infecting different sites in the same host species have speciated in that host by adapting to different sites, or whether species infecting a particular site in one host have given rise to species infecting the same site in different hosts. Results show unequivocally that congeneric species infecting the same site, even of host species belonging to different suborders and occurring on different continents, are more closely related than congeneric species infecting different sites of the same host species. This is interpreted as meaning that speciation has not occurred in one host. Morphological evolution of polystomes has been very slow: few differences between species and even genera have evolved over a period of at least 150 Myr, and this is matched by low substitution rates of nucleotides, and the ambiguous position of species of different genera, depending on whether COI or 28S rDNA sequences are used.

Littlewood, D. T. J., K. Rohde, et al. (1998). "The phylogenetic position of Udonella (Platyhelminthes)1." International Journal for Parasitology **28**(8): 1241.

http://www.sciencedirect.com/science/article/B6T7F-3Y6GX73-F/2/1d4e32882dc2ddf0d6d253622bede025

Phylogenetic analysis of molecular data from complete 18S rRNA and partial 28S rRNA genes, of a variety of platyhelminths, places the enigmatic Udonella caligorum firmly as a monopisthocotylean monogenean. Both maximum parsimony and a modified distance measure, operating under a maximum likelihood model, gave identical solutions for each data set. These data further support morphological evidence from ultrastructural studies indicating the neodermatan affinities of Udonella, namely shared features in sensory receptors, surface tegument, sperm structure and spermiogenesis. The molecular data reject the class Udonellidea and the placement of udonellids as sister-group to the Neodermata. As shown previously with molecular data, the monogeneans appear as a paraphyletic assemblage comprising strongly monophyletic Monopisthocotylea and Polyopisthocotylea. Their relationships with the trematodes and cestodes are not resolved with 28S rDNA or 18S rDNA alone.

Lu, S. Q., A. C. Baruch, et al. (1998). "Molecular comparison of Giardia lamblia isolates." International Journal for Parasitology **28**(9): 1341.

http://www.sciencedirect.com/science/article/B6T7F-3W1RGB1-1D/2/6aa09e0dd72c08f89012724348195d38

Giardia lamblia (also Giardia duodenalis, Giardia intestinalis) isolates have been variably divided into two or three genotypes by different investigators. We have compared the triose phosphate isomerase sequences of the three genotypes (Groups 1, 2, and 3) described by Nash and shown that Groups 1 and 2 are similar, while Group 3 is markedly different from Groups 1 and 2, indicating that Group1/2 and Group 3 correspond to the two major genotypes identified by other investigators. We have also analysed three Chinese isolates and showed that two fit into Group 3, while the third contained a mixture of Groups 1 and 3 isolates. These results confirm the relatedness of G. lamblia isolates from throughout the world, and established the feasibility of using DNA amplification and sequence analysis for detecting mixed isolates.

Masiga, D. K., A. J. Smyth, et al. (1992). "Sensitive detection of trypanosomes in tsetse flies by DNA amplification." International Journal for Parasitology **22**(7): 909.

http://www.sciencedirect.com/science/article/B6T7F-476FF6X-154/2/9ff9371dda2e625023b1703fa1358269

African trypanosome species were identified using the Polymerase Chain Reaction (PCR) by targeting repetitive DNA for amplification. Using oligonucleotide primers designed to anneal specifically to the satellite DNA monomer of each species/subgroup, we were able to accurately identify Trypanosoma simiae, three subgroups of T.congolense, T.brucei and T.vivax. The assay was sensitive and specific, detecting one trypanosome unequivocally and showing no reaction with non-target trypanosome DNA or a huge excess of host DNA. The assay was used to identify developmental stage trypanosomes in the tsetse fly. The use of radioisotopes was not necessary and mixed infections could be detected easily by incorporating more than one set of primers in a single reaction. The use of crude preparations of template made the process very rapid. The methodology should be suitable for large-scale epidemiological studies.

Matsuba, T., C. Sugimoto, et al. (1995). "Expression of a 32 kilodalton Theileria sergenti piroplasm surface protein by recombinant baculoviruses." International Journal for Parasitology **25**(8): 939.

http://www.sciencedirect.com/science/article/B6T7F-3YF49VW-27/2/f83e029b52ec29f78a0d375a4b6ef3bd

Previous studies detected a single amino acid substitution (Ala196 to Gly196) between cDNA clones encoding a 32 kDa antigen (p32) of Theileria sergenti (Chitose stock) obtained from a persistently infected calf. In this study, 2 different recombinant baculoviruses (pAc/p32-Ala196 and pAc/p32-Gly196) were constructed for the expression of p32. Molecular masses of the polypeptides produced in Spodoptera frugiperda cells infected with the recombinant baculoviruses were the same as that of antheutic p32. pAc/p32-Ala196 produced additional polypeptides, with molecular masses higher than 32 kDa, which resulted from differential N-glycosylation as revealed by endo N-glycosidase treatment. The results indicate that a single amino acid substitution may lead to a conformational change in p32 which affected post-translational modification of recombinant products.

Matthews, J. B., A. J. Davidson, et al. (2001). "Immunisation of cattle with recombinant acetylcholinesterase from Dictyocaulus viviparus and with adult worm ES products." International Journal for Parasitology **31**(3): 307.

http://www.sciencedirect.com/science/article/B6T7F-42D811P-B/2/d73f6feb70615674a822328aba6c26ec

Dictyocaulus viviparus causes a serious lung disease of cattle. For over 30 years, a radiation-attenuated larval vaccine has been used with success; however, this vaccine has several disadvantages. A more stable vaccine against D. viviparus, capable of stimulating prolonged protective immunity, would be beneficial. Recent research has been directed at adult worm ES components that may be involved in parasite survival in the host. One component is the secreted enzyme, acetylcholinesterase (AChE), a target for circulating antibody in infected calves. Here, we describe a study where protection was investigated in calves immunised with either native adult ES products or a recombinant parasite AChE. These antigens were administered twice with Freund's incomplete adjuvant. Subsequently, all calves were challenged with 700 L3 and their worm burdens and immune responses compared with those in calves that received an anthelmintic-abbreviated infection and challenge control calves. Significant levels of protection were not obtained in the immunised groups but significant immunity was achieved in the calves that received the anthelmintic abbreviated infection. Antibody responses amongst the groups were different, with significantly higher IgG1 responses in the immune, infected group and in adult ES recipients. Significantly higher IgG2 responses were found in the latter group. Following challenge, the groups that received the abbreviated infection and the fusion protein produced specific antibody that bound the native enzyme. No differences were observed between groups in peripheral blood mononuclear cell responsiveness to either antigen. However, adult ES products appeared to have a mitogenic effect on these cells, whilst the fusion protein exhibited an inhibitory effect. These results suggest that in this form, AChE is not a potential vaccine candidate and that adult ES products, in contrast to previous experiments in guinea pigs, do not contain protective components.

McDiarmid, L., T. Petney, et al. (2000). "Range expansion of the tick Amblyomma triguttatum triguttatum, an Australian vector of Q fever." International Journal for Parasitology **30**(7): 791.

http://www.sciencedirect.com/science/article/B6T7F-40PRGMX-1/2/71358285493327d604f340d5399ecdd0

The tick Amblyomma triguttatum triguttatum has previously been reported from Western Australia, Queensland and New South Wales. A viable population of this species, including all developmental stages, has now been discovered on the southern end of Yorke Peninsula, South Australia. Species determination was carried out morphologically and using 18S and 16S rRNA. The data for 16S rRNA are the first published for this species. Amblyomma t. triguttatum is significant through its involvement in the natural, Australian cycle of Coxiella burnetti, the pathogen causing Q fever. The environment of Yorke Peninsula contains all of the components required for a natural Q fever cycle and three cases of this disease have been reported from this area since 1995. These findings reinforce the need to put in place effective mechanisms to monitor parasite distributions at a time of large scale global change.

Morrison, D. A., S. Bornstein, et al. (2004). "The current status of the small subunit rRNA phylogeny of the coccidia (Sporozoa)." International Journal for Parasitology **34**(4): 501.

http://www.sciencedirect.com/science/article/B6T7F-4B71912-B/2/1711750abc739b647d44cc23b1feb1b8

There is no current comprehensive assessment of the molecular phylogeny of the coccidia, as all recently published papers either deal with subsets of the taxa or sequence data, or provide non-robust analyses. Here, we present a comprehensive and consistent phylogenetic analysis of the available data for the small-subunit ribosomal RNA gene sequence, including a number of taxa not previously studied, based on a Bayesian tree-building analysis and the covariotide model of evolution. The assumptions of the analysis have been rigorously tested, and the benefits and limitations highlighted. Our results provide support for a number of prior conclusions, including the monophyly of the families Sarcocystidae (cyst-forming coccidia) and Eimeriidae (oocyst-forming coccidia), but with bird-host Isospora species in the Eimeriidae and mammal-host species in the Sarcocystidae. However, it is clear that a number of previously reported relationships are dependent on the evolutionary model chosen, such as the placements of Goussia janae, Lankesterella minimia and Caryospora bigenetica. Our results also confirm the monophyly of the subfamilies Toxoplasmatinae and Sarcocystinae, but only some of the previously reported groups within these subfamilies are supported by our analysis. Similarly, only some of the previously reported groups within the Eimeriidae are supported by our analysis, and the genus Eimeria is clearly paraphyletic. There are unambiguous patterns of host-parasite relationship within the coccidia, as most of the well-supported groups have a consistent and restricted range of hosts, with the exception of the Toxoplasmatinae. Furthermore, the previously reported groups for which we found no support all have a diverse range of unrelated hosts, confirming that these are unlikely to be natural groups. The most interesting unaddressed questions may relate to Isospora, which has the fewest available sequences and host-parasite relationships apparently not as straightforward as elsewhere within the suborder.

Nair, S., A. Brockman, et al. (2002). "Rapid genotyping of loci involved in antifolate drug resistance in Plasmodium falciparum by primer extension." International Journal for Parasitology **32**(7): 852.

http://www.sciencedirect.com/science/article/B6T7F-45HFG08-1/2/a2565d108ef6242b94245d9ce5939489

Current methods used to genotype point mutations in Plasmodium falciparum genes involved in resistance to antifolate drugs include restriction digestion of PCR products, allele-specific

amplification or sequencing. Here we demonstrate that known point mutations in dihydrofolate reductase and dihydropteroate synthase can be scored quickly and accurately by single-nucleotide primer extension and detection of florescent products on a capillary sequencer. We use this method to genotype parasites in natural infections from the Thai-Myanmar border. This approach could greatly simplify large-scale screening of resistance mutations of the type required for evaluating and updating antimalarial drug treatment policies. The method can be easily adapted to other P. falciparum genes and will greatly simplify scoring of point mutations in this and other parasitic organisms.

Olivier, C., S. van de Pas, et al. (2001). "Sequence variability in the first internal transcribed spacer region within and among Cyclospora species is consistent with polyparasitism." International Journal for Parasitology **31**(13): 1475.

http://www.sciencedirect.com/science/article/B6T7F-4447KS1-9/2/1a6c4f8e7ec7a3eb7a427c8aef5f1393

Cyclospora cayetanensis is a coccidian parasite which causes severe gastroenteritis in humans. Molecular information on this newly emerging pathogen is scarce. Our objectives were to assess genetic variation within and between human-associated C. cayetanensis and baboon-associated Cyclospora papionis by examining the internal transcribed spacer (ITS) region of the ribosomal RNA operon, and to develop an efficient polymerase chain reaction- (PCR)-based method to distinguish C. cayetanensis from other closely related organisms. For these purposes, we studied C. cayetanensis ITS-1 nucleotide variability in 24 human faecal samples from five geographic locations and C. papionis ITS-1 variability in four baboon faecal samples from Tanzania. In addition, a continuous sequence encompassing ITS-1, 5.8S rDNA and ITS-2 was determined from two C. cayetanensis samples. The results indicate that C. cayetanensis and C. papionis have distinct ITS-1 sequences, but identical 5.8S rDNA sequences. ITS-1 is highly variable within and between samples, but variability does not correlate with geographic origin of the samples. Despite this variability, conserved species-specific ITS-1 sequences were identified and a single-round, C. cayetanensis-specific PCR-based assay with a sensitivity of one to ten oocysts was developed. This consistent and remarkable diversity among Cyclospora spp. ITS-1 sequences argues for polyparasitism and simultaneous transmission of multiple strains.

Pape, M., G. von Samson-Himmelstjerna, et al. (1999). "Characterisation of the beta-tubulin gene of Cylicocyclus nassatus." International Journal for Parasitology **29**(12): 1941.

http://www.sciencedirect.com/science/article/B6T7F-3Y9MD96-8/2/9d2dbb1f81a712e843e67db23c8dbbf5

mRNA and genomic DNA were isolated from adult Cylicocyclus nassatus, and the mRNA was reverse transcribed. The cDNA was PCR amplified using degenerate primers designed according to the alignment of the [beta]-tubulin amino acid sequences of other species. To complete the coding sequence, the 3' end was amplified with the 3'-RACE, and for amplification of the 5' end the SL1-primer was used. The cDNA of the [beta]-tubulin gene of C. nassatus spans 1429 bp and encodes a protein of 448 amino acids. Specific primers were developed from the cDNA sequence to amplify the genomic DNA sequence and to analyse the genomic organisation of the [beta]-tubulin gene. The complete sequence of the genomic DNA of the [beta]-tubulin gene of C. nassatus has a size of 2652 bp and is organised into nine exons and eight introns. The identities with the exons of the gru-1 [beta]-tubulin gene of Haemonchus contortus range between 79% and 97%.

Petersen, E., A. Pollak, et al. (2001). "Recent trends in research on congenital toxoplasmosis." International Journal for Parasitology **31**(2): 115.

http://www.sciencedirect.com/science/article/B6T7F-42G0KDC-2/2/31f30b33e9cf8e290ebafb8419b7e64c

Putland, R. A., S. M. Thomas, et al. (1993). "Analysis of the 18S ribosomal RNA gene of Strongyloides stercoralis." International Journal for Parasitology **23**(1): 149.

http://www.sciencedirect.com/science/article/B6T7F-476HJBR-1SW/2/25a4daa26419b9ff7080bb6e465587ef

The entire 1766 bases of the 18S rRNA gene of Strongyloides stercoralis have been sequenced. The gene has a 38% G + C content. Although it is similar in length to the 18S rRNA gene of Caenorhabditis elegans, the only other completely sequenced nematode 18S rRNA gene, it is only 69% identical. Closely related helminths will need to be sequenced in order to delineate sequences specific for the diagnosis of strongyloidiasis.

Robinson, M. W., E. M. Hoey, et al. (2001). "Characterisation of a [beta]-tubulin gene from the liver fluke, Fasciola hepatica." International Journal for Parasitology **31**(11): 1264.

http://www.sciencedirect.com/science/article/B6T7F-43YSX81-F/2/8be602ce0205e12ea60d83d67bd61a6f

This study represents the first [beta]-tubulin sequence from a trematode parasite, namely, the liver fluke, Fasciola hepatica. PCR of genomic DNA showed that at least one [beta]-tubulin gene from F. hepatica contains no introns. A number of amino acids in the primary sequence of fluke tubulin are different from those described previously in various nematode species and the cestode, Echinococcus multilocularis. [beta]-Tubulin is an important target for benzimidazole anthelmintics, although (with the exception of triclabendazole) they show limited activity against F. hepatica. The amino acid differences in fluke [beta]-tubulin are discussed in relation to the selective toxicity of benzimidazoles against helminths and the mechanism of drug resistance.

Rose, K., J. Curtis, et al. (2004). "Cutaneous leishmaniasis in red kangaroos: isolation and characterisation of the causative organisms." International Journal for Parasitology **34**(6): 655.

http://www.sciencedirect.com/science/article/B6T7F-4C0HSD4-1/2/bacc711af1e5a70bc40cb7fcb502e92b

This is the first report of cutaneous leishmaniasis in kangaroos where infection was acquired within Australia. The diagnosis is based on the clinical criteria used for humans, the lesion histopathology, the detection and isolation of parasites from the lesions, and the analysis of the small subunit ribosomal RNA genes using the polymerase chain reaction. Despite a clear indication that the parasites belong to the genus Leishmania, no assignation to a known Leishmania species could be made using these or other less conserved genetic loci such as the non-transcribed spacer of the mini-exon repeat. As is the case in humans, some but not all animals harbouring lesions had antibodies to the isolated parasites or to several other

Leishmania species. The isolated parasites displayed two well characterised Leishmania glycoconjugates, the lipophosphoglycan and proteophosphoglycan. They were infectious for mouse macrophages in vitro and established long-term infection at 33 [deg]C but not at 37 [deg]C. Our findings raise the possibility of transmission to humans, which may be unrecognised and suggest the possibility that imported species of Leishmania could become endemic in Australia.

Sturm, N. R., N. S. Vargas, et al. (2003). "Evidence for multiple hybrid groups in Trypanosoma cruzi." International Journal for Parasitology **33**(3): 269.

http://www.sciencedirect.com/science/article/B6T7F-47P8MGH-2/2/f54c0ec187e48290fa15e4a8dd5b435c

A role for parasite genetic variability in the spectrum of Chagas disease is emerging but not yet evident, in part due to an incomplete understanding of the population structure of Trypanosoma cruzi. To investigate further the observed genotypic variation at the sequence and chromosomal levels in strains of standard and field-isolated T. cruzi we have undertaken a comparative analysis of 10 regions of the genome from two isolates representing T. cruzi I (Dm28c and Silvio X10) and two from T. cruzi II (CL Brener and Esmeraldo). Amplified regions contained intergenic (non-coding) sequences from tandemly repeated genes. Multiple nucleotide polymorphisms correlated with the T. cruzi I/T. cruzi II classification. Two intergenic regions had useful polymorphisms for the design of classification probes to test on genomic DNA from other known isolates. Two adjacent nucleotide polymorphisms in HSP 60 correlated with the T. cruzi I and T. cruzi II distinction. 1F8 nucleotide polymorphisms revealed multiple subdivisions of T. cruzi II: subgroups IIa and IIc displayed the T. cruzi I pattern; subgroups IId and IIe possessed both the I and II patterns. Furthermore, isolates from subgroups IId and IIe contained the 1F8 polymorphic markers on different chromosome bands supporting a genetic exchange event that resulted in chromosomes V and IX of T. cruzi strain CL Brener. Based on these analyses, T. cruzi I and subgroup IIb appear to be pure lines, while subgroups IIa/IIc and IId/IIe are hybrid lines. These data demonstrate for the first time that IIa/IIc are hybrid, consistent with the hypothesis that genetic recombination has occurred more than once within the T. cruzi lines.

Stwora-Wojczyk, M. M., J. C. Kissinger, et al. (2004). "O-glycosylation in Toxoplasma gondii: Identification and analysis of a family of UDP-GalNAc:polypeptide N-acetylgalactosaminyltransferases." International Journal for Parasitology **34**(3): 309.

http://www.sciencedirect.com/science/article/B6T7F-4BBV1KF-2/2/d6b6d4e222616e9256848709e99f6974

The initiation of mucin-type O-glycosylation is catalysed by a family of UDP-GalNAc:polypeptide N-acetylgalactosaminyltransferases (EC 2.4.1.41). These enzymes are responsible for the transfer of N-acetylgalactosamine from the nucleotide sugar donor, UDP-GalNAc, to the hydroxyl group on specific serine or threonine residues in acceptor proteins. By screening a Toxoplasma gondii cDNA library, three distinct isoforms of the ppGalNAc-T gene family were cloned. Two additional isoforms were identified and partially cloned following analysis of the T. gondii genome sequence database. All of the cloned and identified ppGalNAc-T's are type II membrane proteins that share up to 50% amino acid sequence identity within the conserved catalytic domain. They each contain an N-terminal cytoplasmic domain, a hydrophobic transmembrane domain, and a lumenal domain; the latter consists of stem, catalytic, and lectin-like domains. Moreover, each of this ppGalNAc-T's contains important sequence motifs that are typical for this class of glycosyltransferases. These include a glycosyltransferase 1 motif containing the DXH sequence, a Gal/GalNAc-T motif, and the CLD and QXW sequence motifs located in [alpha]-, [beta]-, and

[gamma]-repeats present within the lectin-like domain. The coding regions of T. gondii ppGalNAc-T1, -T2, and -T3 reside in multiple exons ranging in number from 6 to 10. Our results demonstrate that mucin-type O-glycosylation in T. gondii is catalysed by a multimember gene family, which is evolutionarily conserved from single-celled eukaryotes through nematodes and insects up to mammals. Taken together, this information creates the basis for future studies of the function of the ppGalNAc-T gene family in the pathobiology of this apicomplexan parasite.

Walker, J. and A. Tait (1997). "Ostertagia circumcincta: Isolation of a partial cDNA encoding an unusual member of the mitochondrial processing peptidase subfamily of M16 metallopeptidases." International Journal for Parasitology **27**(11): 1389.

http://www.sciencedirect.com/science/article/B6T7F-3RSP2P5-N/2/c3a2e7e5d6c29a65d3365b4678753889

A reverse-transcriptase polymerase chain reaction (PCR) procedure was used to isolate an Ostertagia circumcincta partial cDNA encoding a protein with general primary sequence features characteristic of members of the mitochondrial processing peptidase (MPP) subfamily of M16 metallopeptidases. The structural relationships of the predicted protein (Oc MPPX) with MPP subfamily proteins from other species (including the model free-living nematode Caenorhabditis elegans) were examined, and Northern analysis confirmed the expression of the Oc mppx gene in adult nematodes.

Ware, J., L. Moran, et al. (2002). "Sequencing and analysis of a 63 kb bacterial artificial chromosome insert from the Wolbachia endosymbiont of the human filarial parasite Brugia malayi." International Journal for Parasitology **32**(2): 159.

http://www.sciencedirect.com/science/article/B6T7F-44V1VBF-3/2/92b95238ab92c6012d8044308d95d5ff

Wolbachia endosymbiotic bacteria are widespread in filarial nematodes and are directly involved in the immune response of the host. In addition, antibiotics which disrupt Wolbachia interfere with filarial nematode development thus, Wolbachia provide an excellent target for control of filariasis. A 63.1 kb bacterial artificial chromosome insert, from the Wolbachia endosymbiont of the human filarial parasite Brugia malayi, has been sequenced using the New England Biolabs Inc. Genome Priming System(TM) transposition kit in conjunction with primer walking methods. The bacterial artificial chromosome insert contains approximately 57 potential ORFs which have been compared by individual protein BLAST analysis with the 35 published complete microbial genomes in the Comprehensive Microbial Resource database at The Institute for Genomic Research and in the NCBI GenBank database, as well as to data from 22 incomplete genomes from the DOE Joint Genome Institute. Twenty five of the putative ORFs have significant similarity to genes from the [alpha]-proteobacteria Rickettsia prowazekii, the most closely related completed genome, as well as to the newly sequenced [alpha]-proteobacteria endosymbiont Sinorhizobium meliloti. The bacterial artificial chromosome insert sequence however has little conserved synteny with the R. prowazekii and S. meliloti genomes. Significant sequence similarity was also found in comparisons with the currently available sequence data from the Wolbachia endosymbiont of Drosophila melanogaster. Analysis of this bacterial artificial chromosome insert provides useful gene density and comparative genomic data that will contribute to whole genome sequencing of Wolbachia from the B. malayi host. This will also lead to a better understanding of the interactions between the endosymbiont and its host and will offer novel approaches and drug targets for elimination of filarial disease.

Yamasaki, H., R. Mineki, et al. (2002). "Characterisation and expression of the Fasciola gigantica cathepsin L gene." International Journal for Parasitology **32**(8): 1031.

http://www.sciencedirect.com/science/article/B6T7F-45RFM7B-1/2/d5e476fbbd10bb4068cf30cfa02b374a

The gene structure of a cathepsin L from Fasciola gigantica was characterised. The gene spans approximately 2.0 kb and comprises four exons and three introns and is a compact gene as in the cases of crustaceous and platyhelminth cathepsins L. Southern blot analysis suggested that a few copies of the genes are sparsely organised in the genome. Of the three intron insertion positions, two of which are in the same position as in the mammalian cathepsin L gene. Phylogenetic analysis revealed that F. gigantica cathepsin L forms a clade with those from Fasciola hepatica, but not with those from Spirometra erinacei and schistosomes. Putative TATA-boxes were found upstream of a transcription initiation site. The sequence analysis of the 5'-upstream of the transcript revealed that the cathepsin L gene is transcribed by cis-splicing fashion. Furthermore, the experiments using recombinant F. gigantica procathepsin L showed that it was processed to an enzymatically active cathepsin L by pH-dependent autocatalysis. However, the pro-peptide deleted cathepsin L showed no enzyme activity, indicating that the pro-region of F. gigantica procathepsin L is essential for the folding and/or refolding of functional cathepsin L. These results are consistent with the observations in mammalian cathepsin L and papain.

International Journal of Antimicrobial Agents (10)

Bamaga, M., D. J. M. Wright, et al. (2002). "Selection of in vitro mutants of pyrazinamide-resistant Mycobacterium tuberculosis." International Journal of Antimicrobial Agents **20**(4): 275.

http://www.sciencedirect.com/science/article/B6T7H-46YV8PF-S/2/55e61eda2a011758c81dd840deef8f68

Mutations within the pncA gene coding for pyrazinamidase of Mycobacterium tuberculosis can cause pyrazinamide (PZA) resistance. The effect of drug concentrations on PZA resistance in a clinical isolate of M. tuberculosis was studied in vitro. Serial passage at gradually increased concentrations of PZA from 200 to 500 [mu]g/ml was performed using BACTEC radiometric method. Thirteen in vitro-selected variant strains were assembled and sequence analysis showed that 12 of the 13 variants had a novel single point mutation within the pncA gene by deletion at nucleotide 381 (G), codon 127. This lead to a frameshift that affected the function of the pyrazinamidase resulting in PZA resistance regardless of different PZA concentrations used. One variant had a silent mutation at nucleotide 6 (G->A) and remains PZA sensitive. We conclude that the mutation location found is an important position for full resistance, at least in this strain. The lack of further mutations even after exposure to higher PZA concentrations implies a critical value for development of resistance--a level exceeded in tissues in clinical treatment regimes.

Boulos, A., J.-M. Rolain, et al. (2004). "Measurement of the antibiotic susceptibility of Coxiella burnetii using real time PCR." International Journal of Antimicrobial Agents **23**(2): 169.

http://www.sciencedirect.com/science/article/B6T7H-4BG8VG4-8/2/26d734046a4577db21d827846accf128

The objective of this study was to determine MICs of antibiotics for two reference strains of Coxiella burnetii using real time quantitative PCR. The method was very sensitive and specific and allowed the evaluation of the doubling time of Nine Mile and Q212 strains: 37 and 15 h, respectively. Dose response curves of antibiotics were used to determine MICs. Those of doxycycline, fluoroquinolone compounds and rifampicin were in the range 1-4 mg/l. Telithromycin was the most effective macrolide compound with MICs of 1-2 mg/l. The results confirmed previous reports on the accuracy of this new method for the determination of the antibiotic susceptibility of C. burnetii and could be used for the screening of new drugs.

Essack, S. Y., L. M. C. Hall, et al. (2004). "Klebsiella pneumoniae isolate from South Africa with multiple TEM, SHV and AmpC [beta]-lactamases." International Journal of Antimicrobial Agents **23**(4): 398.

http://www.sciencedirect.com/science/article/B6T7H-4BY3YK8-2/2/9a3f6dc6a13a39459fadfc2acc0ff39e

Klebsiella pneumoniae 2207, from Durban, was resistant to cefoxitin and [beta]-lactamase inhibitor combinations as well as oxyimino-aminothiazolyl cephalosporins. [beta]-Lactamases with isoelectric points of 5.4, 5.6, 7.6, 8.2 and 8.4 were found. DNA hybridisation identified two BamHI and three HindIII fragments carrying blaTEM, and two SalI fragments carrying blaSHV. At least two genes encoded TEM-1 enzyme; one blaSHV copy encoded SHV-5 but the other determined SHV-23, a novel SHV-5 variant with conservative amino-acid substitutions far from the catalytic site. The pI 8.4 activity was an AmpC-type enzyme. Determinants of the pI 5.6 and 7.6 activities were not identified.

Kanaan, A., I. Cour, et al. (2004). "Significance of nested PCR and quantitative real time PCR for cytomegalovirus detection in renal transplant recipients." International Journal of Antimicrobial Agents **24**(5): 455.

http://www.sciencedirect.com/science/article/B6T7H-4DFT4GY-3/2/0eb2a51fe8e31ee1ebf3d1bfa74c4409

Immunocompromised renal transplant recipients are susceptible to severe cytomegalovirus (CMV) infection that makes its detection important in clinical practice. A total of 536 blood and 536 serum samples from 67 renal transplant recipients who had previously been diagnosed with terminal renal insufficiency were studied for CMV infection. In all samples, serology, shell vial culture, antigenaemia and nested polymerase chain reaction (PCR) in blood and serum were tested, and a real-time quantitative PCR was run on 90 specimens. Sixty-seven blood donors were used as controls. The results show that the quantitative real-time PCR assay could be of great interest for predicting CMV disease, and to monitor the onset of pre-emptive therapy.

Latini, L., M. P. Ronchetti, et al. (1999). "Prevalence of mefE, erm and tet(M) genes in Streptococcus pneumoniae strains from central Italy." International Journal of Antimicrobial Agents **13**(1): 29.

http://www.sciencedirect.com/science/article/B6T7H-3XJK9KC-5/2/00edacef5c06fab050f68f6889682d1a

One hundred and seventy-three Streptococcus pneumoniae strains isolated from surveillance studies conducted in daycare centres were studied. The mefE, erm and tet(M) genes were detected in 16.2, 45.1 and 47.4% of isolates respectively. Agreement between PCR results and antibiotic susceptibility patterns was 100%. Macrolide resistance was due to the presence of erm in 73.6% of strains and to the presence of mefE in the remaining 26.4%. All tetracycline resistant strains carried the tet(M) gene. erm was associated with tet(M) in 98.7% of strains, whereas no isolate carrying mefE carried tet(M). A significant association was found between mefE and serogroup 6 (Perm and tet(M) and serogroup 19 (P<0.00001).

Luzzaro, F., M. Perilli, et al. (2001). "Properties of multidrug-resistant, ESBL-producing Proteus mirabilis isolates and possible role of [beta]-lactam/[beta]-lactamase inhibitor combinations." International Journal of Antimicrobial Agents **17**(2): 131.

http://www.sciencedirect.com/science/article/B6T7H-427JWG1-8/2/8d6f89991b153f14c792316d5f6ca780

At our institution, isolation rates of clinical strains of ESBL-producing Proteus mirabilis increased to 8.8% of all P. mirabilis isolates during the period 1997-1999. To evaluate the susceptibility of ESBL-producing P. mirabilis strains against commonly used drugs, we studied 50 non-duplicated isolates selected on the basis of synergy between clavulanate and [beta]-lactams (ceftazidime, aztreonam, cefotaxime, and ceftriaxone). The presence of ESBL-coding genes was confirmed by colony hybridization with blaTEM-1 and blaSHV-1 probes. Minimum inhibitory concentrations of several antimicrobial agents for each isolate were obtained using the Etest method. All strains were encoding for TEM-derived enzymes. Gene sequencing showed that at least three different genes (TEM-15, TEM-20, and TEM-52) were present. These enzymes have not been previously reported in P. mirabilis. Isolates were characterized by: (a) reduced susceptibility or resistance to third- and fourth-generation cephalosporins (MIC>=2 mg/l), (b) resistance to piperacillin that was abolished by tazobactam (MIC>=256 vs. P. mirabilis by third-generation cephalosporins has been repeatedly observed both at our Institution and elsewhere. Piperacillin-tazobactam, as well as amikacin and meropenem appear to be important therapeutic options for infections due to multidrug-resistant, ESBL-producing P. mirabilis isolates.

Reato, G., A. M. Cuffini, et al. (2004). "Immunomodulating effect of antimicrobial agents on cytokine production by human polymorphonuclear neutrophils." International Journal of Antimicrobial Agents **23**(2): 150.

http://www.sciencedirect.com/science/article/B6T7H-4BG8VG4-1/2/9c2389a9c2f7cc1fd0f938230ef3adf0

It has been previously demonstrated that some antimicrobial agents enhance activities of human polymorphonuclear neutrophils (PMNs). The effect on the release of cytokines in an inflammatory context from PMNs by these antibiotics was evaluated. We studied the effect of the release of some cytokines by human PMNs RT-PCR analysis on a clinical strain of Klebsiella pneumoniae by comparing the effect with that observed in the presence of co-amoxiclav, sanfetrinem, clarithromycin, prulifloxacin and tobramycin. All the drugs tested were capable of modulating PMN synthesis in vitro of pro-inflammatory cytokines IL-8, IL-1[beta], TNF-[alpha] and IL-6, but not that of anti-inflammatory cytokine IL-10. The degree of their stimulatory or inhibitory potency varied with the cytokine examined.

Syrogiannopoulos, G. A., F. Ronchetti, et al. (2000). "Mediterranean clone of penicillin-susceptible, multidrug-resistant serotype 6B Streptococcus pneumoniae in Greece, Italy and Israel." International Journal of Antimicrobial Agents **16**(3): 219.

http://www.sciencedirect.com/science/article/B6T7H-41PP1PH-5/2/88795c988a63bafe8a58a7b54ecef8d7

In 1996, 19 isolates of serotype 6B Streptococcus pneumoniae with a unique resistance pattern were found in carriers attending daycare centres in Patras, Southwestern Greece. These isolates were penicillin susceptible but resistant to chloramphenicol, tetracycline, erythromycin, clindamycin and trimethoprim-sulphamethoxazole. Subsequently, isolates with the same characteristics were found in 23 additional carriers in central and southern Greece in 1997-98 as well as in 19 carriers in central Italy in 1997, and in seven carriers in southern Israel in 1998. Carriers were all children under 6 years of age, attending daycare centres or outpatient hospital visits. The relatedness of the isolates was determined on representative isolates from the three countries by pulsed-field gel electrophoresis of SmaI digests of chromosomal DNA. Most Greek isolates were identical to each other, while isolates from Italy and Israel showed one to three band differences, with all isolates being closely related to each other as well as to the isolates from Greece. We have therefore documented the presence of this unique clone of S. pneumoniae in these three countries and have named this the 'Mediterranean' clone. While isolates appear to have a common origin, their source and direction of spread are unknown. However, isolates from Italy showed the most diversity, suggesting that this clone had been present in that country for a longer period than it had been in Greece.

van der Feltz, M., C. J. C. de Haas, et al. (1996). "Two rapid complementary methods to detect progressive zidovudine resistance mutations in mononuclear cells of HIV-infected patients." International Journal of Antimicrobial Agents **7**(2): 135.

http://www.sciencedirect.com/science/article/B6T7H-3W2YDRV-7/2/752744041523a645f19cffbbe8d56e42

The objective of the study was to evaluate a rapid screening technique for the presence of mutations in the viral reverse transcriptase gene of HIV following prolonged therapy with zidovudine in patients with AIDS. Peripheral blood mononuclear cells (PBMCs) of 14 HIV-infected patients were analyzed by micro-titer point mutation assay (PMA) before therapy with zidovudine and after at least 10 months of treatment. In addition, five of these were analyzed longitudinally. Three nontreated HIV-seropositive individuals were tested as controls. To confirm the validity of the PMA, patients' material was also analyzed with the single strand conformational polymorphism (SSCP) assay. After 10-55 months of treatment, at codons 41, 70, and 215 a shift from predominantly wild type strains to a mixture of wild type and mutant strains (21%-100% mutant sequences) appeared in the majority of patients' PBMCs. At codons 67 and 219, the wild type strain persisted after therapy in all but 3 patients. Most mutations were detected by SSCP as well as by PMA, except for one mutation at codon 41 and one at codon 70. However, when the two mutations were both present, SSCP and PMA were both able to detect these mutations. In conclusion, both PMA and SSCP are rapid and simple methods for screening for mutations causing drug resistance in zidovudine-treated HIV-infected patients. Although PMA is more labor-extensive than SSCP, the advantage of PMA over SSCP is that it permits the quantitative detection of point mutations coding for zidovudine resistance. The application of these assays may improve procedures of monitoring and modifying antiretroviral therapy on an individual basis.

Xiong, Z., D. Zhu, et al. (2004). "A Klebsiella pneumoniae producing three kinds of class A [beta]-lactamases encoded by one single plasmid isolated from a patient in Huashan Hospital,

Shanghai, China." International Journal of Antimicrobial Agents **23**(3): 262.

http://www.sciencedirect.com/science/article/B6T7H-4BN0J57-1/2/a3a0003f9ddbdccc223221a93bad3fc4

A Klebsiella pneumoniae strain was isolated from a sputum specimen of a patient in the intensive care unit in 1999 in Shanghai Huashan Hospital, China. The isolate was confirmed as an extended-spectrum [beta]-lactamase (ESBL) producing strain by double-disk synergy test. The results of susceptibility test showed that it was resistant to most [beta]-lactams (including third generation cephalosporins) and non-[beta]-lactam antimicrobial agents. Transconjugants were obtained at a frequency of 10-4. A plasmid of about 60 kb was obtained from the transconjugant by plasmid extraction. Three major nitrocefin-hydrolysing bands with pIs of 5.4, 8.2 and 8.4, were shown in extracts of the transconjugant. Partial gene amplification products of blaTEM, blaSHV, and CTX-M-1group gene were obtained from the isolate as well as its transconjugant. The entire blaTEM, blaSHV, and blaCTX-M in the transconjugant were amplified by PCR and the PCR products were cloned into a pHSG398 vector. Afterwards, the susceptibility of transformants and activities of [beta]-lactamases of transformants on antibiotics were tested. The PCR products were directly sequenced, analysed and identified as TEM-1, SHV-12, and CTX-M-3 genes. These results confirm that this strain of Klebsiella pneumoniae produces SHV-12, CTX-M-3 ESBLs and TEM-1 [beta]-lactamase, encoded by one single plasmid, which is responsible for the resistance of this strain to most [beta]-lactams.

International Journal of Food Microbiology (30)

Aguado, V., A. I. Vitas, et al. (2004). "Characterization of Listeria monocytogenes and Listeria innocua from a vegetable processing plant by RAPD and REA." International Journal of Food Microbiology **90**(3): 341.

http://www.sciencedirect.com/science/article/B6T7K-49CRCXW-1/2/f1d4b44b29539b7aef1e79abb7c3a4e7

The incidence of Listeria monocytogenes in a vegetable processing plant was investigated over a 23-month period. Frozen ready-to-eat vegetable samples, well as the plant environment, were sampled. The molecular subtyping techniques, Random Amplified Polymorphic DNA (RAPD) and Restriction Endonuclease Analyses (REA), were performed to help investigate the origin and routes of Listeria dissemination.The low and sporadic incidence of L. monocytogenes made it impossible to establish an epidemiological sequence in the processing plant, though a case of cross-contamination between tomato and ratatouille was detected. Listeria innocua subtyping, however, allowed us to determine the prevalence of several strains in vegetables, and their presence on machinery samples suggested the possibility of cross-contamination during processing.The low incidence of L. monocytogenes indicated that the risk of listeriosis transmission by vegetable consumption is low. On the other hand, the isolation of the same strain of L. innocua in several surveys pointed out the risk of colonisation on surfaces and machinery. The persistence of Listeria spp. is a cause for concern as can lead to future contamination of vegetables processed in the plant and to a possible increased risk for health. Therefore, periodic controls for the presence of Listeria spp. and a further review of the cleaning and disinfection procedures used in frozen vegetable plants are recommended.

Bae, S., G. H. Fleet, et al. (2004). "Occurrence and significance of Bacillus thuringiensis on wine grapes." International Journal of Food Microbiology **94**(3): 301.

http://www.sciencedirect.com/science/article/B6T7K-4C7DF6C-2/2/834a5ddbb98ea14555c4d07119c3970c

Wine grapes harvested at different stages during cultivation from several vineyards in New South Wales, Australia, harboured Bacillus thuringiensis at viable populations of 102-106 cfu/g. Commercial preparations of B. thuringiensis had been sprayed onto the grapes as a biological insecticide. B. thuringiensis (101-103 cfu/ml) was isolated from grape juice and fermenting grape juice in a commercial winery. Although B. thuringiensis remained viable when inoculated at 103-104 cfu/ml into grape juice and wine (pH 3.0-6.0), it did not grow. Using in vitro agar culture assays, B. thuringiensis inhibited several grape-associated yeasts and bacteria as well as various species of fungi associated with grape spoilage and ochratoxin A production. B. thuringiensis did not inhibit Saccharomyces cerevisiae in agar culture or during alcoholic fermentation of grape juice. B. thuringiensis inhibited the malolactic bacterium, Oenococcus oeni, in agar culture but not during mixed cultures in a liquid medium.

Baleiras Couto, M. M., J. M. B. M. van der Vossen, et al. (1994). "RAPD analysis: a rapid technique for differentiation of spoilage yeasts." International Journal of Food Microbiology **24**(1-2): 249.

http://www.sciencedirect.com/science/article/B6T7K-4771XB6-1K/2/cc0f4d4ab46fdcc062c61fb6b68836c2

Techniques for the identification of the spoilage yeasts Saccharomyces cerevisiae and members of the Zygosaccharomyces genus from food and beverages sources were evaluated. The use of identification systems based on physiological characteristics resulted often in incomplete identification or misidentification. Also the cellular fatty acid analysis failed on differentiating species within the Zygosaccharomyces genus. However, the Random Amplified Polymorphic DNA (RAPD) assay, using selected 10-mer oligonucleotides, allowed discrimination between all species tested. For this RAPD assay, a simple and reproducible method of DNA isolation from spoilage yeast cells is described.

Croci, L., D. De Medici, et al. (2002). "The survival of hepatitis A virus in fresh produce." International Journal of Food Microbiology **73**(1): 29.

http://www.sciencedirect.com/science/article/B6T7K-448RF02-1/2/6d2cf4eaaf278ff92f3cf6a99a190af6

Fresh produce has been repeatedly implicated as the source of human viral infections, including infection with hepatitis A virus (HAV). The objective of the present study was to evaluate the HAV adsorption capacity of the surface of various fresh vegetables that are generally eaten raw and the persistence of the HAV. To this end, the authors experimentally contaminated samples of lettuce, fennel, and carrot by immersing them in sterile distilled water supplemented with an HAV suspension until reaching a concentration of 5 log tissue culture infectious dose (TCID50)/ml. After contamination, the samples were stored at 4 [deg]C and analysed at 0, 2, 4, 7, and 9 days. To detect the HAV, RT-nested-PCR was used; positive samples were subjected to the quantitative determination using cell cultures. The three vegetables differed in terms of their adsorption capacity. The highest quantity of virus was consistently detected for lettuce, for which only a slight decrease was observed over time (HAV TITRE=4.44+/-0.22 log TCID50/ml at day 0 vs. 2.46+/-0.17 log TCID50/ml at day 9, before washing). The virus remained vital through the last

day of storage. For the other two vegetables, a greater decrease was observed, and complete inactivation had occurred at day 4 for carrot and at day 7 for fennel. For all three vegetables, washing does not guarantee a substantial reduction in the viral contamination.

Daniel, H. M. and W. Meyer (2003). "Evaluation of ribosomal RNA and actin gene sequences for the identification of ascomycetous yeasts." International Journal of Food Microbiology **86**(1-2): 61.

http://www.sciencedirect.com/science/article/B6T7K-4909G6G-1/2/e7973d040f034adc587983addbd45302

Highly similar gene sequences of the 5' region of the large subunit (LSU) are commonly interpreted to predict the organism's identity. However, it was recognised that closely related taxa do not always show sufficiently diverged D1/D2 LSU sequences to differentiate between them. The effectiveness of species separation using D1/D2 LSU sequences, small subunit (SSU) sequences and actin gene sequences was determined by pair-wise comparisons. The LSU data showed coinciding similarities among and within species. The actin data resolved all investigated species. Examples strengthened the value of almost complete SSU sequences for species separation. The larger number of differences in the highly conserved actin gene, compared to the overall more variable LSU gene, is due to the tolerance of protein coding genes to synonymous nucleotide changes. In contrast, the pairing in secondary structures of the rRNA, ensuring the functionality of the molecule, relies on longer and uninterrupted sequence sections. In conclusion, D1/D2 LSU sequences are not specific enough to identify closely related taxa. The actin gene is a better marker in these cases. However, because of the availability of a large database of fungal D1/D2 LSU sequences, this gene region is currently still the preferred target for sequence-based identification.

Dubois, E., G. Merle, et al. (2004). "Diversity of enterovirus sequences detected in oysters by RT-heminested PCR." International Journal of Food Microbiology **92**(1): 35.

http://www.sciencedirect.com/science/article/B6T7K-4BT19BR-1/2/02e87754070f3fc4e8ffbadbc877cfa6

Oysters harvested in western France, from five sites associated with outbreaks of food-borne norovirus gastroenteritis between February 2000 and March 2001, were assayed for enterovirus RNA by reverse transcriptase-heminested polymerase chain reaction (RT-heminested PCR). Forty percent (21/52) of shellfish samples (pool of seven oysters) were contaminated by enteroviruses. Infectious coxsackieviruses serotype A21 were isolated from three of these positive samples. Amplicons corresponding to 65 base sequences in the 5' untranslated region of the enteroviral genome were analyzed by direct sequencing. Interpretable results were obtained from 18 amplicons, but mixtures of sequences confused the results from 3 samples. Sequences isolated from samples from the different sites were different but similarities were observed between sequences detected in shellfish from two sites at different dates. Sequences were also compared to sequences of human, bovine and porcine enteroviruses. Both human and animal origins of enterovirus contamination of shellfish seemed likely.

Giraffa, G., C. Andrighetto, et al. (2004). "Genotypic and phenotypic diversity of Lactobacillus delbrueckii subsp. lactis strains of dairy origin." International Journal of Food Microbiology **91**(2): 129.

http://www.sciencedirect.com/science/article/B6T7K-49D236Y-

4/2/de111942102af32efa30b5e1845cb635

Eighty-nine strains of Lactobacillus delbrueckii subsp. lactis isolated from Italian hard and semi-hard cheeses and artisan starter cultures were characterised by phenotypic and genotypic methods. Phenotypic diversity was evaluated by studying biochemical characteristics (i.e. acidifying and peptidase activities) of technological interest. Genotypic diversity was evidenced by RAPD-PCR and pulsed field gel electrophoresis (PFGE). Phenotypic characterisation indicated a wide variability of the acidifying activity within Lact. delbrueckii subsp. lactis. Although the data was variable, it allowed us to evidence groups of strains with different acidifying properties, especially in terms of acidification intensity. Concerning peptidase activity, Lact. delbrueckii subsp. lactis showed a homogeneously high x-prolil-dipeptidil-aminopeptidase activity and a considerable but more heterogeneous lysil-aminopeptidase activity. The increased resolution obtained by the use of two molecular typing techniques, i.e. RAPD-PCR and PFGE, allowed to widen the level of strain heterogeneity. Technological and ecological pressures are determinant in selecting Lact. delbrueckii subsp. lactis sub-populations which are more functional to the different cheese technologies.

Giraffa, G., C. Lazzi, et al. (2003). "Molecular typing of Lactobacillus delbrueckii of dairy origin by PCR-RFLP of protein-coding genes." International Journal of Food Microbiology **82**(2): 163.

http://www.sciencedirect.com/science/article/B6T7K-46HJKYC-5/2/df6fd9e32669384273d1ff605000a67d

Thirty-five strains of Lactobacillus delbrueckii subsp. lactis and subsp. bulgaricus isolated from dairy products were typed by restriction fragment length polymorphism (RFLP) of protein-coding genes. The strains were analysed by RFLP of PCR amplified, infragenic fragments of the following housekeeping genes: [beta]-galactosidase, lactose permease, and proline dipeptidase. Sequencing of the variable regions of the 16S rDNA was then performed on a reduced number of strains. PCR-RFLP analysis evidenced wide strain heterogeneity. Strains were grouped into genotypes according to both subspecies assignment and infra-species genetic polymorphism. This polymorphism was related to the presence of microbial groups within subspecies populations. The low infra-species sequence polymorphism detected in the variable region of the 16S rRNA gene did not enable to group the strains with the same sensitivity reached by PCR-RFLP of protein-coding genes. PCR-RFLP of protein-coding genes applied to L. delbrueckii seems a promising tool to evaluate microbial diversity within bacterial subpopulations. Differences among bacterial subpopulations based upon molecular heterogeneity in protein-coding genes would enable to better understand the role of strains from different ecological niches.

Hazeleger, W., C. Arkesteijn, et al. (1994). "Detection of the coccoid form of Campylobacter jejuni in chicken products with the use of the polymerase chain reaction." International Journal of Food Microbiology **24**(1-2): 273.

http://www.sciencedirect.com/science/article/B6T7K-4771XB6-1N/2/6de766b0c80ea8cfbc8f9ef6d3a84b3e

Detection of the coccoid form of Campylobacter jejuni with the use of the polymerase chain reaction (PCR) was examined. Coccoid cells of this pathogen, formed at different temperatures, showed different detection characteristics in the PCR. For spirals and cocci formed at 4[deg]C and 12[deg]C, the detection limit was about 2 x 103 cells/PCR. However, for detection of coccoid cells formed at 25[deg]C and 37[deg]C, at least 2 x 104 cells per PCR were needed. PCR was also performed on homogenates in peptone saline solution and enrichment broths of chicken

meat and chicken liver that were artificially contaminated with cocci formed at 4[deg]C. PCR-products of these samples could not be demonstrated clearly.

in't Veld, P. H., W. S. Ritmeester, et al. (2001). "Detection of genes encoding for enterotoxins and determination of the production of enterotoxins by HBL blood plates and immunoassays of psychrotrophic strains of Bacillus cereus isolated from pasteurised milk." International Journal of Food Microbiology **64**(1-2): 63.

http://www.sciencedirect.com/science/article/B6T7K-42BSPWM-7/2/6b9c2c361d579c26771a787fb5cb14f7

The presence of genes for the production of the three components of the HBL enterotoxin complex and enterotoxin-T in Bacillus cereus was evaluated by PCR tests for strains isolated from milk. In addition enterotoxin production of B. cereus was evaluated by means of the HBL blood agar plate and two commercially available toxin tests. All three genes for the HBL enterotoxin complex were detected in 55% of the 86 strains tested, the enterotoxin-T gene was detected in 62% of the strains. A few strains showed a weak reaction in the PCR tests for the L1 or L2 components of the HBL enterotoxin complex. Many strains that were found to contain the genes for the HBL complex gave negative or doubtful results in the HBL blood agar plate test. All strains that contain the L2 part of the HBL complex showed a titer of at least 8 in the Oxoid RPLA test. Two strains that did not contain the L2 part of the HBL enterotoxin complex gave high titers (=64) in the RPLA test.

Kitazawa, H., T. Itoh, et al. (1996). "Induction of IFN-[gamma] and IL-1[alpha] production in macrophages stimulated with phosphopolysaccharide produced by Lactococcus lactis ssp. cremoris." International Journal of Food Microbiology **31**(1-3): 99.

http://www.sciencedirect.com/science/article/B6T7K-3W2T595-7/2/8a2b407e57f67cb118b26797c95ddb2b

The induction of interferon (IFN) and interleukin-1 (IL-1) production in murine macrophages by a phosphopolysaccharide, produced by a dairy lactic acid bacteria, Lactococcus lactis ssp. cremoris, was investigated. When the phosphopolysaccharide was added into macrophage cultures at concentrations from 1 to 200 [mu]g/ml, substantial IFN titers (6.2-79.2 IU/ml) were detected. Using the reverse transcription-polymerase chain reaction (RT-PCR), the expression of mRNA encoding IFN-[gamma] was verified in spleen macrophage cultures. Macrophages stimulated with the phosphopolysaccharide also produced IL-1[alpha] at a concentration of 50 [mu]g/ml. This study showed for the first time that phosphopolysaccharide derived from a dairy lactic acid bacterium can induce IFN-[gamma] and IL-1[alpha] production in macrophages. These findings strongly suggest that the phosphopolysaccharide is a type of 'biological response modifier' and the fermented dairy foods containing Lactococcus lactis ssp. cremoris can be designated as a physiologically functional food.

Knutsen, A. K., M. Torp, et al. (2004). "Phylogenetic analyses of the Fusarium poae, Fusarium sporotrichioides and Fusarium langsethiae species complex based on partial sequences of the translation elongation factor-1 alpha gene." International Journal of Food Microbiology **95**(3): 287.

http://www.sciencedirect.com/science/article/B6T7K-4C9HPBB-1/2/6c39cb6b3592d31fca3eac0b5ecadaad

Phylogenetic relationships between four Fusarium species were studied using parts of the nuclear translation elongation factor-1 alpha (EF-1[alpha]) gene as a phylogenetic marker. Sequences from 12 isolates of Fusarium poae, 10 isolates of Fusarium sporotrichioides and 12 isolates of Fusarium langsethiae yielded 4, 5 and 5 haplotypes, respectively. In addition, we included one isolate of Fusarium kyushuense. The aligned sequences were subjected to neighbor-joining (NJ), maximum parsimony and maximum likelihood (ML) analyses. The results from the different analyses were highly concordant. The EF-1[alpha]-based phylogenies support the classification of F. langsethiae as a separate taxon in the section Sporotrichiella of Fusarium, as the closest sister taxon to F. sporotrichioides, while F. kyushuense is the sister taxon to F. poae. This corresponds well with the ability of F. langsethiae and F. sporotrichioides to produce T-2 and HT-2 toxins. In contrast, morphological characters indicate a closer relationship between F. langsethiae and F. poae on the one hand, and between F. sporotrichioides and F. kyushuense on the other hand.

Kosiak, E. B., A. Holst-Jensen, et al. (2005). "Morphological, chemical and molecular differentiation of Fusarium equiseti isolated from Norwegian cereals." International Journal of Food Microbiology **99**(2): 195.

http://www.sciencedirect.com/science/article/B6T7K-4DS98YD-2/2/452ab637395bb8e22e53d536f97f855d

The morphological variation, secondary metabolite profiles and restriction fragment length polymorphisms (RFLPs) of PCR amplified intergenic spacer (IGS) ribosomal DNA (rDNA) were studied in 27 isolates of Fusarium equiseti, 25 isolated from Norwegian cereals and 2 from soil obtained from the IBT culture collection (BioCentrum, Technical University of Denmark). All 27 isolates were tested for production of fusarochromanone (FUSCHR), zearalenone (ZEA) and the trichothecenes: 15-monoacetoxy-scirpentriol (MAS), diacetoxy-scirpenol (DAS), T-2 and HT-2 toxins, T2-triol, neosolaniol (NEO), deoxynivalenol (DON), nivalenol (NIV) and 4-acetylnivalenol (Fus-X). The trichothecenes were analysed by GC-MS in a selected ion monitoring mode, while FUSCHR was determined by ion pair HPLC with fluorometric detection and production of ZEA by TLC. For amplification of IGS rDNA primers CNL12 and CNS1 were applied. IGS rDNA was digested with the four restriction enzymes: AvaII, CfoI, EcoRI and Sau3A. In addition, we sequenced the IGS rDNA region of three of the Norwegian isolates. There were two morphological types among the Norwegian strains of F. equiseti, type I with short apical cells (dominating) and type II with long apical cells, with four haplotypes identified based on the RFLP data. Variation in secondary metabolite profiles within and between the morphological groups was observed and the levels of produced toxins were: FUSCHR 3000-42,500 and 25-30 ng/g, NIV 20-2500 and 120-700 ng/g, FUS-X 20-15,000 and 0 ng/g, DAS 30-7500 and 0-600 ng/g, and MAS 10-600 and 0-500 ng/g, for strains with short and long apical cells, respectively. NEO was detected in 16/27 strains tested (all morphotype I). All but four strains of type I (these four lacked a restriction site for EcoRI) had identical RFLP profiles. The isolates of type II had two haplotypes. The IGS sequence similarity data indicated differences between these morphotypes corresponding to two separate lineages apparently at the species level.

Lindqvist, R. (1997). "Preparation of PCR samples from food by a rapid and simple centrifugation technique evaluated by detection of Escherichia coli O157:H7." International Journal of Food Microbiology **37**(1): 73.

http://www.sciencedirect.com/science/article/B6T7K-3RJNRBN-9/2/5a9d03d4a417f6e9363d85ea0e20b4c4

A sample treatment method based on buoyant density centrifugation which separates bacteria

from food, concentrates bacteria and removes PCR inhibitors is described. The method involves a one minute centrifugation of food homogenate layered over a gradient medium (Percoll(R) or BacXtractor(TM)) in Eppendorf tubes, followed by a single wash step. The small scale of this treatment makes it possible to process many samples in a short time. To evaluate the method beef and minced beef samples, spiked with strains of Escherichia coli O157;H7, were treated and then analysed by PCR aimed at verocytotoxin- (VT1 and VT2) and eae-genes. The detection limits in 1:10 (w/v) beef and minced beef homogenates were 125-250 cfu ml-1 (1250-2500 cfu g-1) and 1000 cfu ml-1 (1 x 104 cfu g-1), respectively. The enrichment of spiked samples in buffered peptone water at 37 [deg]C for 6 hours before buoyant density centrifugation and PCR, allowed 0.5 cfu g-1 beef and 5 cfu g-1 minced beef to be detected. This combination of enrichment and buoyant density centrifugation was also used for analysis of 43 beef samples from a consignment in which E. coli O157:H7 had been detected, and detected VT-genes in all 43 samples. E. coli O157:H7 was also separated and detected in spiked samples of milk, lettuce, shrimps, and blue cheese at arbitrary concentrations of 3000 cfu ml-1. The present sample preparation method has the potential to be applicable to many other combinations of bacteria and food, and in connection with other detection methods than PCR as well.

Martinez-Cuesta, M. C., T. Requena, et al. (2001). "Use of a bacteriocin-producing transconjugant as starter in acceleration of cheese ripening." International Journal of Food Microbiology **70**(1-2): 79.

http://www.sciencedirect.com/science/article/B6T7K-44724S4-9/2/a7d857954d81811cdc6306131ae18b98

The non-conjugative 46 kb plasmid that encodes the biosynthesis of lacticin 3147 in Lactococcus lactis IFPL105 has been transferred to the starter L. lactis IFPL359, used in goat's milk cheesemaking. The accelerating effect exerted on proteolysis and development of sensory characteristics of semi-hard cheese by the bacteriocin-producing transconjugant L. lactis IFPL3593 (Lac+ Bac+ Imm+), which is able to induce cell lysis in starter adjuncts with high peptidase activity, has been studied. It has been demonstrated that the use of IFPL3593 as starter accelerates cheese ripening as it increases the level of amino nitrogen correlated with early cell lysis of adjuncts. The fact that the bacteriocin-producing microorganism used is immune to the bacteriocin, allowed proper acidification of the curd without altering the cheese-making process.

Mayer, Z., A. Bagnara, et al. (2003). "Quantification of the copy number of nor-1, a gene of the aflatoxin biosynthetic pathway by real-time PCR, and its correlation to the cfu of Aspergillus flavus in foods." International Journal of Food Microbiology **82**(2): 143.

http://www.sciencedirect.com/science/article/B6T7K-46FXSDH-2/2/3e8843db592d5e80a0a098dade336983

A real-time PCR system directed against the nor-1 gene of the aflatoxin biosynthetic pathway as a target sequence has been applied to detect an aflatoxinogenic A. flavus strain in plant-type foods like maize, pepper and paprika. The system is based on the TaqMan(R) fluorescent probe technology. The copy numbers of the nor-1 gene were compared to conventional cfu data obtained from the same set of samples. In general, a good correlation between nor-1 gene copy number and the cfu data was observed; however, the nor-1 copy numbers were always higher. It was shown that the system is specific for nor-1 containing species.

Meng, J., S. Zhao, et al. (1998). "Virulence genes of Shiga toxin-producing Escherichia coli isolated from food, animals and humans." International Journal of Food Microbiology **45**(3): 229.

http://www.sciencedirect.com/science/article/B6T7K-3VCVFHR-8/2/96c11d3ef685c609690190b4a2243152

The presence of virulence genes, encoding enterohemorrhagic Escherichia coli (EHEC)-hemolysin (EHEC-hlyA), intimin (eae), and Shiga toxins 1 (stx1) and 2 (stx2), in 178 isolates of pathogenic E. coli, was determined using the polymerase chain reaction with primers specific for each virulence gene. The tested organisms were 120 isolates of E. coli O157:H7 from human patients, cattle, sheep and foods, 16 non-O157:H7 EHEC isolates from patients suffering from hemorrhagic colitis or hemolytic uremic syndrome, 15 non-O157:H7 Shiga toxin-producing E. coli (STEC) isolates from cattle and foods, 26 isolates of enteropathogenic E. coli (EPEC), enteroinvasive E. coli (EIEC) and enterotoxigenic E. coli (ETEC), and an E. coli K12 strain. Results revealed that all isolates of O157:H7 carried EHEC-hlyA, eae, and one or both stx genes; 15 of the 16 non-O157:H7 EHEC isolates had EHEC-hlyA, but all possessed eae and one or both stx genes; only seven of the 15 non-O157 STEC isolated from cattle and foods contained both EHEC-hlyA and eae genes. The EPEC, EIEC, ETEC, and the E. coli K12 strain did not carry these virulence genes, except eight EPEC isolates were positive for eae. Results suggest that a combination of EHEC-hlyA and eae genes could serve as markers to differentiate EHEC from less pathogenic STEC, and other pathogenic or non-pathogenic E. coli.

Meng, J., S. Zhao, et al. (1996). "Polymerase chain reaction for detecting Escherichia coli O157:H7." International Journal of Food Microbiology **32**(1-2): 103.

http://www.sciencedirect.com/science/article/B6T7K-3W2T5DW-1B/2/144f0e6429266a27fb2545ede63d0792

Escherichia coli O157:H7 is known as an important cause of hemorrhagic colitis and hemolytic uremic syndrome. Real-time procedures that are sensitive for detecting small populations of this bacterium in food are lacking and needed. An expression library was constructed by ligation of BamHI-EcoRI DNA fragments of E. coli O157:H7 to plasmid vector pUC19 and transformation of recombinant plasmids to E. coli JM109. A clone that contained a specific DNA fragment of E. coli O157:H7 was identified by colony immunoblot assay using monoclonal antibody MAb 4E8C12 that uniquely links to E. coli O157:H7 and a few other serotypes of verotoxin-producing E. coli. The DNA sequence of the clone consisted of 110 bp of 5' region of enterohemorrhagic E. coli (EHEC) eae gene and a 688 bp DNA fragment adjacent to 5' end of the eae gene, including an unknown function gene encoding 156 amino acids. A pair of oligonucleotide primers was synthesized based on the sequence of the 688 bp fragment. The primers were used in a polymerase chain reaction (PCR) to amplify a target DNA of 633 bp. The primers amplified 1 ng of DNA from 67 strains of E. coli O157:H7, two strains of E. coli O157:NM, and 7 of 11 E. coli O55:H7 and O55:NM strains, but not 50 ng of DNA from 34 strains of 29 other E. coli serotypes and 25 strains of 8 other bacterial species. Annealing temperatures from 60 to 63 [deg]C could be used for the PCR without loss of specificity. The minimum amount of target DNA detected by the PCR was 5 pg. When a boiling method and GeneReleaser were used, the PCR was able to detect as few as 25 and 38 CFU of E. coli O157:H7, respectively, in 3 h.

Ng, P. J., G. H. Fleet, et al. "Pesticides as a source of microbial contamination of salad vegetables." International Journal of Food Microbiology **In Press, Corrected Proof**
http://www.sciencedirect.com/science/article/B6T7K-4FFX9CR-4/2/b934eca2346750c8bd7e49fada74ad04

Ten commercially available pesticides (insecticides, herbicides and fungicides), used during the production of vegetable produce, were examined as potential sources of microbial contaminants. As purchased, none of the pesticides showed the presence of viable microorganisms (Pseudomonas, Salmonella and Escherichia coli. Listeria monocytogenes did not survive after inoculation into any of the pesticides.Pesticides were reconstituted in different sources of agricultural water (bore, dam and river) and examined for survival and growth of microorganisms naturally present in these waters. On storage at 30 [deg]C for 48 h, nine of the pesticides supported the growth of bacterial species present in these waters. Predominant species in the pesticide solutions, before and after storage, varied according to the source, but species of Pseudomonas, Acinetobacter and Aeromonas and various coliforms exhibited significant growth. Unless managed properly (reconstituted in potable water, and used without lengthy storage), pesticides could contribute to the microbial load of vegetable produce, thereby affecting their shelf-life and public health safety.

Nogva, H. K. and D. Lillehaug (1999). "Detection and quantification of Salmonella in pure cultures using 5'-nuclease polymerase chain reaction." International Journal of Food Microbiology **51**(2-3): 191.

http://www.sciencedirect.com/science/article/B6T7K-3XK0WG0-D/2/f393c83634f473d6b5aad0f28dc149fb

Advances in detection and quantification assays based on nucleic acids conceivably will revolutionize the ability to quickly and specifically detect and quantify microorganisms in foods. Among these assays, the polymerase chain reaction (PCR) assay and the TaqMan(TM) PCR Detection System (Perkin-Elmer) probably are among the most promising. Since a 5'-nuclease PCR renders possible the automated and direct detection and quantification of PCR products (Holland et al., 1991. Proc. Natl. Acad. Sci. USA 88, 7276-7280), microorganisms in foods can be detected and quantified indirectly within a few hours through analysis of the microbial DNA or RNA sequences present. In the present report we have adapted a 5'-nuclease-based kit for the quantification of Salmonella.

Olier, M., S. Rousseaux, et al. (2004). "Screening of glutamate decarboxylase activity and bile salt resistance of human asymptomatic carriage, clinical, food, and environmental isolates of Listeria monocytogenes." International Journal of Food Microbiology **93**(1): 87.

http://www.sciencedirect.com/science/article/B6T7K-4BVRVDR-1/2/7832ad24e928b8d9fd750561ce600f6f

Following consumption, stomach acidity is the first major barrier encountered by the food-borne pathogen Listeria monocytogenes. Analysis of low pH sensitivity and glutamate decarboxylase (GAD) acid resistance system of 14 isolates of L. monocytogenes carried asymptomatically by humans showed that levels of GAD activity were subjected to strain variation. Similar variations were observed for strains responsible for 18 cases of listeriosis, whereas in comparison, 13 strains isolated from food and food-processing plant environments showed lower GAD activity. Following survival of the stomach barrier, L. monocytogenes also has to resist bile salts encountered in the small intestines. Analysis revealed that all strains tested were able to grow in the presence of bile salts with concentrations as high as those encountered in the small intestines and had previously identified bile salt hydrolase (BSH) activity. Strain variation was observed but there was no relationship between the origin of the strains and the ability to degrade bile salts.

Ostlie, H. M., L. Eliassen, et al. (2004). "Phenotypic and PCR-based characterization of the microflora in Norvegia cheese during ripening." International Journal of Food Microbiology **94**(3): 287.

http://www.sciencedirect.com/science/article/B6T7K-4C4W2J8-2/2/8101aeb7b424d37c03123a1d2da5c55f

Microbiological sampling of Norvegia cheese from three cheese factories was done during ripening. The evolution of aerobic mesophilic bacteria, lactococci, lactobacilli, enterococci, presumptive leuconostoc and pediococci was investigated after 30, 90, 180 and 270 days of ripening. Isolates (135) of non-starter lactic acid bacteria (NSLAB) from nine Norvegia cheeses after 90, 180 and 270 days of ripening were examined. The isolates were tested by physiological and biochemical assays, species-specific PCR and 16S rDNA sequencing. After 90 days of ripening Leuconostoc spp., most probably from the starter, and the NSLAB specie Lactobacillus paracasei dominated among the isolates, however, after longer ripening Lb. paracasei dominated. The development and evolution of the microflora in Norvegia varied according to dairy and ripening time.

Pedersen, L. H., P. Skouboe, et al. (1997). "Detection of Penicillium species in complex food samples using the polymerase chain reaction." International Journal of Food Microbiology **35**(2): 169.

http://www.sciencedirect.com/science/article/B6T7K-3RH0CXW-B/2/3400d2dc8530b2996c8d859761baeffd

Rapid identification of filamentous fungi is becoming increasingly important in food mycology both for monitoring the production process and for the identification of food spoilers. This paper describes the development and trial of two specifie PCR primer sets. A 336 bp fragment from species belonging to Penicillium subgenus Penicillium was amplified by the primers ITS 212d and ITS 549. The other primer set, ITS 183 and ITS 401 specifically identified two species. Penicillium roqueforti and P. carneum, both known as spoilers in the bread industry, by amplification of a 300 bp fragment. The future perspectives of PCR based identification of filamentous fungi in food are discussed.

Rijpens, N. and L. Herman (2004). "Comparison of selective and nonselective primary enrichments for the detection of Listeria monocytogenes in cheese." International Journal of Food Microbiology **94**(1): 15.

http://www.sciencedirect.com/science/article/B6T7K-4C56P5F-1/2/31bd3f9aad72cd50e759f6eef4f3386d

A completely selective enrichment procedure was compared with two partially nonselective ones for the detection of Listeria monocytogenes in cheeses. After enrichment for approximately 48 h, the enrichment media were streaked on selective agars and presumptive Listeria colonies were confirmed using PCR. In some cases, PCR was also performed directly on the enrichment broth. The conventional, completely selective enrichment procedure was not always the best choice for the detection of stressed L. monocytogenes in cheeses. Especially in the case of semi-hard cheeses from pasteurized milk and soft cheeses of the blue veined and the red smear types, the methods that incorporated a nonselective enrichment step gave better results than the completely selective method. For mold ripened, soft cheeses, the results were highly dependent on the brand of cheese and time of sampling, but the best results were obtained using the completely selective enrichment procedure.

Rijpens, N., L. Herman, et al. (1999). "Rapid detection of stressed Salmonella spp. in dairy and egg products using immunomagnetic separation and PCR." International Journal of Food Microbiology **46**(1): 37.

http://www.sciencedirect.com/science/article/B6T7K-3VKT477-4/2/0199a00f94e46d04610ef9a99e680e8f

The rapid detection of an average of 5.9 stressed Salmonella cells in 25 g of food product using immunomagnetic separation (IMS) and PCR is described. For pasteurised egg yolk, egg yolk powder, ice-cream, whole egg, egg white and cheeses made from pasteurised milk PCR was applied after 16 h of preenrichment in buffered peptone water (BPW) using IMS and alkaline lysis as sample preparation method. For whole egg and egg white the BPW was supplemented with iron. For milk powder, and raw milk cheeses, the 16-h preenrichment in BPW was followed by IMS and a 4-h enrichment in Rappaport-Vassiliadis broth. In the latter case, PCR was applied on the enrichment medium after centrifugation and alkaline lysis. For PCR the primers ST11 and ST15 (Aabo et al., 1993) producing a fragment of 429 bp were used. An internal PCR control, designed to be co-amplified with the target DNA using the same primers but producing a smaller fragment of 240 bp, was used.

Schnerr, H., L. Niessen, et al. (2001). "Real time detection of the tri5 gene in Fusarium species by LightCycler(TM)-PCR using SYBR(R)Green I for continuous fluorescence monitoring." International Journal of Food Microbiology **71**(1): 53.

http://www.sciencedirect.com/science/article/B6T7K-44CMXVM-6/2/3abed1e0a2b67a80f2aa567792abf524

LightCycler(TM) technology combines rapid in vitro amplification of DNA with real time detection and quantification of the amount of target molecules present in a sample. The system enables a 35-cycle PCR with 32 samples do be completed in 45 min, including quantification and identification of the product. It is therefore well suited for routine analysis of large numbers of samples in quality control and for defining HACCP concepts. Based on PCR primers specific to the tri5 gene, a quantitative group specific assay was established for Fusarium species producing trichothecenes. In the assay, SYBR(R)Green I was used as fluorescent dye enabling real time detection of PCR products. Characterisation of the amplicons was achieved by melting point analysis (85+/-0.1 [deg]C). Nonspecific products such as primer dimers could readily be distinguished from the product by their lower melting points. Composition of the amplification buffer was optimised and various hot start methods were tested in order to achieve the highest sensitivity of the assay. Uracil DNA glycosylase was added to prevent amplification of nonspecific products due to DNA carryover. The spectrum of species detected was generally in accordance with the results found in conventional PCR using the Tox5 primer pair. Reproducibility in six parallel experiments of the assay was determined to be 98% in the range between 0.05 and 6 ng of purified Fusarium graminearum DNA. The assay was used to analyse 30 wheat samples contaminated with toxigenic Fusarium spp. Contamination ranged from 0% to 78% as revealed by mycological analysis, and this is compared with results from the LightCycler(TM). This is the first report on the use of the LightCycler(TM) system in combination with SYBR(R)Green I for the quantification and identification of fungal DNA in pure cultures and sample material.

Schroeder, C. M., D. G. White, et al. (2003). "Isolation of antimicrobial-resistant Escherichia coli from retail meats purchased in Greater Washington, DC, USA." International Journal of Food

Microbiology **85**(1-2): 197.

http://www.sciencedirect.com/science/article/B6T7K-47DTB1D-1/2/ff5280e2554f7c4ae1fb199ab987b3f4

Four hundred and seventy-two generic Escherichia coli isolates were recovered from ground and whole retail beef, chicken, pork, and turkey obtained from Greater Washington, DC, USA during the years 1998 to 2000. Many of the isolates displayed resistance to tetracycline (59%), sulfamethoxazole (45%), streptomycin (44%), cephalothin (38%) and ampicillin (35%). Resistance was also observed, but to a lesser extent, to gentamicin (12%), nalidixic acid (8%), chloramphenicol (6%), ceftiofur (4%) and ceftriaxone (1%). Sixteen percent of the isolates displayed resistance to one antimicrobial, followed by 23% to two, 23% to three, 12% to four, 7% to five, 3% to six, 2% to seven and 2% to eight. Three E. coli isolates were shown to possess Shiga toxin genes (stx2) via PCR; all were O non-typeable and were recovered from ground beef samples purchased on the same day at the same supermarket. One of the Shiga toxin-producing E. coli (STEC) isolates was susceptible to each of the antimicrobials tested, whereas one displayed resistance to cephalothin and sulfamethoxazole, and one displayed resistance to ampicillin, cephalothin, gentamicin, streptomycin, sulfamethoxazole and tetracycline. Findings from this study indicate that retail raw meats may often be contaminated with antimicrobial-resistant E. coli.

Thisted Lambertz, S., R. Lindqvist, et al. (2000). "A combined culture and PCR method for detection of pathogenic Yersinia enterocolitica in food." International Journal of Food Microbiology **57**(1-2): 63.

http://www.sciencedirect.com/science/article/B6T7K-409VGKY-7/2/6895857ade009fea1a3c5f2b2fbb8adb

A combined method based on traditional culturing, buoyant density centrifugation, (BDC), and polymerase chain reaction (PCR) techniques for detection and identification of pathogenic Y. enterocolitica in food was developed and evaluated. An internal control, which was added in each PCR-tube and co-amplified by the same primer pair as the pathogen, monitored false-negative PCR results. The sample preparation step, BDC, was used to remove PCR inhibiting food substances and to concentrate the Y. enterocolitica cells. Single PCR with a chromosomal gene (ail) as target was chosen for screening the samples. The method was tested on naturally and artificially contaminated food samples. In three different food samples, processed meat (brawn), unprocessed beef and minced pork, inoculated with 10 cfu pathogenic Y. enterocolitica per gram, Y. enterocolitica was detected and cultural bacteria indicated within 18 h of enrichment.

Vogel, B. F., L. V. Jorgensen, et al. (2001). "Diversity of Listeria monocytogenes isolates from cold-smoked salmon produced in different smokehouses as assessed by Random Amplified Polymorphic DNA analyses." International Journal of Food Microbiology **65**(1-2): 83.

http://www.sciencedirect.com/science/article/B6T7K-42M1DDR-B/2/ec2a457be279e0ec1c0f9be146a5c07e

One hundred and forty-eight Listeria monocytogenes isolates originating from vacuum packed cold-smoked salmon produced in 10 different Danish smokehouses were compared by Random Amplified Polymorphic DNA (RAPD) profiling. A total of 16 different reproducible RAPD profiles were obtained using a standardised RAPD analysis by four primers separately. The grouping of the 148 strains was exactly the same for the four primers used. For a sub-set of 20 strains typed

by Pulsed Field Gel Electrophoresis (PFGE), only one strain was allocated into a different group as compared to the grouping by RAPD typing. Different RAPD types dominated in products from different smokehouses. Some identical RAPD types were isolated in several smokehouses. In each of four smokehouses, one particular RAPD type could be repeatedly isolated from products. Each smokehouse/product carried its own specific RAPD type and this may indicate a possible persistence of closely related strains of L. monocytogenes in smokehouses.

Wernars, K., P. Boerlin, et al. (1996). "The WHO multicenter study on Listeria monocytogenes subtyping: Random amplification of polymorphic DNA (RAPD)." International Journal of Food Microbiology **32**(3): 325.

http://www.sciencedirect.com/science/article/B6T7K-3W2V4C8-7/2/02bcc956526001f75b5517ef60abfd6f

As part of a WHO multicenter study on Listeria monocytogenes subtyping methods the random amplification of polymorphic DNA (RAPD)-technique was evaluated. Six participants were asked to use a standard protocol to analyse a set of 80 L. monocytogenes strains. This set contained 22 groups of epidemiologically linked isolates and 11 pairs of duplicate strains. Using three different 10-mer primers, the median reproducibility of the RAPD-results obtained by the six participants was 86.5% (range 0-100%). Failure in reproducibility was mainly due to results obtained with one particular primer. The number of epidemiological groups found to be homogeneous varied from 1-22 (median 16). However, for some groups an inhomogeneity was found by the majority of participants. The overall correlation between the results from the different participants ranged from 32 to 85%.

International Journal of Medical Microbiology(3)

Prager, R., S. Annemuller, et al. (2005). "Diversity of virulence patterns among shiga toxin-producing Escherichia coli from human clinical cases -- need for more detailed diagnostics." International Journal of Medical Microbiology **295**(1): 29.

http://www.sciencedirect.com/science/article/B7GW0-4FFN4SH-1/2/d567cd93b9ba7d876d68da29e1ec4566

Intestinal infections due to shiga toxin-producing Escherichia coli bacteria (STEC) reveal a broad range of clinical symptoms and a large scale of virulence properties of the respective pathogens. The question whether all STEC variants or only a particular group of them need to be considered for clinical and epidemiological purposes was answered throughout this study. Using the PCR technique for the identification of 25 different virulence-associated genes, 266 E. coli strains belonging to 81 different E. coli serotypes from various clinical origins were investigated. A great genetic diversity of the virulence properties and a broad range of virulence marker combinations have been identified. However, distinct virulence marker combinations (e.g. Stx2/LEE/pO157 as well as Stx2dac/pO113) were found to be associated with the same notified clinical symptoms (e.g. HUS). Such an association speaks either for the "shiga toxin-only concept" or for several redundant, but clinically or epidemiologically important virulence properties.

Swiderek, H., H. Claus, et al. (2005). "Evaluation of custom-made DNA microarrays for multilocus sequence typing of Neisseria meningitidis." International Journal of Medical Microbiology **295**(1): 39.

http://www.sciencedirect.com/science/article/B7GW0-4F97397-4/2/a190909fba2cd1d814a1c80e2c231c69

Multilocus sequence typing (MLST) has become the gold standard for typing of a variety of bacterial and fungal micro-organisms. Others recently reported the successful use of the tiling DNA array technology to sequence-type Staphylococcus aureus. We now evaluated microarrays based on polymorphism-directed oligonucleotide design for typing of Neisseria meningitidis. The rationale behind this approach was to minimize the number of microarray probes by exploiting the comprehensive knowledge of polymorphisms combined in the Neisseria MLST website. Initial experiments using model oligonucleotides of 28-32 base-pairs in length revealed that the hybridization protocols used were highly specific. However, despite of several optimization steps, the rate of misidentification of oligonucleotides remained >1.8% in consecutive validation experiments using arrays representing the genetic diversity at three MLST loci. We assume that the high density of polymorphic sites and the extensive GC-content variations at N. meningitidis MLST loci hinder the successful implementation of MLST microarrays based on polymorphism-directed oligonucleotide design.

Wiese, J., J. H. Helbig, et al. (2004). "Evaluation of different primers for DNA fingerprinting of Legionella pneumophila serogroup 1 strains by polymerase chain reaction." International Journal of Medical Microbiology **294**(6): 401.

http://www.sciencedirect.com/science/article/B7GW0-4DN99XW-6/2/d1d686410420f7766874bcccd7318eed

A DNA fingerprinting method for the characterization of Legionella pneumophila serogroup 1 strains was established. This method was based on the DNA extraction using Chelex 100 and subsequent PCR analysis using primers under conditions of low stringency. Sixteen single primers were tested for the typing of the 10 epidemiologically unrelated reference strains of L. pneumophila serogroup 1 as well as patient isolates and environmental strains isolated from the water system of a hospital where patients with legionellosis were treated. In addition, a combination of two primers (Lpm-1 and Lpm-2) originally established for the specific detection of Legionella strains was tested. The PCR results were compared with two further subtyping methods, i.e. monoclonal antibody analysis and pulsed-field gel electrophoresis. The type strains Philadelphia 1, Knoxville 1, Allentown 1, Benidorm 0303E, Bellingham 1, and France 5811 could be distinguished clearly in experiments using all of the primers. Depending on the primer used, Heysham 1 and Oxford 4032E showed different DNA profiles. The strains Olda and Camperdown 1 were nearly indistinguishable. In contrast, the analysis by PFGE and MAb subtyping revealed distinct types for all 10 reference strains. The discrimination of the patient isolates from two suspected cases of nosocomial legionellosis and environmental isolates was not possible with the 16 single primers used in the study. However, the PCR assay with the combination of Lpm-1 and Lpm-2 as well as the PFGE and MAb analysis were able to differentiate distinct types. The use of the sequence-specific primers under low-stringency annealing conditions allowed both simultaneous gene detection as well as epidemiological typing of Legionella strains.

Block, J., M. Drost, et al. (2003). "Use of insulin-like growth factor-I during embryo culture and treatment of recipients with gonadotropin-releasing hormone to increase pregnancy rates following the transfer of in vitro-produced embryos to heat-stressed, lactating cows." J Anim Sci **81**(6): 1590-1602.

http://jas.fass.org/cgi/content/abstract/81/6/1590

An experiment was conducted to determine whether pregnancy rates following the transfer of in vitro-produced embryos to heat-stressed cows could be improved by 1) culturing embryos in the presence of IGF-I and 2) treating recipients with GnRH. Lactating Holstein cows (n = 260) were synchronized using a timed ovulation protocol. Embryos were produced in vitro and cultured with or without 100 ng/mL of IGF-I. On d 7 after anticipated ovulation (d 0), a single embryo was transferred to all recipients with a palpable corpus luteum (n = 210). A subset of recipients (n = 164) was injected with either GnRH or placebo on d 11. Plasma progesterone concentrations on d 0 and 7 were used to determine the synchrony of recipients. Pregnancy was diagnosed at d 53 and 81 by rectal palpation. Among all recipients, transfer of IGF-I-treated embryos increased pregnancy rate at d 53 ($P < 0.05$) and tended to increase pregnancy rate at d 81 ($P < 0.06$). Calving rate also tended to be higher for recipients that received IGF-I-treated embryos ($P < 0.07$). Among the subset of synchronized recipients (n = 190), pregnancy rate at d 53 and d 81 and calving rate were higher ($P < 0.05$) for IGF-I-treated embryos. The GnRH tended to increase pregnancy rate at d 53 for all recipients ($P < 0.08$) and the subset of synchronized recipients ($P < 0.10$). There were no effects of GnRH ($P > 0.10$) for pregnancy rate at d 81 and calving rate. The overall proportion of male calves was 64.3%. There was no effect ($P > 0.10$) of embryo treatment or GnRH on the birth weight or sex ratio of calves. Results of this experiment indicate that treatment of embryos with IGF-I can improve pregnancy and calving rates following transfer of in vitro-produced embryos. Further research is necessary to determine whether the treatment of recipients with GnRH is a practical approach to increase pregnancy rates following in vitro embryo transfer.

Chowdhury, E. H., H. Kuribara, et al. (2003). "Detection of corn intrinsic and recombinant DNA fragments and Cry1Ab protein in the gastrointestinal contents of pigs fed genetically modified corn Bt11." J Anim Sci **81**(10): 2546-2551.

http://jas.fass.org/cgi/content/abstract/81/10/2546

Genetically modified corn has been approved as an animal feed in several countries, but information about the fate of genetically modified DNA and protein in vivo is insufficient. Genetically modified corn Bt11 is developed by inserting a recombinant DNA sequence encoding insecticidal Cry1Ab protein from Bacillus thuringiensis subsp. kurstaki. We examined the presence of corn intrinsic and recombinant cry1Ab gene by PCR, and the Cry1Ab protein by immunological tests in the gastrointestinal contents of five genetically modified corn Bt11-fed and five nongenetically modified corn-fed pigs. Fragments of corn zein (242 bp), invertase (226 bp) and of ribulose-1,5-bisphosphate carboxylase/oxygenase genes (1,028 bp) were detected in the gastrointestinal contents of both Bt11 and nongenetically modified corn-fed pigs. Fragments of recombinant cry1Ab gene (110 bp and 437 bp) were detected in the gastrointestinal contents of the Bt11-fed pigs but not in the control pigs. Neither corn intrinsic nor cry1Ab gene fragments were detected in the peripheral blood by PCR. The gastrointestinal contents were positive for Cry1Ab protein by ELISA, immunochromatography, and immunoblot; however, these methods did not work for blood and precluded conclusions about any potential absorption of the protein. These results suggest that ingested corn DNA and Cry1Ab protein were not totally degraded in the gastrointestinal tract, as shown by their presence in a form detectable by PCR or immunological tests.

Ciobanu, D. C., J. W. M. Bastiaansen, et al. (2004). "New alleles in calpastatin gene are associated with meat quality traits in pigs." J Anim Sci **82**(10): 2829-2839.

http://jas.fass.org/cgi/content/abstract/82/10/2829

Suggestive QTL affecting raw firmness scores and average Instron force, tenderness, juiciness, and chewiness on cooked meat were mapped to pig chromosome 2 using a three-generation intercross between Berkshire and Yorkshire pigs. Based on its function and location, the calpastatin (CAST) gene was considered to be a good candidate for the observed effects. Several missense and silent mutations were identified in CAST and haplotypes covering most of the coding region were constructed and used for association analyses with meat quality traits. Results demonstrated that one CAST haplotype was significantly associated with lower Instron force and cooking loss and higher juiciness and, therefore, this haplotype is associated with higher eating quality. Some of the sequence variation identified may be associated with differences in phosphorylation of CAST by adenosine cyclic 3', 5'-monophosphate-dependent protein kinase and may in turn explain the meat quality phenotypic differences. The beneficial haplotype was present in all the commercial breeds tested and may provide significant improvements for the pig industry and consumers because it can be used in marker-assisted selection to produce naturally tender and juicy pork without additional processing steps.

Flint, A. F., P. L. Chapman, et al. (2003). "Fertility assessment through heterospermic insemination of flow-sorted sperm in cattle." J Anim Sci **81**(7): 1814-1822.

http://jas.fass.org/cgi/content/abstract/81/7/1814

The ability to assess fertility of bovine sperm accurately and rapidly would be very useful for research and applications to the cattle industry. Sperm motility and other in vitro tests of sperm normality are only partially correlated with fertility, and lengthy breeding trials are expensive and time consuming. Heterospermic insemination by mixing sperm from more than one male provides an in vivo method to assess relative fertility among bulls that can be economical and rapid. Sperm that had been flow-sorted and cryopreserved from four groups of four bulls were inseminated in all combinations of three bulls within groups into nonsuperovulated heifers or superovulated heifers. Embryos were collected nonsurgically between d 13.5 and 20 following estrus and evaluated for paternity by genotyping. Following determination of paternity, a heterospermic index was created for each bull using a maximum likelihood function. These indices ranged from 0.22 {+/-} 0.15 to 2.43 {+/-} 0.43 (mean = 1.00, with a higher value indicative of greater fertility). In all four groups, either the high- or low-fertility bull was identified ($P < 0.05$) using a total of 25 to 36 genotypable embryos from nonsuperovulated heifers. The heterospermic rankings of bulls were similar for single and superovulated heifers for one group of bulls, but dissimilar for a second group. Heterospermic insemination followed by genotyping of embryos proved to be efficacious for rapidly ranking fertility of flow-sorted sperm from bulls when females were not superovulated, but results were less clear when females were superovulated.

Muroya, S., I. Nakajima, et al. (2003). "Amino acid sequences of multiple fast and slow troponin T isoforms expressed in adult bovine skeletal muscles." J Anim Sci **81**(5): 1185-1192.

http://jas.fass.org/cgi/content/abstract/81/5/1185

Multiple nucleotide sequences of complementary DNA (cDNA) of bovine troponin T (TnT)

isoforms expressed in the adult skeletal muscles were determined to facilitate the elucidation of the TnT degradation progress during postmortem aging of muscles. Fresh muscle samples were excised from the lingual, masseter, pectoralis, diaphragm, psoas major, longissimus thoracis, spinnalis, semitendinosus, semimembranosus, and biceps femoris muscles of three Holstein cows within 1 h of slaughter. Complementary DNA fragments of fast and slow TnT isoforms expressed in each muscle were amplified by reverse-transcribed PCR. Consequently, four major fragments of fast TnT and two fragments of slow TnT, all of which contained the complete coding region, were obtained. The sequence determination of these fragments revealed that at least eight and two isoforms were generated by the alternative splicing from bovine fast and slow TnT messenger RNA, respectively. In the fast TnT isoforms, five small variable exons were observed; three of these five exons were in the amino (N)-terminal region. The calculated molecular weight of fast and slow TnT isoforms ranged from 29,816 to 32,125 and from 30,166 to 31,284, respectively. The deduced amino acid sequences revealed that the N-terminal region of all the TnT isoforms was extremely glutamic acid-rich. Reverse-transcribed PCR analysis revealed that expression of each of these isoforms was distributed in a fast or slow muscle-specific manner. Given that TnT degradation has been reported to accompany a decrease in glutamic acid content in the conventional 30-kDa degradation product, the sequence data suggested that the 30-kDa fragment seem to be generated by the proteolytic removal of the glutamic acid-rich N-terminal ends. The multiplicity of TnT isoforms may result in a complicated pattern of TnT degradation on SDS-PAGE gel during beef aging.

Neil, J. E., S. E. Vleck, et al. (2002). "Rapid communication: physical and linkage mapping of the porcine calcitonin (CALC) gene." J Anim Sci **80**(6): 1700-1701.

http://jas.fass.org

Page, B. T., E. Casas, et al. (2002). "Evaluation of single-nucleotide polymorphisms in CAPN1 for association with meat tenderness in cattle." J Anim Sci **80**(12): 3077-3085.

http://jas.fass.org/cgi/content/abstract/80/12/3077

Micromolar calcium activated neutral protease (CAPN1) was evaluated as a candidate gene for a quantitative trait locus (QTL) on BTA29 affecting meat tenderness by characterization of nucleotide sequence variation in the gene. Single-nucleotide polymorphisms (SNP) were identified by sequencing all 22 exons and 19 of the 21 introns in two sires (Piedmontese x Angus located at the U.S. Meat Animal Research Center in Clay Center, NE; Jersey x Limousin located at AgResearch in New Zealand) of independent resource populations previously shown to be segregating meat tenderness QTL on BTA29. The majority of the 38 SNP were found in introns or were synonymous substitutions in the coding regions, with two exceptions. Exons 14 and 9 contained SNP that were predicted to alter the protein sequence by the substitution of isoleucine for valine in Domain III of the protein, and alanine for glycine in Domain II of the protein. The resource populations were genotyped for these two SNP in addition to six intronic polymorphisms and two silent substitutions. Analysis of genotypes and shear force values in both populations revealed a difference between paternal CAPN1 alleles in which the allele encoding isoleucine at position 530 and glycine at position 316 associated with decreased meat tenderness (increased shear force values) relative to the allele encoding valine at position 530 and alanine at position 316 ($P < 0.05$). The association of maternal alleles with meat tenderness phenotypes is consistent with the hypothesis of CAPN1 as the gene underlying the QTL effect in two independent resource populations and presents the possibility of using these markers for selective breeding to reduce the numbers of animals with unfavorable meat tenderness traits.

Rexroad, C. E., 3rd, R. L. Coleman, et al. (2002). "Rapid communication: Thirty-eight polymorphic microsatellite markers for mapping in rainbow trout." J Anim Sci **80**(2): 541-542.

http://jas.fass.org

J Biol Rhythms (4)

Dey, J., A. J. F. Carr, et al. (2005). "The tau Mutation in the Syrian Hamster Differentially Reprograms the Circadian Clock in the SCN and Peripheral Tissues." J Biol Rhythms **20**(2): 99-110.

http://jbr.sagepub.com/cgi/content/abstract/20/2/99

The hypothalamic suprachiasmatic nuclei (SCN), the principal circadian oscillator in mammals, are synchronized to the solar day by the light-dark cycle, and in turn, they coordinate circadian oscillations in peripheral tissues. The tau mutation in the Syrian hamster is caused by a point mutation leading to a deficiency in the ability of Casein Kinase 1{epsilon} to phosphorylate its targets, including circadian PER proteins. How this accelerates circadian period in neural tissues is not known, nor is its impact on peripheral circadian oscillators established. We show that this mutation has no effect on per mRNA expression nor the nuclear accumulation of PER proteins in the SCN. It does, however, accelerate the clearance of PER proteins from the nucleus to an extent sufficient to explain the shortened circadian period of behavioral rhythms. The mutation also has novel, unanticipated consequences for circadian timing in the periphery, including tissue-specific phase advances and/or reduced amplitude of circadian gene expression. The results suggest that the tau mutation accelerates a specific phase, during mid-late subjective night of the SCN circadian feedback loop, rather than cause a global compression of the entire cycle. This reprogrammed output from the clock is associated with peripheral desynchrony, which in turn could account for impaired growth and metabolic efficiency of the mutant.

Fu, Z., M. Inaba, et al. (2002). "Molecular Cloning and Circadian Regulation of Cryptochrome Genes in Japanese Quail (Coturnix coturnix japonica)." J Biol Rhythms **17**(1): 14-27.

http://jbr.sagepub.com/cgi/content/abstract/17/1/14

The circadian system is thought to have three components: input, pacemaker (internal clock), and output. Cryptochromes (Cry) are important clock genes, and recent findings indicate that these genes not only act as circadian photoreceptors but are also essential components in the negative feedback of the circadian system. As a first step toward understanding the avian circadian system, the authors tried to clone Japanese quail homologs of mammalian Crys and analyze their expression patterns in different circumstances. Partial cDNAs of qCry1 and qCry2, which are homologs of mammalian Cry1 and Cry2, respectively, were obtained and their gene expressions were analyzed. Both qCry1 and qCry2 mRNAs were present in all the tissues examined. The oscillation patterns of the qCry1 transcripts were tissue specific and generally showed robust changes between daytime and nighttime; except for lung and testis tissues (which showed no detectable changes between daytime and nighttime), daytime levels were higher in all of the tissues examined. This rapid oscillation in qCry1 persisted through constant darkness or constant illumination, indicating that an endogenous clock controls these changes. In contrast, the expression of qCry2 did not oscillate in any tissue examined. In addition, in tissues of the pineal

gland and eye, unexpected light exposure in the dark period was able to block the decrease in qCry1 transcripts or induce its expression. These findings, in conjunction with the established roles of CRYs in other species, led the authors to propose that in the circadian system, qCRYs may play important roles similar to the known roles of CRYs of other species, such as acting as circadian photoreceptors and as components of the circadian system.

Kolker, D. E., H. Fukuyama, et al. (2003). "Aging Alters Circadian and Light-Induced Expression of Clock Genes in Golden Hamsters." J Biol Rhythms **18**(2): 159-169.

http://jbr.sagepub.com/cgi/content/abstract/18/2/159

Aging alters numerous aspects of circadian biology, including the amplitude of rhythms generated by the suprachiasmatic nuclei (SCN) of the hypothalamus, the site of the central circadian pacemaker in mammals, and the response of the pacemaker to environmental stimuli such as light. Although previous studies have described molecular correlates of these behavioral changes, to date only 1 study in rats has attempted to determine if there are age-related changes in the expression of genes that comprise the circadian clock itself. We used in situ hybridization to examine the effects of age on the circadian pattern of expression of a subset of the genes that comprise the molecular machinery of the circadian clock in golden hamsters. Here we report that age alters the 24-h expression profile of Clock and its binding partner Bmal1 in the hamster SCN. There is no effect of age on the 24-h profile of either Per1 or Per2 when hamsters are housed in constant darkness. We also found that light pulses, which induce smaller phase shifts in old animals than in young, lead to decreased induction of Per1, but not of Per2, in the SCN of old hamsters.

Lambert, C. M., K. K. Machida, et al. (2005). "Analysis of the Prokineticin 2 System in a Diurnal Rodent, the Unstriped Nile Grass Rat (Arvicanthis niloticus)." J Biol Rhythms **20**(3): 206-218.

http://jbr.sagepub.com/cgi/content/abstract/20/3/206

Prokineticin 2 (PK2) is a putative output molecule from the SCN. PK2 RNA levels are rhythmic in the mouse SCN, with high levels during the day, and PK2 administration suppresses nocturnal locomotor activity in rats. The authors examined the PK2 system in a diurnal rodent, Arvicanthis niloticus, to determine whether PK2 or PK2 receptors differ between diurnal and nocturnal species. The major transcript variant of A. niloticus PK2 (AnPK2) encodes a 26-residue signal peptide followed by the presumed mature peptide of 81 residues. Within the grass rat signal sequence, polymorphic sequences and amino acid substitutions were observed relative to mouse and laboratory rats, but the hydrophobic core and cleavage site of the signal sequence were preserved. The mature PK2 peptide is identical among A. niloticus, rat, and mouse. AnPK2 mRNA is rhythmically expressed in the SCN, with peak RNAlevels occurring in the morning, preceding peaks of Per1 and Per2 as in mouse SCN. Analysis of prokineticin receptor 2 (PKR2) sequences revealed polymorphisms among the grass rats studied. PKR2 mRNAwas expressed in the SCN and paraventricular nuclei of the thalamus and hypothalamus. While further analysis is necessary, there is no clear evidence indicating that a difference in the PK2 ligand/receptor system accounts for diurnality in this rodent species. These data contribute to a growing body of evidence suggesting that the key to diurnality lies downstream of the SCN in A. niloticus.

Boutet, P., D. Boulanger, et al. (2004). "Delayed Neutrophil Apoptosis in Bovine Subclinical Mastitis." J Dairy Sci **87**(12): 4104-4114.

http://jds.fass.org/cgi/content/abstract/87/12/4104

Bovine subclinical mastitis can be defined as a moderated inflammatory disease characterized by a persistent accumulation of neutrophils in milk. As GMCSF-mediated delay of neutrophil apoptosis contributes to the accumulation of inflammatory cells at the site of inflammation in many human diseases, we sought to determine whether subclinical mastitis in cows is also associated with a GMCSF-dependent increase in milk-neutrophil survival. We first addressed the hypothesis that GMCSF delays bovine neutrophil apoptosis by activation of the signal transducer and activator of transcription (STAT) family members STAT3 and STAT5, which are critical regulators of the expression of various Bcl-2 family proteins. Granulocyte-macrophage colony-stimulating factor significantly delayed apoptosis of blood neutrophils obtained from healthy cows. In these cells, GMCSF activated STAT5, but not STAT3, and induced an increase in the mRNA of the antiapoptotic Bcl-2 member, Bcl-xL. Granulocyte-macrophage colony-stimulating factor-dependent STAT5 activation and up-regulation of Bcl-xL mRNA were blocked by the Jak inhibitor, AG-490. This inhibition was associated with abrogation of the prosurvival effect of GMCSF, demonstrating a key role for STAT5 in delayed neutrophil apoptosis. We further found that GMCSF expression was increased in milk cells from cows affected with subclinical mastitis. Neutrophils from these cows demonstrated a significant delay of apoptosis as compared with neutrophils obtained from healthy cows and were unresponsive to GMCSF. Active STAT5 complexes were detected in these neutrophils. Finally, in the presence of AG-490, apoptosis was induced and a time-dependent down-regulation of Bcl-xL mRNA was observed in milk neutrophils from mastitis-affected cows. These results indicate that neutrophil survival is enhanced in milk of subclinical mastitis-affected cows and suggest a role for a GMCSF-activated STAT5 signaling pathway in this phenomenon. This pathway could thus represent a target for the control of persistent accumulation of neutrophils in the bovine mammary gland.

Koike, S., J. Pan, et al. (2003). "Kinetics of In Sacco Fiber-Attachment of Representative Ruminal Cellulolytic Bacteria Monitored by Competitive PCR." J Dairy Sci **86**(4): 1429-1435.

http://jds.fass.org/cgi/content/abstract/86/4/1429

Stems of orchardgrass hay in nylon bags were incubated in the rumens of three ruminally fistulated sheep to monitor the rate and extent of fiber attachment by the representative ruminal cellulolytic bacteria via competitive polymerase chain reaction. After incubation for 5 min, the numbers of Fibrobacter succinogenes and the two ruminococcal species attached to stems were 105 and 104/g dry matter (DM) of stem, respectively. At 10 min, the numbers of all three species attached to stems increased 10-fold. Thereafter, attached cell numbers of the three species gradually increased and peaked at 24 h (109/g DM for F. succinogenes and 107/g DM for Ruminococcus flavefaciens) or 48 h (106/g DM for Ruminococcus albus). On the other hand, cell numbers of all three species in the whole digesta were constant over 24 h. Changes in the rate of in sacco neutral detergent fiber disappearance of hay stem, which showed a linear increase up to 96 h, were not synchronized with changes in cellulolytic bacterial mass. These results suggest that sufficient numbers of cells of the three cellulolytic species to move to new plant fragments are present at the start of incubation, the initial attachment to new plant matter is mostly accomplished within 10 min and then bacterial growth and fibrolytic action follow. F. succinogenes was most dominant, both in the whole rumen digesta and on the suspended hay stems, demonstrating the ecological and functional significance of this species in ruminal fiber

digestion.

Maudet, C. and P. Taberlet (2002). "Holstein's Milk Detection in Cheeses Inferred from Melanocortin Receptor 1 (MC1R) Gene Polymorphism." J Dairy Sci **85**(4): 707-715.

http://jds.fass.org/cgi/content/abstract/85/4/707

For some French Registered Designation of Origin (RDO) cheeses Prim'Holstein's milk is not allowed for cheese making (e.g., Reblochon, Abondance, and Beaufort cheeses). To find molecular markers for Prim'Holstein's milk detection in RDO cheese, four genes affecting coat color in cattle (c-kit, MGH, TYRP1, and MC1R) have been sequenced for three mountain breeds and the Prim'Holstein breed. Only the MC1R gene (E-locus) has shown variation between the four breeds. Among the 25 French and Italian breeds sequenced for the MC1R gene, only the Vosgienne breed has presented the same allele as the black Prim'-Holstein breed (ED). A quick and easy DNA-based method to detect Holstein's milk in RDO cheese is proposed based on ED allele detection. A DNA extraction from cheese, a preamplification of the gene and a competitive oligonuleotide priming PCR on MC1R mutations were performed. Using an automated sequencer, differences in fluorescence and fragment size reveal the allele type. This simple approach provides good reproducibility and is shown to be relatively sensitive, with a detection limit of about 1% of Holstein's milk in milk curd.

McKenna, S. L. B., G. P. Keefe, et al. (2004). "Cow-Level Prevalence of Paratuberculosis in Culled Dairy Cows in Atlantic Canada and Maine." J Dairy Sci **87**(11): 3770-3777.

http://jds.fass.org/cgi/content/abstract/87/11/3770

The prevalence of Mycobacterium avium ssp. paratuberculosis (Mptb) in culled dairy cattle in Eastern Canada and Maine was determined to be 16.1% (95% confidence interval 13.8 to 18.3%) based on a systematic random sample of abattoir cattle. Mesenteric lymph nodes and ileum from 984 cows were examined by histologic and bacteriologic methods. Histological testing was far less sensitive than bacteriologic methods for detecting infected cattle. A seasonal pattern of positive cows was also detected, with the highest proportion of cows being Mptb-positive in June (42.5%). Overall, body condition score was not associated with prevalence of Mptb isolation.

Yahyaoui, M. H., A. Angiolillo, et al. (2003). "Characterization and Genotyping of the Caprine {kappa}-Casein Variants." J Dairy Sci **86**(8): 2715-2720.

http://jds.fass.org/cgi/content/abstract/86/8/2715

{kappa} -Casein ({kappa}-CN) is the milk protein that determines the size and specific function of milk micelles, and its cleavage by chymosin is responsible for milk coagulation. We have previously detected and characterized four variants of the goat{kappa} -CN in Spanish, French, and Italian breeds by screening the major part of the coding region in exon 4. Here we have sequenced and analyzed the full coding region of the{kappa} -CN gene which includes exons 3 and 4. No additional mutations were found, with exception of a single nucleotide substitution in exon 3, which had no amino acid change. However, the analysis of the association between the different mutations resulted in two new variants designated{kappa} -CN F and G. The novel variants are present in the Italian breeds Teramana, Girgentana, and Sarda (variant F). A protocol for rapid simultaneous genotyping of all known{kappa} -CN variants using the primer

extension method was described, and a total of 210 animals from nine European breeds were genotyped. Alleles A and B are the most frequent variants occuring in the majority of breeds with highest prevalence of the B variant, except for the Canaria breed where the A allele is more frequent. Sequence data suggest that the F variant is the original type of caprine{kappa} -CN, other alleles being derived from this type following two different trunks by successive mutations.

J Forensic Sci (2)

Krenke, B. E., A. Tereba, et al. (2002). "Validation of a 16-locus fluorescent multiplex system." J Forensic Sci **47**(4): 773-85.

STR multiplexes have been indispensable for the efficient genotyping of forensic samples. The PowerPlex 16 System contains the coreCODIS loci, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D21S11, CSF1PO, FGA, THOI, TPOX, vWA, the sex determinant locus, amelogenin, and two pentanucleotide STR loci, Penta D and Penta E. This multiplex satisfies the locus requirements for most national databases and is the most efficient currently available system due to its single PCR amplification. To provide the groundwork for judicial acceptance, including the publication of primer sequences, and to evaluate laboratory-to-laboratory variation, a developmental validation for casework on this commercially available system was performed in 24 laboratories and produced the following conclusions. Amplification was reliable on a variety of thermal cyclers and product could be analyzed on either an ABI PRISM 310 Genetic Analyzer or an ABI PRISM 377 DNA Sequencer. Genotyping using single source samples was consistent between 0.25 and 2 ng of input DNA template with a few laboratories obtaining complete genotypes at 0.0625 ng. However, heterozygote allele imbalance (<60% peak height balance) caused by stochastic effects was observed at a rate of 13% with 0.125 ng DNA and 22% at 0.0625 ng DNA. Mixture analyses were done using a total of 1 ng of DNA template. Most alleles were detected in mixtures of 4 to 1 and some minor alleles were detected in mixtures of 19 to 1. Optimum amplification cycle number was dependent on the sensitivity of the detection instrument used and could also be adjusted to accommodate larger amounts of DNA on solid supports such as FTA paper. Reaction conditions including volume, annealing temperature, and concentrations of primer, AmpliTaq Gold, and magnesium were shown to be optimal yet robust enough to withstand moderate variations without affecting genotype analysis. Environmental, matrix and standard source analyses revealed an ability to obtain complete genotypes in all sample types except those exposed to 80 degrees C for 12-48 days. Finally, comparison of genotype results from the PowerPlex 16 System with other commercially available systems on non-probative reference and forensic samples showed consistent results.

Nelson, M. S., E. N. Levedakou, et al. (2002). "Detection of a primer-binding site polymorphism for the STR locus D16S539 using the Powerplex 1.1 system and validation of a degenerate primer to correct for the polymorphism." J Forensic Sci **47**(2): 345-9.

Quality assurance samples submitted from the NCSBI as part of a contract with TBTG to

outsource DNA Database samples showed unexpected discrepancies for the locus D16S539 when all other loci yielded identical results. Discrepancies observed included allele drop out and an imbalance in sister alleles with samples returned from TBTG. This led to a comprehensive review of the technical procedures used between the two laboratories to determine the cause of the discrepancies noted for the locus D16S539, since both laboratories were using the PowerPlex 1.1 typing kit from the Promega Corporation. The NCSBI and the TBTG utilize different extraction methods (organic extraction vs. FTA) and amplification conditions (AmpliTaq vs AmpliTaq Gold), respectively, so the exact cause of discrepancy observed was not immediately apparent. Experiments at the NCSBI associated the observed allele drop out and the imbalance of the sister alleles with the use of AmpliTaq Gold and a hot start procedure. Sequencing data revealed that a point mutation resides on the D16S539 primer-binding site that reaches polymorphic levels in African-American populations. This led to the development of a degenerate primer by the Promega Corporation to detect "missing" alleles when AmpliTaq Gold is used. The degenerate primer was then thoroughly tested to show its efficacy in detecting the "true" D16S539 profile when used.

J Med Virol (1)

Castle, P. E., M. Schiffman, et al. (2002). "Comparisons of HPV DNA detection by MY09/11 PCR methods." J Med Virol **68**(3): 417-23.

Two modifications to the original L1 consensus primer human papillomavirus (HPV) PCR method, MY09-MY011, using AmpliTaq DNA polymerase (MY-Taq), were evaluated for HPV DNA detection on clinical specimens from a cohort study of cervical cancer in Costa Rica. First, HPV DNA testing of 2978 clinical specimens by MY09-MY011 primer set, using AmpliTaq Gold DNA polymerase (MY-Gold) were compared with MY-Taq testing. There was 86.8% total agreement (kappa = 0.72, 95%CI = 0.70-75) and 69.6% agreement among positives between MY-Gold and MY-Taq. MY-Gold detected 38% more HPV infections (P < 0.0001) and 45% more cancer-associated (high-risk) HPV types (P < 0.0001) than MY-Taq, including 12 of the 13 high-risk HPV types. Analyses of discordant results using cytologic diagnoses and detection of HPV DNA by the Hybrid Capture 2 Test suggested that MY-Gold preferentially detected DNA positive specimens with lower HPV viral loads compared with MY-Taq. In a separate analysis, PGMY09-PGMY11 (PGMY-Gold), a redesigned MY09/11 primer set, was compared with MY-Gold for HPV DNA detection (n = 439). There was very good agreement between the two methods (kappa = 0.83; 95%CI = 0.77-0.88) and surprisingly no significant differences in HPV detection (P = 0.41). In conclusion, we found MY-Gold to be a more sensitive assay for the detection of HPV DNA than MY-Taq. Our data also suggest that studies reporting HPV DNA detection by PCR need to report the type of polymerase used, as well as other assay specifics, and underscore the need for worldwide standards of testing.

J Soc Biol (1)

Kloosterman, A. D. and P. Kersbergen (2003). "Efficacy and limits of genotyping low copy number (LCN) DNA samples by multiplex PCR of STR loci." J Soc Biol **197**(4): 351-9.

In this study, we have evaluated the efficacy and the validity of the AmpFISTR SGM plus multiplex PCR typing system when Low Copy Number (LCN) amounts of DNA are processed. The characteristics of SGM plus profiles produced under LCN conditions were studied on the basis of heterozygote balance, between loci balance and stutter proportion based on profiles that were obtained from a variety of mock casework samples. These experiments clearly showed that LCN DNA profiles carry their own characteristic features, which must be taken into account during interpretation. Herewith, we confirmed the data of recent other studies that a comprehensive interpretation strategy is dependent upon multiple replication of the PCR using the same extract together with the proper use of extraction and amplification controls. The limitations of LCN DNA analysis were further studied in a series of single cell PCR experiments using an amplification regime of 34 PCR cycles. The allele dropout phenomenon was demonstrated to its full extent when single cells were analysed. However, the "consensus profile" which was obtained from separate single cell PCR experiments matched the actual profile of the cell donor. Single cell PCR experiments also showed that a further increase of the number of PCR cycles did not result in enhanced sensitivity and had a highly negative effect on the balance of this multiplex PCR system which hampered correct interpretation of the profile. Also, the potential of LCN typing in analysing mixtures of DNA was investigated. It was clearly shown that LCN typing had no advantages over 28 cycles amplification in the detection of the minor component of DNA-mixtures. In addition to the 34 cycles PCR amplification regime, the utility of a new approach that involved reamplification of the 28 cycle SGM plus PCR products with an extra 6 PCR cycles after the addition of fresh AmpliTaq Gold DNA Polymerase was investigated. This approach provides the scientist with an extra typing result that enhances the reliability of the consensus profile, which is commonly retrieved from two separate 34 cycle PCR results. Furthermore, the 28 + 6 cycles approach may be used to screen LCN samples for their potential to produce a 34 PCR cycle profile. Finally and as a last resort the 28 + 6 cycles approach can be used in those cases where no further extract from the crime sample is available. Finally, the potential of LCN typing was demonstrated in typing samples from non-probative and actual casework examples. From a high proportion of samples that failed to demonstrate SGM plus typing results using the standard protocol of 28 cycles, at least partial profiles could be obtained after LCN methods were used. For example, LCN typing was applied in a case where 10-year old samples from bones and teeth that were retrieved from a mass grave had to be identified. This study resulted in the positive identification of a number of victims by comparing the LCN DNA profiles with the profiles from putative relatives. The value of LCN DNA typing was further demonstrated in a strangulation case. The throat of the victim was sampled and only after 34 PCR cycles were we able to reveal that the evidential sample contained a distinct mixture of the victim's own DNA and the DNA of the defendant.

J. Antimicrob. Chemother. (13)

Aubouy, A., S. Jafari, et al. (2003). "DHFR and DHPS genotypes of Plasmodium falciparum isolates from Gabon correlate with in vitro activity of pyrimethamine and cycloguanil, but not with sulfadoxine-pyrimethamine treatment efficacy." J. Antimicrob. Chemother. **52**(1): 43-49.

http://jac.oupjournals.org/cgi/content/abstract/52/1/43

Objectives: To assess the relationship between the presence of DHFR and DHPS mutations in Plasmodium falciparum, parasite in vitro resistance, and in vivo efficacy of sulfadoxine-pyrimethamine (SP) treatment. Patients and methods: Measurement of SP treatment efficacy in malaria-infected children in Gabon was combined with in vitro tests of susceptibility to pyrimethamine and cycloguanil, and molecular genotyping at several DHFR and DHPS loci of parasites isolated before treatment. DHFR was studied at codons 108, 51, and 59, whereas DHPS gene was typed at positions 436, 437, 540 and 581. Results: SP treatment was effective in 86% of children by day 28. Seventy-five percent of isolates were in vitro resistant to pyrimethamine and 65.5% to cycloguanil. No mutation was detected at codons 540 and 581 of the DHPS gene. Most isolates (71.8%) presented with the triple mutant DHFR genotype, whereas 64.3% combined at least three DHFR and one DHPS mutations. The increase in the number of DHFR mutations was associated with an increase in in vitro resistance to pyrimethamine and cycloguanil; three DHFR mutations conferred pyrimethamine and to a lesser extent cycloguanil resistance. Treatment failures only occurred with isolates presenting at least two DHFR mutations (S108N and C59R) and one DHPS mutation (S436A or A437G), but SP treatment of infections with such parasites gave treatment success in 82.0% of children. Conclusions: DHFR mutations that lead to high-level in vitro resistance to pyrimethamine plus 1-2 DHPS mutations are not sufficient to induce in vivo failure of SP treatment in young children from Gabon.

Boyd, D. A., P. Kibsey, et al. (2004). "Enterococcus faecium N03-0072 carries a new VanD-type vancomycin resistance determinant: characterization of the VanD5 operon." J. Antimicrob. Chemother. **54**(3): 680-683.

http://jac.oupjournals.org/cgi/content/abstract/54/3/680

Objectives: To genotypically characterize the vancomycin resistance mechanism of Enterococcus faecium N03-0072, which was negative by PCR for the currently known van genotypes. Methods: PCR was used to amplify the entire vancomycin resistance operon and the complete nucleotide sequence was determined by dideoxy cycle sequencing. Results: Analysis revealed a VanD-type operon with 94% nucleotide identity to the VanD4 operon and 90% nucleotide identity to the VanD1/D3 operons. A set of universal primers was designed in order to identify all current vanD variants by PCR. Conclusions: E. faecium N03-0072 carries a new VanD-type operon, designated VanD5.

Fernandes, C. J., L. A. Fernandes, et al. (2005). "Cefoxitin resistance as a surrogate marker for the detection of methicillin-resistant Staphylococcus aureus." J. Antimicrob. Chemother. **55**(4): 506-510.

http://jac.oupjournals.org/cgi/content/abstract/55/4/506

Objectives: To evaluate the usefulness of cefoxitin when used as a surrogate marker for the detection of methicillin resistance. Patients and methods: Eight hundred and seventy-one strains of Staphylococcus aureus, collected from eight tertiary referral centres serving diverse socio-economic populations, were included in the study using NCCLS disc diffusion and the agar dilution methods. Results: Using cefoxitin and NCCLS criteria for disc diffusion, the sensitivity and specificity for recognizing methicillin resistance were both 100%. Similar results were obtained when the strains were tested by the agar dilution method. The cefoxitin MICs for methicillin-susceptible strains were [&le] 4 mg/L. Conclusions: Testing with cefoxitin as a surrogate marker for the detection of methicillin resistance was very accurate with both disc diffusion and agar dilution methods. Such testing clearly distinguished methicillin-resistant strains of S. aureus from methicillin-susceptible strains.

Hayes, J. R., D. D. Wagner, et al. (2005). "Distribution of streptogramin resistance determinants among Enterococcus faecium from a poultry production environment of the USA." J. Antimicrob. Chemother. **55**(1): 123-126.

http://jac.oupjournals.org/cgi/content/abstract/55/1/123

Objectives: The impact of agricultural use of antimicrobials on the present and future efficacy of therapeutic drugs in human medicine is a growing public concern. Quinupristin/dalfopristin has been approved to treat human disease caused by vancomycin-resistant Enterococcus faecium and is related to virginiamycin, a streptogramin complex that has long been used in USA agriculture poultry production. Methods: Streptogramin-resistant isolates of E. faecium from poultry production environments on the eastern seaboard were recovered without selection for streptogramin resistance and examined using ribotyping to evaluate clonal bias. Colony PCR screening for the previously described streptogramin resistance determinants erm(A), erm(B), msr(C), vgb(A), vat(D) and vat(E) was performed to determine the prevalence of streptogramin resistance mechanisms from these environments. Results: The collection of E. faecium isolates was unevenly distributed among 28 ribogroups and did not cluster geographically. The most prevalent ribogroups was composed of isolates that possessed diverse antimicrobial resistance profiles. Of the 127 isolates examined, 63% were resistant to quinupristin/dalfopristin. The resistance determinants erm(A) and erm(B) were observed among 6% and 10%, respectively, of streptogramin-resistant isolates. msr(C) was detected in a single isolate that was resistant to macrolide and lincosamide antimicrobials. The streptogramin B hydrolase vgb(A) and the streptogramin A acetyltransferases genes vat(D) and vat(E) were not detected in any of the E. faecium isolates. Conclusions: These results indicate that there is widespread resistance to streptogramin antimicrobials among E. faecium throughout the poultry production region in this study and that the mechanisms of resistance to streptogramin antimicrobials within this population remain largely uncharacterized.

Ho, P. L., R. C. W. Wong, et al. (2004). "Application of a multiplex pbp2b and pbp2x PCR for prediction of penicillin resistance in Streptococcus pneumoniae." J. Antimicrob. Chemother. **53**(5): 890-891.

http://jac.oupjournals.org

Junker, L. M. and A. G. Hay (2004). "Effects of triclosan incorporation into ABS plastic on biofilm communities." J. Antimicrob. Chemother. **53**(6): 989-996.

http://jac.oupjournals.org/cgi/content/abstract/53/6/989

Objective: This study compared the attached biofilm populations on acrylonitrile-butadiene-styrene (ABS) plastic with and without the incorporation of the antimicrobial triclosan [5-chloro-2-(2,4-dichlorophenoxy) phenol] after 1-3 weeks of exposure to drinking water. Methods: Biofilms were cultivated on triclosan-incorporated (TP) and control plastics (CP) in continuous flow culture reactors with drinking water as the growth medium and inoculum. After 1-3 weeks of exposure, the plastics were removed and the biofilms aseptically harvested. The attached communities were examined with respect to direct cell counts, culturability, triclosan resistance and community composition. Results: Based on these analyses, no significant differences were observed between the populations attached to TP and CP surfaces. Results from both a bioavailability assay and gas chromatography mass spectrometry analyses, revealed that only trace amounts of triclosan desorbed from the plastic. The lack of biofilm community difference, coupled with this

limited desorption of triclosan from the TP indicates that the ABS plastic studied was no more effective at controlling bacterial populations than the control plastic because the antimicrobial was not bioavailable. Conclusions: These results call into question the long-term utility of triclosan incorporation into ABS plastic and highlight the need for proof of efficacy regarding the antimicrobial properties of such materials.

Kerrn, M. B., T. Klemmensen, et al. (2002). "Susceptibility of Danish Escherichia coli strains isolated from urinary tract infections and bacteraemia, and distribution of sul genes conferring sulphonamide resistance." J. Antimicrob. Chemother. **50**(4): 513-516.

http://jac.oupjournals.org/cgi/content/abstract/50/4/513

Antibiotic resistance of urinary tract pathogens has increased worldwide. Our aim was to provide information regarding resistance patterns of Escherichia coli in urinary tract infections (UTIs) and E. coli bacteraemia in Denmark. The overall resistance ranged from: ampicillin 20-47%, mecillinam 0-7%, trimethoprim 10-28%, sulfamethizole 22-47% and nitrofurantoin 0-3%. In strains with sulfamethizole MICs > 2048 mg/L, 97% carried sulI, sulII or both genes, with sulII being the most common. Among the sulI gene-positive strains, 96% were intI 1 gene positive.

Kugelberg, E., S. Lofmark, et al. (2005). "Reduction of the fitness burden of quinolone resistance in Pseudomonas aeruginosa." J. Antimicrob. Chemother. **55**(1): 22-30.

http://jac.oupjournals.org/cgi/content/abstract/55/1/22

Objectives: Quinolone resistance in the opportunistic pathogen Pseudomonas aeruginosa is commonly caused by mutations that alter the target molecules DNA gyrase/topoisomerase IV, or cause activation of various efflux systems. We have analysed the effect of quinolone resistance caused by DNA gyrase/topoisomerase IV mutations on bacterial fitness. Methods: Norfloxacin-resistant mutants were isolated and by DNA sequencing the mutations conferring resistance were identified. Mutant fitness was determined by measuring growth rates in vitro. Mutants with reduced growth rates were serially passaged to obtain growth-compensated mutants. The level of DNA supercoiling was determined by isolating plasmid DNA from the susceptible, resistant and compensated mutants and comparing the topoisomer distribution patterns by gel electrophoresis in the presence of chloroquine. Results: Low-level resistance (4-48 mg/L) was caused by single mutations in gyrA or gyrB. Among these strains, three out of eight mutants showed lower fitness, whereas high-level resistant (>256 mg/L) mutants with double mutations in gyrA and parC, parE, nfxB or unknown genes all showed a reduced fitness. Slow-growing resistant mutants with a gyrA mutation had decreased DNA supercoiling. After serial passage in laboratory medium, mutant fitness was increased by compensatory mutation(s) that restored supercoiling to normal levels. The compensatory mutation(s) was not located in any of the genes (gyrAB, topA, parCE, hupB, fis, hupN, himAD or PA5348) that were expected to affect supercoiling. Conclusions: Our results show that no cost' and compensatory mutations are common in quinolone-resistant P. aeruginosa.

Lee, R. E. B., T. T. Liu, et al. (2005). "Genome-wide expression profiling of the response to ciclopirox olamine in Candida albicans." J. Antimicrob. Chemother. **55**(5): 655-662.

http://jac.oupjournals.org/cgi/content/abstract/55/5/655

Objectives: The aim of this study was to identify changes in the gene expression profile of Candida albicans upon exposure to the hydroxypyridone anti-infective agent ciclopirox olamine in an effort to better understand its mechanism of action. Methods: C. albicans SC5314 was exposed to either medium alone or ciclopirox olamine at a concentration equivalent to the IC50 (0.24 mg/L) for 3 h. RNA was isolated and gene expression profiles were compared using DNA microarrays. Differential expression of select genes was confirmed by real-time reverse transcription (RT)-PCR. Mutants disrupted for CDR2 and both CDR1 and CDR2, as well as a clinical isolate overexpressing CDR1 and CDR2, were examined for changes in susceptibility to ciclopirox olamine. Results: A total of 49 genes were found to be responsive to ciclopirox olamine, including 36 up-regulated genes and 13 down-regulated genes. These included genes involved in small molecule transport (HGT11, HXT5, ENA22, PHO84, CDR4), iron uptake (FRE30, FET34, FTR1, FTR2, SIT1) and cell stress (SOD1, SOD22, CDR1, DDR48). Mutants disrupted for CDR2 and both CDR1 and CDR2, as well as a clinical isolate overexpressing CDR1 and CDR2, showed no change in susceptibility to ciclopirox olamine compared with the respective parent. Conclusions: Consistent with the hypothesis that ciclopirox olamine acts as an iron chelator, it induced changes in expression of many genes involved in iron uptake. Despite induction of the multidrug efflux pump genes CDR1 and, to a lesser extent, CDR2 by ciclopirox olamine, these genes do not affect susceptibility to this agent.

Merlino, J., J. Watson, et al. (2002). "Detection and expression of methicillin/oxacillin resistance in multidrug-resistant and non-multidrug-resistant Staphylococcus aureus in Central Sydney, Australia." J. Antimicrob. Chemother. **49**(5): 793-801.

http://jac.oupjournals.org/cgi/content/abstract/49/5/793

Ninety clinical Staphylococcus aureus isolates from separate patients were examined phenotypically and genotypically for susceptibility to methicillin/oxacillin. Thirty were methicillin/oxacillin susceptible and 60 were methicillin and oxacillin resistant (MRSA). The 60 MRSA isolates examined were subdivided into two groups according to their antibiotic profiles and comprised 30 non-multidrug-resistant (NMDR) isolates, resistant to less than two non-{beta}-lactam antibiotics, and 30 multidrug-resistant (MDR) isolates, resistant to three or more non-{beta}-lactam antibiotics. Phenotypic and genotypic analysis of methicillin/oxacillin showed that despite use of the guidelines published by the NCCLS for the testing of S. aureus susceptibility to methicillin/oxacillin, MIC values of some NMDR MRSA isolates fell below the NCCLS-recommended breakpoints. Etest strips failed to detect two NMDR MRSA isolates tested with oxacillin and four tested with methicillin. Lowering the NCCLS-recommended oxacillin screen agar concentration from 6 to 2 mg/L and temperature of incubation to 30{degrees}C, improved the specificity and sensitivity of NMDR MRSA detection from 87% to 100%. On PFGE analysis these NMDR MRSA strains were genotypically different. Genotypic tests, such as multiplex PCR for the mecA/nuc genes and DNA hybridization for the mecA gene, or phenotypic monoclonal antibody-based tests to detect penicillin-binding protein 2a (PBP2a) offer advantages for problematic isolates in detecting or confirming low-level phenotypic heterogeneous mecA expression of oxacillin and methicillin resistance in NMDR MRSA.

Plante, I., D. Centron, et al. (2003). "An integron cassette encoding erythromycin esterase, ere(A), from Providencia stuartii." J. Antimicrob. Chemother. **51**(4): 787-790.

http://jac.oupjournals.org/cgi/content/abstract/51/4/787

We have mapped the variable region of the two class 1 integrons found in the multiresistant strain Providencia stuartii 1723. Integron 1 contains a new arrangement of gene cassettes, aacA4-aadB-aadA1, conferring resistance to all aminoglycosides used for clinical treatment. Integron 2

contains a variant of the gene cassette ere(A), coding for an erythromycin esterase, whose nucleotide sequence shares 93.7% DNA identity with ere(A) from Escherichia coli BM2195 plasmid pIP1100.

Poulsen, A. B., R. Skov, et al. (2003). "Detection of methicillin resistance in coagulase-negative staphylococci and in staphylococci directly from simulated blood cultures using the EVIGENE MRSA Detection Kit." J. Antimicrob. Chemother. **51**(2): 419-421.

http://jac.oupjournals.org/cgi/content/abstract/51/2/419

The EVIGENE MRSA Detection Kit was evaluated on coagulase-negative staphylococci (CoNS) from agar plates and on staphylococci directly from positive spiked blood cultures. For the CoNS study, a total of 242 isolates were tested, and of these 237 gave valid test results. For the 237 valid tests, all gave correct mecA classification. For the blood culture procedure, a collection of 51 mecA-positive Staphylococcus aureus, 21 mecA-negative S. aureus, 31 mecA-positive CoNS and 28 mecA-negative CoNS were used for the simulated blood cultures. For the S. aureus strains, all gave valid test results and correct mecA classification. One of the MRSA isolates gave a very faint nuc signal, and another four isolates gave results close to the cut-off of the kit; however, these were still clearly positive when read by the naked eye. For the CoNS isolates, 51 of the 59 strains gave valid results. All of these 51 strains gave correct mecA status. Thus the EVIGENE MRSA Detection Kit can provide fast and accurate determination of methicillin resistance in CoNS. This preliminary study of the blood culture procedure indicates that it is possible to achieve determination of methicillin resistance in staphylococci 8 h after positivity of the blood culture, making same-day detection of methicillin resistance possible.

Yan, J.-J., J.-J. Wu, et al. (2004). "Plasmid-mediated 16S rRNA methylases conferring high-level aminoglycoside resistance in Escherichia coli and Klebsiella pneumoniae isolates from two Taiwanese hospitals." J. Antimicrob. Chemother. **54**(6): 1007-1012.

http://jac.oupjournals.org/cgi/content/abstract/54/6/1007

Objectives: This study was conducted to investigate the occurrence of 16S rRNA methylases that confer high-level aminoglycoside resistance in Klebsiella pneumoniae and Escherichia coli isolates from two Taiwanese hospitals and the characteristics of these isolates. Methods: A total of 1624 K. pneumoniae and 2559 E. coli isolates consecutively collected over an 18 month period from a university hospital and seven E. coli and eight K. pneumoniae isolates that were resistant to amikacin from a district hospital were analysed. Two 16S rRNA methylase genes, armA and rmtB, were detected by PCR-based assays. {beta}-Lactamase characteristics were determined by phenotypic and genotypic methods. Results: Overall, 28 armA-positive and seven rmtB-positive isolates were identified, and extended-spectrum {beta}-lactamases (ESBLs) were detected in 33 (94.3%) isolates. The prevalence rates of armA and rmtB at the university hospital were 0.9% (n=15) and 0.3% (n=5) in K. pneumoniae and 0.4% (n=10) and 0.04% (n=1) in E. coli. CTX-M-3, CTX-M-14, SHV-5-like ESBLs, and CMY-2 were detected alone or in combination in 21, 6, 11, and 2, respectively, of the 28 armA-positive isolates. CTX-M-14 was detected in six of the seven rmtB-positive isolates. Fingerprinting of conjugative plasmids revealed the dissemination of closely related plasmids containing both armA and blaCTX-M-3. PFGE suggests that armA and rmtB spread by both horizontal transfer and clonal spread. Conclusions: This is the first report of the emergence of 16S rRNA methylases in Enterobacteriaceae in Taiwan. The spread of the multidrug-resistant isolates producing both ESBLs and 16S rRNA methylases may become a clinical problem.

J. Bacteriol. (47)

Anton, N., M. V. Mendes, et al. (2004). "Identification of PimR as a Positive Regulator of Pimaricin Biosynthesis in Streptomyces natalensis." J. Bacteriol. **186**(9): 2567-2575.

http://jb.asm.org/cgi/content/abstract/186/9/2567

Sequencing of the DNA region on the left fringe of the pimaricin gene cluster revealed the presence of a 3.6-kb gene, pimR, whose deduced product (1,198 amino acid residues) was found to have amino acid sequence homology with bacterial regulatory proteins. Database comparisons revealed that PimR represents the archetype of a new class of regulators, combining a Streptomyces antibiotic regulatory protein (SARP)-like N-terminal section with a C-terminal half homologous to guanylate cyclases and large ATP-binding regulators of the LuxR family. Gene replacement of pimR from Streptomyces natalensis chromosome results in a complete loss of pimaricin production, suggesting that PimR is a positive regulator of pimaricin biosynthesis. Gene expression analysis by reverse transcriptase PCR (RT-PCR) of the pimaricin gene cluster revealed that S. natalensis {Delta}PimR shows no expression at all of the cholesterol oxidase-encoding gene pimE, and very low level transcription of the remaining genes of the cluster except for the mutant pimR gene, thus demonstrating that this regulator activates the transcription of all the genes belonging to the pimaricin gene cluster but not its own transcription.

Barragan, M. J. L., M. Carmona, et al. (2004). "The bzd Gene Cluster, Coding for Anaerobic Benzoate Catabolism, in Azoarcus sp. Strain CIB." J. Bacteriol. **186**(17): 5762-5774.

http://jb.asm.org/cgi/content/abstract/186/17/5762

We report here that the bzd genes for anaerobic benzoate degradation in Azoarcus sp. strain CIB are organized as two transcriptional units, i.e., a benzoate-inducible catabolic operon, bzdNOPQMSTUVWXYZA, and a gene, bzdR, encoding a putative transcriptional regulator. The last gene of the catabolic operon, bzdA, has been expressed in Escherichia coli and encodes the benzoate-coenzyme A (CoA) ligase that catalyzes the first step in the benzoate degradation pathway. The BzdA enzyme is able to activate a wider range of aromatic compounds than that reported for other previously characterized benzoate-CoA ligases. The reduction of benzoyl-CoA to a nonaromatic cyclic intermediate is carried out by a benzoyl-CoA reductase (bzdNOPQ gene products) detected in Azoarcus sp. strain CIB extracts. The bzdW, bzdX, and bzdY gene products show significant similarity to the hydratase, dehydrogenase, and ring-cleavage hydrolase that act sequentially on the product of the benzoyl-CoA reductase in the benzoate catabolic pathway of Thauera aromatica. Benzoate-CoA ligase assays and transcriptional analyses based on lacZ-reporter fusions revealed that benzoate degradation in Azoarcus sp. strain CIB is subject to carbon catabolite repression by some organic acids, indicating the existence of a physiological control that connects the expression of the bzd genes to the metabolic status of the cell.

Bass, K. A. and D. W. Hecht (2002). "Isolation and Characterization of cLV25, a Bacteroides fragilis Chromosomal Transfer Factor Resembling Multiple Bacteroides sp. Mobilizable Transposons." J. Bacteriol. **184**(7): 1895-1904.

http://jb.asm.org/cgi/content/abstract/184/7/1895

Horizontal DNA transfer contributes significantly to the dissemination of antibiotic resistance genes in Bacteroides fragilis. To further our understanding of DNA transfer in B. fragilis, we isolated and characterized a new transfer factor, cLV25. cLV25 was isolated from B. fragilis LV25 by its capture on the nonmobilizable Escherichia coli-Bacteroides shuttle vector pGAT400{Delta}BglII. Similar to other Bacteroides sp. transfer factors, cLV25 was mobilized in E. coli by the conjugative plasmid R751. Using Tn1000 mutagenesis and deletion analysis of cLV25, two mobilization genes, bmgA and bmgB, were identified, whose predicted proteins have similarity to DNA relaxases and mobilization proteins, respectively. In particular, BmgA and BmgB were homologous to MocA and MocB, respectively, the two mobilization proteins of the B. fragilis mobilizable transposon Tn4399. A cis-acting origin of transfer (oriT) was localized to a 353-bp region that included nearly all of the intergenic region between bmgB and orf22 and overlapped with the 3' end of orf22. This oriT contained a putative nic site sequence but showed no significant similarity to the oriT regions of other transfer factors, including Tn4399. Despite the lack of sequence similarity between the oriTs of cLV25 and Tn4399, a mutation in the cLV25 putative DNA relaxase, bmgA, was partially complemented by Tn4399. In addition to the functional cross-reaction with Tn4399, a second distinguishing feature of cLV25 is that predicted proteins have similarity to proteins encoded not only by Tn4399 but by several Bacteroides sp. transfer factors, including NBU1, NBU2, CTnDOT, Tn4555, and Tn5520.

Bierne, H., C. Garandeau, et al. (2004). "Sortase B, a New Class of Sortase in Listeria monocytogenes." J. Bacteriol. **186**(7): 1972-1982.

http://jb.asm.org/cgi/content/abstract/186/7/1972

Sortases are transamidases that covalently link proteins to the peptidoglycan of gram-positive bacteria. The genome of the pathogenic bacterium Listeria monocytogenes encodes two sortases genes, srtA and srtB. The srtA gene product anchors internalin and some other LPXTG-containing proteins to the listerial surface. Here, we focus on the role of the second sortase, SrtB. Whereas SrtA acts on most of the proteins in the peptidoglycan fraction, SrtB appears to target minor amounts of surface polypeptides. We identified one of the SrtB-anchored proteins as the virulence factor SvpA, a surface-exposed protein which does not contain the LPXTG motif. Therefore, as in Staphylococcus aureus, the listerial SrtB represents a second class of sortase in L. monocytogenes, involved in the attachment of a subset of proteins to the cell wall, most likely by recognizing an NXZTN sorting motif. The {Delta}srtB mutant strain does not have defects in bacterial entry, growth, or motility in tissue-cultured cells and does not show attenuated virulence in mice. SrtB-mediated anchoring could therefore be required to anchor surface proteins involved in the adaptation of this microorganism to different environmental conditions.

Cabrol, S., A. Olliver, et al. (2003). "Transcription of Quorum-Sensing System Genes in Clinical and Environmental Isolates of Pseudomonas aeruginosa." J. Bacteriol. **185**(24): 7222-7230.

http://jb.asm.org/cgi/content/abstract/185/24/7222

Quorum sensing (QS)-based transcriptional responses in Pseudomonas aeruginosa have been defined on the basis of increases in transcript levels of QS-controlled genes such as lasB and aprA following the hierarchical transcriptional increases of central controllers such as the lasR gene. These increases occur at high bacterial concentrations such as early-stationary-phase growth in vitro. However, the extent to which the increases occur in a variety of clinical and environmental isolates has not been determined nor is there extensive information on allelic

variation in lasR genes. An analysis of the sequences of the lasR gene among 66 clinical and environmental isolates showed that 81% have a sequence either identical to that of strain PAO1 or with a silent mutation, 15% have nucleotide changes resulting in amino acid changes, and 5% have an insertion sequence in the lasR gene. Using real-time PCR to quantify transcript levels of lasR, lasB, and aprA in the early log and early stationary phases among 35 isolates from bacteremia and pneumonia cases and the environment, we found most (33 of 35) strains had increases in lasR transcripts in early stationary phase but with a very wide range of final transcript levels per cell. There was a strong correlation (r2 = 0.84) between early-log- and early-stationary-phase transcript levels in all strains, but this finding remained true only for the 50% of strains above the median level of lasR found in early log phase. There were significant ($P < 0.05$) but weak-to-modest correlations of lasR transcript levels with aprA (r2 = 0.2) and lasB (r2 = 0.5) transcript levels, but again this correlation occurred only in the 50% of P. aeruginosa strains with the highest levels of lasR transcripts in early stationary phase. There were no differences in distribution of lasR alleles among the bacteremia, pneumonia, or environmental isolates. Overall, only about 50% of P. aeruginosa strains from clinical and environmental sources show a lasR-dependent increase in the transcription of aprA and lasB genes, indicating that for about 50% of clinical isolates this regulatory system may not play a significant role in pathogenesis.

Chen, T., Y. Hosogi, et al. (2004). "Comparative Whole-Genome Analysis of Virulent and Avirulent Strains of Porphyromonas gingivalis." J. Bacteriol. **186**(16): 5473-5479.

http://jb.asm.org/cgi/content/abstract/186/16/5473

We used Porphyromonas gingivalis gene microarrays to compare the total gene contents of the virulent strain W83 and the avirulent type strain, ATCC 33277. Signal ratios and scatter plots indicated that the chromosomes were very similar, with approximately 93% of the predicted genes in common, while at least 7% of them showed very low or no signals in ATCC 33277. Verification of the array results by PCR indicated that several of the disparate genes were either absent from or variant in ATCC 33277. Divergent features included already reported insertion sequences and ragB, as well as additional hypothetical and functionally assigned genes. Several of the latter were organized in a putative operon in W83 and encoded enzymes involved in capsular polysaccharide synthesis. Another cluster was associated with two paralogous regions of the chromosome with a low G+C content, at 41%, compared to that of the whole genome, at 48%. These regions also contained conserved and species-specific hypothetical genes, transposons, insertion sequences, and integrases and were located adjacent to tRNA genes; thus, they had several characteristics of pathogenicity islands. While this global comparative analysis showed the close relationship between W83 and ATCC 33277, the clustering of genes that are present in W83 but divergent in or absent from ATCC 33277 is suggestive of chromosomal islands that may have been acquired by lateral gene transfer.

Choi, J. Y., C. D. Sifri, et al. (2002). "Identification of Virulence Genes in a Pathogenic Strain of Pseudomonas aeruginosa by Representational Difference Analysis." J. Bacteriol. **184**(4): 952-961.

http://jb.asm.org/cgi/content/abstract/184/4/952

Pseudomonas aeruginosa is an opportunistic pathogen that may cause severe infections in humans and other vertebrates. In addition, a human clinical isolate of P. aeruginosa, strain PA14, also causes disease in a variety of nonvertebrate hosts, including plants, Caenorhabditis elegans, and the greater wax moth, Galleria mellonella. This has led to the development of a multihost pathogenesis system in which plants, nematodes, and insects have been used as adjuncts to animal models for the identification of P. aeruginosa virulence factors. Another approach to

identifying virulence genes in bacteria is to take advantage of the natural differences in pathogenicity between isolates of the same species and to use a subtractive hybridization technique to recover relevant genomic differences. The sequenced strain of P. aeruginosa, strain PAO1, has substantial differences in virulence from strain PA14 in several of the multihost models of pathogenicity, and we have utilized the technique of representational difference analysis (RDA) to directly identify genomic differences between P. aeruginosa strains PA14 and PAO1. We have found that the pilC, pilA, and uvrD genes in strain PA14 differ substantially from their counterparts in strain PAO1. In addition, we have recovered a gene homologous to the ybtQ gene from Yersinia, which is specifically present in strain PA14 but absent in strain PAO1. Mutation of the ybtQ homolog in P. aeruginosa strain PA14 significantly attenuates the virulence of this strain in both G. mellonella and a burned mouse model of sepsis to levels comparable to those seen with PAO1. This suggests that the increased virulence of P. aeruginosa strain PA14 compared to PAO1 may relate to specific genomic differences identifiable by RDA.

Correia, F. F., A. R. Plummer, et al. (2003). "Two Paralogous Families of a Two-Gene Subtilisin Operon Are Widely Distributed in Oral Treponemes." J. Bacteriol. **185**(23): 6860-6869.

http://jb.asm.org/cgi/content/abstract/185/23/6860

Certain oral treponemes express a highly proteolytic phenotype and have been associated with periodontal diseases. The periodontal pathogen Treponema denticola produces dentilisin, a serine protease of the subtilisin family. The two-gene operon prcA-prtP is required for expression of active dentilisin (PrtP), a putative lipoprotein attached to the treponeme's outer membrane or sheath. The purpose of this study was to examine the diversity and structure of treponemal subtilisin-like proteases in order to better understand their distribution and function. The complete sequences of five prcA-prtP operons were determined for Treponema lecithinolyticum, "Treponema vincentii," and two canine species. Partial operon sequences were obtained for T. socranskii subsp. 04 as well as 450- to 1,000-base fragments of prtP genes from four additional treponeme strains. Phylogenetic analysis demonstrated that the sequences fall into two paralogous families. The first family includes the sequence from T. denticola. Treponemes possessing this operon family express chymotrypsin-like protease activity and can cleave the substrate N-succinyl-alanyl-alanyl-prolyl-phenylalanine-p-nitroanilide (SAAPFNA). Treponemes possessing the second paralog family do not possess chymotrypsin-like activity or cleave SAAPFNA. Despite examination of a range of protein and peptide substrates, the specificity of the second protease family remains unknown. Each of the fully sequenced prcA and prtP genes contains a 5' hydrophobic leader sequence with a treponeme lipobox. The two paralogous families of treponeme subtilisins represent a new subgroup within the subtilisin family of proteases and are the only subtilisin lipoprotein family. The present study demonstrated that the subtilisin paralogs comprising a two-gene operon are widely distributed among treponemes.

Cummings, C. A., M. M. Brinig, et al. (2004). "Bordetella Species Are Distinguished by Patterns of Substantial Gene Loss and Host Adaptation." J. Bacteriol. **186**(5): 1484-1492.

http://jb.asm.org/cgi/content/abstract/186/5/1484

Pathogens of the bacterial genus Bordetella cause respiratory disease in humans and animals. Although virulence and host specificity vary across the genus, the genetic determinants of this diversity remain unidentified. To identify genes that may underlie key phenotypic differences between these species and clarify their evolutionary relationships, we performed a comparative analysis of genome content in 42 Bordetella strains by hybridization of genomic DNA to a microarray representing the genomes of three Bordetella species and by subtractive hybridization. Here we show that B. pertussis and B. parapertussis are predominantly

differentiated from B. bronchiseptica by large, species-specific regions of difference, many of which encode or direct synthesis of surface structures, including lipopolysaccharide O antigen, which may be important determinants of host specificity. The species also exhibit sequence diversity at a number of surface protein-encoding loci, including the fimbrial major subunit gene, fim2. Gene loss, rather than gene acquisition, accompanied by the proliferation of transposons, has played a fundamental role in the evolution of the pathogenic bordetellae and may represent a conserved evolutionary mechanism among other groups of microbial pathogens.

Di Lorenzo, M., M. Stork, et al. (2003). "Complete Sequence of Virulence Plasmid pJM1 from the Marine Fish Pathogen Vibrio anguillarum Strain 775." J. Bacteriol. **185**(19): 5822-5830.

http://jb.asm.org/cgi/content/abstract/185/19/5822

The virulence plasmid pJM1 enables the fish pathogen Vibrio anguillarum, a gram-negative polarly flagellated comma-shaped rod bacterium, to cause a highly fatal hemorrhagic septicemic disease in salmonids and other fishes, leading to epizootics throughout the world. The pJM1 plasmid 65,009-nucleotide sequence, with an overall G+C content of 42.6%, revealed genes and open reading frames (ORFs) encoding iron transporters, nonribosomal peptide enzymes, and other proteins essential for the biosynthesis of the siderophore anguibactin. Of the 59 ORFs, approximately 32% were related to iron metabolic functions. The plasmid pJM1 confers on V. anguillarum the ability to take up ferric iron as a complex with anguibactin from a medium in which iron is chelated by transferrin, ethylenediamine-di(o-hydroxyphenyl-acetic acid), or other iron-chelating compounds. The fatDCBA-angRT operon as well as other downstream biosynthetic genes is bracketed by the homologous ISV-A1 and ISV-A2 insertion sequences. Other clusters on the plasmid also show an insertion element-flanked organization, including ORFs homologous to genes involved in the biosynthesis of 2,3-dihydroxybenzoic acid. Homologues of replication and partition genes are also identified on pJM1 adjacent to this region. ORFs with no known function represent approximately 30% of the pJM1 sequence. The insertion sequence elements in the composite transposon-like structures, corroborated by the G+C content of the pJM1 sequence, suggest a modular composition of plasmid pJM1, biased towards acquisition of modules containing genes related to iron metabolic functions. We also show that there is considerable microheterogeneity in pJM1-like plasmids from virulent strains of V. anguillarum isolated from different geographical sources.

Duthy, T. G., P. A. Manning, et al. (2002). "Identification and Characterization of Assembly Proteins of CS5 Pili from Enterotoxigenic Escherichia coli." J. Bacteriol. **184**(4): 1065-1077.

http://jb.asm.org/cgi/content/abstract/184/4/1065

This study investigated the role of three genes comprising part of the operon which encodes CS5 pili from enterotoxigenic Escherichia coli. In-frame gene deletions were constructed, and the effects on biogenesis of the pili were examined. A deletion in csfB abolished CsfA major subunit accumulation in the periplasm, which could be restored by trans-complementation with a complete copy of the csfB gene. Localization studies using an antibody against CsfB showed that this protein was periplasmically located, and thus CsfB is likely to function as the specific chaperone for CsfA. An in-frame deletion mutation in the csfE gene resulted in pili approximately three times longer than those of the wild-type strain, thereby indicating a role for CsfE in pilus length regulation. Localization studies using an antibody generated against CsfE showed low-level CsfE accumulation in the outer membranes. Modulation of csfE expression in trans did not reduce the mean length of the pilus below that of the wild type, which indicated that CsfE is not rate-limiting for termination of pilus assembly. Interestingly, a deletion in the csfF gene also resulted in an elongated pilus morphology identical to that of the csfE deletion strain. However,

unlike CsfE, CsfF was shown to be rate-limiting for termination of assembly, since overexpression of CsfF in a csfF deletion strain resulted in a significant decrease in the mean length of the pilus compared to that of the wild type. When the same construct was introduced into the wild-type strain, pilus expression was abolished. Since CsfF bears significant homology to the proposed CsfB chaperone, CsfF was predicted to act as the specific chaperone for CsfE. A double deletion in the csfB and csfF genes was shown to abolish the periplasmic accumulation of both CsfA and CsfD pilins, which could be restored individually only when the strain was trans-complemented with a wild-type copy of csfB or csfF, respectively. Therefore, CsfF may chaperone not only CsfE but also CsfD. A model for CS5 biogenesis is also proposed based on these and previous observations.

Eberhardt, C., L. Kuerschner, et al. (2003). "Probing the Catalytic Activity of a Cell Division-Specific Transpeptidase In Vivo with {beta}-Lactams." J. Bacteriol. **185**(13): 3726-3734.

http://jb.asm.org/cgi/content/abstract/185/13/3726

Penicillin-binding protein 3 (PBP3; also called FtsI) is a transpeptidase that catalyzes cross-linking of the peptidoglycan cell wall in the division septum of Escherichia coli. To determine whether the catalytic activity of PBP3 is activated during division, we assayed acylation of PBP3 with three {beta}-lactams (cephalexin, aztreonam, and piperacillin) in growing cells. Acylation of PBP3 with cephalexin, but not aztreonam or piperacillin, appeared to be stimulated by cell division. Specifically, cephalexin acylated PBP3 about 50% faster in a population of dividing cells than in a population of filamentous cells in which division was inhibited by inactivation or depletion of FtsZ, FtsA, FtsQ, FtsW, or FtsN. However, in a simpler in vitro system using isolated membranes, acylation with cephalexin was not impaired by depletion of FtsW or FtsN. A conflicting previous report that the ftsA3(Ts) allele interferes with acylation of PBP3 was found to be due to the presence of a thermolabile PBP3 in the strain used in that study. The new findings presented here are discussed in light of the hypothesis that the catalytic activity of PBP3 is stimulated by interaction(s) with other division proteins. We suggest that there might be allosteric activation of substrate binding.

Fernandez-Mora, M., J. L. Puente, et al. (2004). "OmpR and LeuO Positively Regulate the Salmonella enterica Serovar Typhi ompS2 Porin Gene." J. Bacteriol. **186**(10): 2909-2920.

http://jb.asm.org/cgi/content/abstract/186/10/2909

The Salmonella enterica serovar Typhi ompS2 gene codes for a 362-amino-acid outer membrane protein that contains motifs common to the porin superfamily. It is expressed at very low levels compared to the major OmpC and OmpF porins, as observed for S. enterica serovar Typhi OmpS1, Escherichia coli OmpN, and Klebsiella pneumoniae OmpK37 quiescent porins. A region of 316 bp, between nucleotides -413 and -97 upstream of the transcriptional start point, is involved in negative regulation, as its removal resulted in a 10-fold increase in ompS2 expression in an S. enterica serovar Typhi wild-type strain. This enhancement in expression was not observed in isogenic mutant strains, which had specific deletions of the regulatory ompB (ompR envZ) operon. Furthermore, ompS2 expression was substantially reduced in the presence of the OmpR D55A mutant, altered in the major phosphorylation site. Upon random mutagenesis, a mutant where the transposon had inserted into the upstream regulatory region of the gene coding for the LeuO regulator, showed an increased level of ompS2 expression. Augmented expression of ompS2 was also obtained upon addition of cloned leuO to the wild-type strain, but not in an ompR isogenic derivative, consistent with the notion that the transposon insertion had increased the cellular levels of LeuO and with the observed dependence on OmpR. Moreover, LeuO and OmpR bound in close proximity, but independently, to the 5' upstream regulatory region. Thus,

the OmpR and LeuO regulators positively regulate ompS2.

Fuangthong, M., A. F. Herbig, et al. (2002). "Regulation of the Bacillus subtilis fur and perR Genes by PerR: Not All Members of the PerR Regulon Are Peroxide Inducible." J. Bacteriol. **184**(12): 3276-3286.

http://jb.asm.org/cgi/content/abstract/184/12/3276

PerR is a ferric uptake repressor (Fur) homolog that functions as the central regulator of the inducible peroxide stress response in Bacillus subtilis. PerR has been previously demonstrated to regulate the mrgA, katA, ahpCF, hemAXCDBL, and zosA genes. We now demonstrate that PerR also mediates both the repression of its own gene and that of fur. Whereas PerR-mediated repression of most target genes can be elicited by either manganese or iron, repression of perR and fur is selective for manganese. Genetic studies indicate that repression of PerR regulon genes by either manganese or iron requires PerR and is generally independent of Fur. Indeed, in a fur mutant, iron-mediated repression is enhanced. Unexpectedly, repression of the fur gene by manganese appears to require both PerR and Fur, but only PerR binds to the fur regulatory region in vitro. The fur mutation appears to act indirectly by affecting cellular metal ion pools and thereby affecting PerR-mediated repression. While many components of the perR regulon are strongly induced by hydrogen peroxide, little, if any, induction of fur and perR could be demonstrated. Thus, not all components of the PerR regulon are components of the peroxide stimulon. We suggest that PerR exists in distinct metallated forms that differ in DNA target selectivity and in sensitivity to oxidation. This model is supported by the observation that the metal ion composition of the growth medium can greatly influence the transcriptional response of the various PerR regulon genes to hydrogen peroxide.

Gill, R. T., E. Katsoulakis, et al. (2002). "Genome-Wide Dynamic Transcriptional Profiling of the Light-to-Dark Transition in Synechocystis sp. Strain PCC 6803." J. Bacteriol. **184**(13): 3671-3681.

http://jb.asm.org/cgi/content/abstract/184/13/3671

We report the results of whole-genome transcriptional profiling of the light-to-dark transition with the model photosynthetic prokaryote Synechocystis sp. strain PCC 6803 (Synechocystis). Experiments were conducted by growing Synechocystis cultures to mid-exponential phase and then exposing them to two cycles of light/dark conditions, during which RNA samples were obtained. These samples were probed with a full-genome DNA microarray (3,169 genes, 20 samples) as well as a partial-genome microarray (88 genes, 29 samples). We concluded that (i) 30-min sampling intervals accurately captured transcriptional dynamics throughout the light/dark transition, (ii) 25% of the Synechocystis genes (783 genes) responded positively to the presence of light, and (iii) the response dynamics varied greatly for individual genes, with a delay of up to 120 to 150 min for some genes. Four classes of genes were identified on the basis of their dynamic gene expression profiles: class I (108 genes, 30-min response time), class II (279 genes, 60 to 90 min), class III (258 genes, 120 to 150 min), and class IV (138 genes, 180 min). The dynamics of several transcripts from genes involved in photosynthesis and primary energy generation are discussed. Finally, we applied Fisher discriminant analysis to better visualize the progression of the overall transcriptional program throughout the light/dark transition and to determine those genes most indicative of the lighting conditions during growth.

Graupner, M., H. Xu, et al. (2002). "The Pyrimidine Nucleotide Reductase Step in Riboflavin and F420

Biosynthesis in Archaea Proceeds by the Eukaryotic Route to Riboflavin." J. Bacteriol. **184**(7): 1952-1957.

http://jb.asm.org/cgi/content/abstract/184/7/1952

The Methanococcus jannaschii gene MJ0671 was cloned and overexpressed in Escherichia coli, and its gene product was tested for its ability to catalyze the pyridine nucleotide-dependent reduction of either 2,5-diamino-6-ribosylamino-4(3H)-pyrimidinone 5'-phosphate (compound 3) to 2,5-diamino-6-ribitylamino-4(3H)-pyrimidinone 5'-phosphate (compound 4) or 5-amino-6-ribosylamino-2,4(1H,3H)-pyrimidinedione 5'-phosphate (compound 7) to 5-amino-6-ribitylamino-2,4(1H,3H)-pyrimidinedione 5'-phosphate (compound 5). Only compound 3 was found to serve as a substrate for the enzyme. NADPH and NADH functioned equally well as the reductants. This specificity for the reduction of compound 3 was also confirmed by using cell extracts of M. jannaschii and Methanosarcina thermophila. Thus, this step in riboflavin biosynthesis in these archaea is the same as that found in yeasts. The absence of the other genes in the biosynthesis of riboflavin in Archaea is discussed.

Hildmann, C., M. Ninkovic, et al. (2004). "A New Amidohydrolase from Bordetella or Alcaligenes Strain FB188 with Similarities to Histone Deacetylases." J. Bacteriol. **186**(8): 2328-2339.

http://jb.asm.org/cgi/content/abstract/186/8/2328

The full-length gene encoding the histone deacetylase (HDAC)-like amidohydrolase (HDAH) from Bordetella or Alcaligenes (Bordetella/Alcaligenes) strain FB188 (DSM 11172) was cloned using degenerate primer PCR combined with inverse-PCR techniques and ultimately expressed in Escherichia coli. The expressed enzyme was biochemically characterized and found to be similar to the native enzyme for all properties examined. Nucleotide sequence analysis revealed an open reading frame of 1,110 bp which encodes a polypeptide with a theoretical molecular mass of 39 kDa. Interestingly, peptide sequencing disclosed that the N-terminal methionine is lacking in the mature wild-type enzyme, presumably due to the action of methionyl aminopeptidase. Sequence database searches suggest that the new amidohydrolase belongs to the HDAC superfamily, with the closest homologs being found in the subfamily assigned acetylpolyamine amidohydrolases (APAH). The APAH subfamily comprises enzymes or putative enzymes from such diverse microorganisms as Pseudomonas aeruginosa, Archaeoglobus fulgidus, and the actinomycete Mycoplana ramosa (formerly M. bullata). The FB188 HDAH, however, is only moderately active in catalyzing the deacetylation of acetylpolyamines. In fact, FB188 HDAH exhibits significant activity in standard HDAC assays and is inhibited by known HDAC inhibitors such as trichostatin A and suberoylanilide hydroxamic acid (SAHA). Several lines of evidence indicate that the FB188 HDAH is very similar to class 1 and 2 HDACs and contains a Zn2+ ion in the active site which contributes significantly to catalytic activity. Initial biotechnological applications demonstrated the extensive substrate spectrum and broad optimum pH range to be excellent criteria for using the new HDAH from Bordetella/Alcaligenes strain FB188 as a biocatalyst in technical biotransformations, e.g., within the scope of human immunodeficiency virus reverse transcriptase inhibitor synthesis.

Huang, L., M. P. McCluskey, et al. (2002). "Global Gene Expression Profiles of the Cyanobacterium Synechocystis sp. Strain PCC 6803 in Response to Irradiation with UV-B and White Light." J. Bacteriol. **184**(24): 6845-6858.

http://jb.asm.org/cgi/content/abstract/184/24/6845

We developed a transcript profiling methodology to elucidate expression patterns of the cyanobacterium Synechocystis sp. strain PCC 6803 and used the technology to investigate changes in gene expression caused by irradiation with either intermediate-wavelength UV light (UV-B) or high-intensity white light. Several families of transcripts were altered by UV-B treatment, including mRNAs specifying proteins involved in light harvesting, photosynthesis, photoprotection, and the heat shock response. In addition, UV-B light induced the stringent response in Synechocystis, as indicated by the repression of ribosomal protein transcripts and other mRNAs involved in translation. High-intensity white light- and UV-B-mediated expression profiles overlapped in the down-regulation of photosynthesis genes and induction of heat shock response but differed in several other transcriptional processes including those specifying carbon dioxide uptake and fixation, the stringent response, and the induction profile of the high-light-inducible proteins. These two profile comparisons not only corroborated known physiological changes but also suggested coordinated regulation of many pathways, including synchronized induction of D1 protein recycling and a coupling between decreased phycobilisome biosynthesis and increased phycobilisome degradation. Overall, the gene expression profile analysis generated new insights into the integrated network of genes that adapts rapidly to different wavelengths and intensities of light.

Hugler, M., C. O. Wirsen, et al. (2005). "Evidence for Autotrophic CO2 Fixation via the Reductive Tricarboxylic Acid Cycle by Members of the {varepsilon} Subdivision of Proteobacteria." J. Bacteriol. **187**(9): 3020-3027.

http://jb.asm.org/cgi/content/abstract/187/9/3020

Based on 16S rRNA gene surveys, bacteria of the {varepsilon} subdivision of proteobacteria have been identified to be important members of microbial communities in a variety of environments, and quite a few have been demonstrated to grow autotrophically. However, no information exists on what pathway of autotrophic carbon fixation these bacteria might use. In this study, Thiomicrospira denitrificans and Candidatus Arcobacter sulfidicus, two chemolithoautotrophic sulfur oxidizers of the {varepsilon} subdivision of proteobacteria, were examined for activities of the key enzymes of the known autotrophic CO2 fixation pathways. Both organisms contained activities of the key enzymes of the reductive tricarboxylic acid cycle, ATP citrate lyase, 2-oxoglutarate:ferredoxin oxidoreductase, and pyruvate:ferredoxin oxidoreductase. Furthermore, no activities of key enzymes of other CO2 fixation pathways, such as the Calvin cycle, the reductive acetyl coenzyme A pathway, and the 3-hydroxypropionate cycle, could be detected. In addition to the key enzymes, the activities of the other enzymes involved in the reductive tricarboxylic acid cycle could be measured. Sections of the genes encoding the {alpha}- and {beta}-subunits of ATP citrate lyase could be amplified from both organisms. These findings represent the first direct evidence for the operation of the reductive tricarboxylic acid cycle for autotrophic CO2 fixation in {varepsilon}-proteobacteria. Since {varepsilon}-proteobacteria closely related to these two organisms are important in many habitats, such as hydrothermal vents, oxic-sulfidic interfaces, or oilfields, these results suggest that autotrophic CO2 fixation via the reductive tricarboxylic acid cycle might be more important than previously considered.

Jacobi, S., R. Schade, et al. (2004). "Characterization of the Alternative Sigma Factor {sigma}54 and the Transcriptional Regulator FleQ of Legionella pneumophila, Which Are Both Involved in the Regulation Cascade of Flagellar Gene Expression." J. Bacteriol. **186**(9): 2540-2547.

http://jb.asm.org/cgi/content/abstract/186/9/2540

We cloned and analyzed Legionella pneumophila Corby homologs of rpoN (encoding {sigma}54) and fleQ (encoding {sigma}54 activator protein). Two other genes (fleR and pilR) whose products

have a {sigma}54 interaction domain were identified in the genome sequence of L. pneumophila. An rpoN mutant strain was nonflagellated and expressed very small amounts of the FlaA (flagellin) protein. Like the rpoN mutant, the fleQ mutant strain of L. pneumophila was also nonflagellated and expressed only small amounts of FlaA protein compared to the amounts expressed by the wild type. In this paper we show that the {sigma}54 factor and the FleQ protein are involved in regulation of flagellar gene operons in L. pneumophila. RpoN and FleQ positively regulate the transcription of FliM and FleN, both of which have a {sigma}54-dependent promoter consensus sequence. However, they seemed to be dispensable for transcription of flaA, fliA, or icmR. Our results confirmed a recently described model of the flagellar gene regulation cascade in L. pneumophila (K. Heuner and M. Steinert, Int. J. Med. Microbiol. 293:133-145, 2003). Flagellar gene regulation was found to be different from that of Enterobacteriaceae but seems to be comparable to that described for Pseudomonas or Vibrio spp.

Kazmierczak, M. J., S. C. Mithoe, et al. (2003). "Listeria monocytogenes {sigma}B Regulates Stress Response and Virulence Functions." J. Bacteriol. **185**(19): 5722-5734.

http://jb.asm.org/cgi/content/abstract/185/19/5722

While the stress-responsive alternative sigma factor {sigma}B has been identified in different species of Bacillus, Listeria, and Staphylococcus, the {sigma}B regulon has been extensively characterized only in B. subtilis. We combined biocomputing and microarray-based strategies to identify {sigma}B-dependent genes in the facultative intracellular pathogen Listeria monocytogenes. Hidden Markov model (HMM)-based searches identified 170 candidate {sigma}B-dependent promoter sequences in the strain EGD-e genome sequence. These data were used to develop a specialized, 208-gene microarray, which included 166 genes downstream of HMM-predicted {sigma}B-dependent promoters as well as selected virulence and stress response genes. RNA for the microarray experiments was isolated from both wild-type and {Delta}sigB null mutant L. monocytogenes cells grown to stationary phase or exposed to osmotic stress (0.5 M KCl). Microarray analyses identified a total of 55 genes with statistically significant {sigma}B-dependent expression under the conditions used in these experiments, with at least 1.5-fold-higher expression in the wild type over the sigB mutant under either stress condition (51 genes showed at least 2.0-fold-higher expression in the wild type). Of the 55 genes exhibiting {sigma}B-dependent expression, 54 were preceded by a sequence resembling the {sigma}B promoter consensus sequence. Rapid amplification of cDNA ends-PCR was used to confirm the {sigma}B-dependent nature of a subset of eight selected promoter regions. Notably, the {sigma}B-dependent L. monocytogenes genes identified through this HMM/microarray strategy included both stress response genes (e.g., gadB, ctc, and the glutathione reductase gene lmo1433) and virulence genes (e.g., inlA, inlB, and bsh). Our data demonstrate that, in addition to regulating expression of genes important for survival under environmental stress conditions, {sigma}B also contributes to regulation of virulence gene expression in L. monocytogenes. These findings strongly suggest that {sigma}B contributes to L. monocytogenes gene expression during infection.

Leski, T. A. and A. Tomasz (2005). "Role of Penicillin-Binding Protein 2 (PBP2) in the Antibiotic Susceptibility and Cell Wall Cross-Linking of Staphylococcus aureus: Evidence for the Cooperative Functioning of PBP2, PBP4, and PBP2A." J. Bacteriol. **187**(5): 1815-1824.

http://jb.asm.org/cgi/content/abstract/187/5/1815

Ceftizoxime, a beta-lactam antibiotic with high selective affinity for penicillin-binding protein 2 (PBP2) of Staphylococcus aureus, was used to select a spontaneous resistant mutant of S. aureus strain 27s. The stable resistant mutant ZOX3 had an increased ceftizoxime MIC and a

decreased affinity of its PBP2 for ceftizoxime and produced peptidoglycan in which the proportion of highly cross-linked muropeptides was reduced. The pbpB gene of ZOX3 carried a single C-to-T nucleotide substitution at nucleotide 1373, causing replacement of a proline with a leucine at amino acid residue 458 of the transpeptidase domain of the protein, close to the SFN conserved motif. Experimental proof that this point mutation was responsible for the drug-resistant phenotype, and also for the decreased PBP2 affinity and reduced cell wall cross-linking, was provided by allelic replacement experiments and site-directed mutagenesis. Disruption of pbpD, the structural gene of PBP4, in either the parental strain or the mutant caused a large decrease in the highly cross-linked muropeptide components of the cell wall and in the mutant caused a massive accumulation of muropeptide monomers as well. Disruption of pbpD also caused increased sensitivity to ceftizoxime in both the parental cells and the ZOX3 mutant, while introduction of the plasmid-borne mecA gene, the genetic determinant of the beta-lactam resistance protein PBP2A, had the opposite effects. The findings provide evidence for the cooperative functioning of two native S. aureus transpeptidases (PBP2 and PBP4) and an acquired transpeptidase (PBP2A) in staphylococcal cell wall biosynthesis and susceptibility to antimicrobial agents.

Lobocka, M. B., D. J. Rose, et al. (2004). "Genome of Bacteriophage P1." J. Bacteriol. **186**(21): 7032-7068.

http://jb.asm.org/cgi/content/abstract/186/21/7032

P1 is a bacteriophage of Escherichia coli and other enteric bacteria. It lysogenizes its hosts as a circular, low-copy-number plasmid. We have determined the complete nucleotide sequences of two strains of a P1 thermoinducible mutant, P1 c1-100. The P1 genome (93,601 bp) contains at least 117 genes, of which almost two-thirds had not been sequenced previously and 49 have no homologs in other organisms. Protein-coding genes occupy 92% of the genome and are organized in 45 operons, of which four are decisive for the choice between lysis and lysogeny. Four others ensure plasmid maintenance. The majority of the remaining 37 operons are involved in lytic development. Seventeen operons are transcribed from {sigma}70 promoters directly controlled by the master phage repressor C1. Late operons are transcribed from promoters recognized by the E. coli RNA polymerase holoenzyme in the presence of the Lpa protein, the product of a C1-controlled P1 gene. Three species of P1-encoded tRNAs provide differential controls of translation, and a P1-encoded DNA methyltransferase with putative bifunctionality influences transcription, replication, and DNA packaging. The genome is particularly rich in Chi recombinogenic sites. The base content and distribution in P1 DNA indicate that replication of P1 from its plasmid origin had more impact on the base compositional asymmetries of the P1 genome than replication from the lytic origin of replication.

Louvel, H., I. Saint Girons, et al. (2005). "Isolation and Characterization of FecA- and FeoB-Mediated Iron Acquisition Systems of the Spirochete Leptospira biflexa by Random Insertional Mutagenesis." J. Bacteriol. **187**(9): 3249-3254.

http://jb.asm.org/cgi/content/abstract/187/9/3249

The specific mechanisms by which Leptospira spp. acquire iron from their ecological niches are unknown. A major factor contributing to our ignorance of spirochetal biology is the lack of methods for genetic analysis of these organisms. In this study, we have developed a system for random transposon mutagenesis of Leptospira biflexa using a mariner transposon, Himar1. To demonstrate the validity of Himar1 in vivo transposon mutagenesis in L. biflexa, a screen of mutants for clones impaired in amino acid biosynthesis was first performed, enabling the identification of tryptophan and glutamate auxotrophs. To investigate iron transporters, 2,000 L.

biflexa transposon mutants were screened onto media with and without hemin, thus allowing the identification of five hemin-requiring mutants, and the putative genes responsible for this phenotype were identified. Three mutants had distinct insertions in a gene encoding a protein which shares homology with the TonB-dependent receptor FecA, involved in ferric citrate transport. We also identified two mutants with a Himar1 insertion into a feoB-like gene, the product of which is required for ferrous iron uptake in many bacterial organisms. Interestingly, the growth inhibition exhibited by the fecA and feoB mutants was relieved by deferoxamine, suggesting the presence of a ferric hydroxamate transporter. These results confirm the importance of iron for the growth of Leptospira and its ability to use multiple iron sources.

Lupo, D. and R. Ghosh (2004). "The Reaction Center H Subunit Is Not Required for High Levels of Light-Harvesting Complex 1 in Rhodospirillum rubrum Mutants." J. Bacteriol. **186**(17): 5585-5595.

http://jb.asm.org/cgi/content/abstract/186/17/5585

The gene (puhA) encoding the H subunit of the reaction center (RC) was deleted by site-directed interposon mutagenesis by using a kanamycin resistance cassette lacking transcriptional terminators to eliminate polar effects in both the wild-type strain Rhodospirillum rubrum S1 and the carotenoid-less strain R. rubrum G9. The puhA interposon mutants were incapable of photoheterotrophic growth but grew normally under aerobic chemoheterotrophic conditions. Absorption spectroscopy and sodium dodecyl sulfate-polyacrylamide gel electrophoresis showed that the RCs were absent. In minimal medium and also in modified medium containing succinate and fructose, the light-harvesting 1 complex (LH1) levels of the S1-derived mutants were about 70 to 100% of the wild-type levels in the same media. The correct assembly of LH1 in the membrane and the pigment-pigment interaction were confirmed by near-infrared circular dichroism spectroscopy. LH1 formation was almost absent when the carotenoid-less G9-derived puhA mutants were grown in standard minimal medium, suggesting that carotenoids may stabilize LH1. In the fructose-containing medium, however, the LH1 levels of the G9 mutants were 70 to 100% of the parental strain levels. Electron micrographs of thin sections of R. rubrum revealed photosynthetic membranes in all mutants grown in succinate-fructose medium. These studies indicate that the H subunit of the RC is necessary neither for maximal formation of LH1 nor for photosynthetic membrane formation but is essential for functional RC assembly.

Lupp, C. and E. G. Ruby (2004). "Vibrio fischeri LuxS and AinS: Comparative Study of Two Signal Synthases." J. Bacteriol. **186**(12): 3873-3881.

http://jb.asm.org/cgi/content/abstract/186/12/3873

Vibrio fischeri possesses two acyl-homoserine lactone quorum-sensing systems, ain and lux, both of which are involved in the regulation of luminescence gene expression and are required for persistent colonization of the squid host, Euprymna scolopes. We have previously demonstrated that the ain system induces luminescence at cell densities that precede lux system activation. Our data suggested that the ain system both relieves repression and initially induces the lux system, thereby achieving sequential induction of gene expression by these two systems. Analysis of the V. fischeri genome revealed the presence of a putative third system based on the enzyme LuxS, which catalyzes the synthesis of the Vibrio harveyi autoinducer 2 (AI-2). In this study, we investigated the impact of V. fischeri LuxS on luminescence and colonization competence in comparison to that of the ain system. Similar to the ain system, inactivation of the AI-2 system decreased light production in culture, but not in the squid host. However, while an ainS mutant produces no detectable light in culture, a luxS mutant expressed approximately 70% of wild-type luminescence levels. A mutation in luxS alone did not compromise symbiotic competence of V. fischeri; however, levels of colonization of an ainS luxS double mutant were reduced to 50% of

the already diminished level of ainS mutant colonization, suggesting that these two systems regulate colonization gene expression synergistically through a common pathway. Introduction of a luxO mutation into the luxS and ainS luxS background could relieve both luminescence and colonization defects, consistent with a model in which LuxS, like AinS, regulates gene expression through LuxO. Furthermore, while luxS transcription appeared to be constitutive and the AI-2 signal concentration did not change dramatically, our data suggest that ainS transcription is autoregulated, resulting in an over 2,000-fold increase in signal concentration as culture density increased. Taken together, these data indicate that V. fischeri LuxS affects both luminescence regulation and colonization competence; however, its quantitative contribution is small when compared to that of the AinS signal.

McNemar, M. D. and W. A. Fonzi (2002). "Conserved Serine/Threonine Kinase Encoded by CBK1 Regulates Expression of Several Hypha-Associated Transcripts and Genes Encoding Cell Wall Proteins in Candida albicans." J. Bacteriol. **184**(7): 2058-2061.

http://jb.asm.org/cgi/content/abstract/184/7/2058

The opportunistic fungal pathogen, Candida albicans, is reported to have several potential virulence factors. A potentially significant factor is the ability to undergo morphological transition from yeast to hypha. This alteration of form is accompanied by many changes within the cell, including alterations in gene expression and cell wall composition. We have isolated a gene that encodes a highly conserved serine/threonine kinase that appears to be involved in the regulation of proteins associated with the cell wall. We have assigned the designation CBK1 (cell wall biosynthesis kinase 1) to this gene. Mutants lacking CBK1 form large aggregates of round cells under all growth conditions and lack the ability to undergo morphological differentiation. Additionally, these mutants show an altered pattern of expression of several transcripts encoding proteins associated with the cell wall. The results suggest that the kinase encoded by CBK1 plays a general role in the maintenance and alteration of the cell wall of C. albicans in all morphologies.

Michan, C., M. Manchado, et al. (2002). "SoxRS Down-Regulation of rob Transcription." J. Bacteriol. **184**(17): 4733-4738.

http://jb.asm.org/cgi/content/abstract/184/17/4733

Rob is regarded as a constitutively expressed protein, although little is known about how rob gene is regulated. We show here by reverse transcription-PCR that the transcriptional levels of rob are strongly down-regulated in response to the superoxide-generating agent paraquat (PQ). Repression reached a maximum of 20-fold after 10 min exposure at 10 {micro}M PQ. The magnitude of rob repression was comparable to that of induction quantified for the most sensitive SoxS targets. {beta}-Galactosidase expression with the rob2:lacZ transcriptional fusion indicates that down-regulation of rob expression takes place, at least in part, at the level of transcription initiation. Moreover, ca. 50% of the rob mRNA was degraded in <1 min after the addition of rifampin to inhibit transcription. This intrinsic short half-life, which is of obvious benefit for a rapid down-regulation after transcription ceases, was unaffected by the addition of PQ. No repression was observed in a soxR-null strain, indicating that the rob transcript level might be negatively modulated by the intracellular amounts of SoxS protein. Gel retardation assays support the idea that in vivo SoxS would block rob transcription directly.

Millikan, D. S. and E. G. Ruby (2004). "Vibrio fischeri Flagellin A Is Essential for Normal Motility and for

Symbiotic Competence during Initial Squid Light Organ Colonization." J. Bacteriol. **186**(13): 4315-4325.

http://jb.asm.org/cgi/content/abstract/186/13/4315

The motile bacterium Vibrio fischeri is the specific bacterial symbiont of the Hawaiian squid Euprymna scolopes. Because motility is essential for initiating colonization, we have begun to identify stage-specific motility requirements by creating flagellar mutants that have symbiotic defects. V. fischeri has six flagellin genes that are uniquely arranged in two chromosomal loci, flaABCDE and flaF. With the exception of the flaA product, the predicted gene products are more similar to each other than to flagellins of other Vibrio species. Immunoblot analysis indicated that only five of the six predicted proteins were present in purified flagella, suggesting that one protein, FlaF, is unique with respect to either its regulation or its function. We created mutations in two genes, flaA and flaC. Compared to a flaC mutant, which has wild-type flagellation, a strain having a mutation in the flaA gene has fewer flagella per cell and exhibits a 60% decrease in its rate of migration in soft agar. During induction of light organ symbiosis, colonization by the flaA mutant is impaired, and this mutant is severely outcompeted when it is presented to the animal as a mixed inoculum with the wild-type strain. Furthermore, flaA mutant cells are preferentially expelled from the animal, suggesting either that FlaA plays a role in adhesion or that normal motility is an advantage for retention within the host. Taken together, these results show that the flagellum of V. fischeri is a complex structure consisting of multiple flagellin subunits, including FlaA, which is essential both for normal flagellation and for motility, as well as for effective symbiotic colonization.

Motin, V. L., A. M. Georgescu, et al. (2002). "Genetic Variability of Yersinia pestis Isolates as Predicted by PCR-Based IS100 Genotyping and Analysis of Structural Genes Encoding Glycerol-3-Phosphate Dehydrogenase (glpD)." J. Bacteriol. **184**(4): 1019-1027.

http://jb.asm.org/cgi/content/abstract/184/4/1019

A PCR-based genotyping system that detects divergence of IS100 locations within the Yersinia pestis genome was used to characterize a large collection of isolates of different biovars and geographical origins. Using sequences derived from the glycerol-negative biovar orientalis strain CO92, a set of 27 locus-specific primers was designed to amplify fragments between the end of IS100 and its neighboring gene. Geographically diverse members of the orientalis biovar formed a homogeneous group with identical genotype with the exception of strains isolated in Indochina. In contrast, strains belonging to the glycerol-positive biovar antiqua showed a variety of fingerprinting profiles. Moreover, strains of the biovar medievalis (also glycerol positive) clustered together with the antiqua isolates originated from Southeast Asia, suggesting their close phylogenetic relationships. Interestingly, a Manchurian biovar antiqua strain Nicholisk 51 displayed a genotyping pattern typical of biovar orientalis isolates. Analysis of the glycerol pathway in Y. pestis suggested that a 93-bp deletion within the glpD gene encoding aerobic glycerol-3-phosphate dehydrogenase might account for the glycerol-negative phenotype of the orientalis biovar. The glpD gene of strain Nicholisk 51 did not possess this deletion, although it contained two nucleotide substitutions characteristic of the glpD version found exclusively in biovar orientalis strains. To account for this close relationship between biovar orientalis strains and the antiqua Nicholisk 51 isolate, we postulate that the latter represents a variant of this biovar with restored ability to ferment glycerol. The fact that such a genetic lesion might be repaired as part of the natural evolutionary process suggests the existence of genetic exchange between different Yersinia strains in nature. The relevance of this observation on the emergence of epidemic Y. pestis strains is discussed.

Ohta, S., K. Tsuchida, et al. (2002). "Presence of a Characteristic D-D-E Motif in IS1 Transposase." J. Bacteriol. **184**(22): 6146-6154.

http://jb.asm.org/cgi/content/abstract/184/22/6146

Transposases encoded by various transposable DNA elements and retroviral integrases belong to a family of proteins with three conserved acidic amino acids, D, D, and E, constituting the D-D-E motif that represents the active center of the proteins. IS1, one of the smallest transposable elements in bacteria, encodes a transposase which has been thought not to belong to the family of proteins with the D-D-E motif. In this study, we found several IS1 family elements that were widely distributed not only in eubacteria but also in archaebacteria. The alignment of the transposase amino acid sequences from these IS1 family elements showed that out of 14 acidic amino acids present in IS1 transposase, three (D, D, and E) were conserved in corresponding positions in the transposases encoded by all the elements. Comparison of the IS1 transposase with other proteins with the D-D-E motif revealed that the polypeptide segments surrounding each of the three acidic amino acids were similar. Furthermore, the deduced secondary structures of the transposases encoded by IS1 family elements were similar to one another and to those of proteins with the D-D-E motif. These results strongly suggest that IS1 transposase has the D-D-E motif and thus belongs to the family of proteins with the D-D-E motif. In fact, mutant IS1 transposases with an amino acid substitution for each of the three acidic amino acids possibly constituting the D-D-E motif were not able to promote transposition of IS1, supporting this hypothesis. The D-D-E motif identified in IS1 transposase differs from those in the other proteins in that the polypeptide segment between the second D and third E in IS1 transposase is the shortest, 24 amino acids in length. Because of this difference, the presence of the D-D-E motif in IS1 transposase has not been discovered for some time.

Pande, S., A. Makela, et al. (2002). "The Bacteriophage T4 Transcription Activator MotA Interacts with the Far-C-Terminal Region of the {sigma}70 Subunit of Escherichia coli RNA Polymerase." J. Bacteriol. **184**(14): 3957-3964.

http://jb.asm.org/cgi/content/abstract/184/14/3957

Transcription from bacteriophage T4 middle promoters uses Escherichia coli RNA polymerase together with the T4 transcriptional activator MotA and the T4 coactivator AsiA. AsiA binds tightly within the C-terminal portion of the {sigma}70 subunit of RNA polymerase, while MotA binds to the 9-bp MotA box motif, which is centered at -30, and also interacts with {sigma}70. We show here that the N-terminal half of MotA (MotANTD), which is thought to include the activation domain, interacts with the C-terminal region of {sigma}70 in an E. coli two-hybrid assay. Replacement of the C-terminal 17 residues of {sigma}70 with comparable {sigma}38 residues abolishes the interaction with MotANTD in this assay, as does the introduction of the amino acid substitution R608C. Furthermore, in vitro transcription experiments indicate that a polymerase reconstituted with a {sigma}70 that lacks C-terminal amino acids 604 to 613 or 608 to 613 is defective for MotA-dependent activation. We also show that a proteolyzed fragment of MotA that contains the C-terminal half (MotACTD) binds DNA with a KD(app) that is similar to that of full-length MotA. Our results support a model for MotA-dependent activation in which protein-protein contact between DNA-bound MotA and the far-C-terminal region of {sigma}70 helps to substitute functionally for an interaction between {sigma}70 and a promoter -35 element.

Persson, A., K. Jacobsson, et al. (2002). "Variable Surface Protein Vmm of Mycoplasma mycoides subsp. mycoides Small Colony Type." J. Bacteriol. **184**(13): 3712-3722.

http://jb.asm.org/cgi/content/abstract/184/13/3712

A variable surface protein, Vmm, of the bovine pathogen Mycoplasma mycoides subsp. mycoides small colony type (M. mycoides SC) has been identified and characterized. Vmm was specific for the SC biotype and was expressed by 68 of 69 analyzed M. mycoides SC strains. The protein was found to undergo reversible phase variation at a frequency of 9 x 10-4 to 5 x 10-5 per cell per generation. The vmm gene was present in all of the 69 tested M. mycoides SC strains and encodes a lipoprotein precursor of 59 amino acids (aa), where the mature protein was predicted to be 36 aa and was anchored to the membrane by only the lipid moiety, as no transmembrane region could be identified. DNA sequencing of the vmm gene region from ON and OFF clones showed that the expression of Vmm was regulated at the transcriptional level by dinucleotide insertions or deletions in a repetitive region of the promoter spacer. Vmm-like genes were also found in four closely related mycoplasmas, Mycoplasma capricolum subsp. capricolum, M. capricolum subsp. capripneumoniae, Mycoplasma sp. bovine serogroup 7, and Mycoplasma putrefaciens. However, Vmm could not be detected in whole-cell lysates of these species, suggesting that the proteins encoded by the vmm-like genes lack the binding epitope for the monoclonal antibody used in this study or, alternatively, that the Vmm-like proteins were not expressed.

Sakamoto, K., S. Ishimaru, et al. (2004). "The Escherichia coli argU10(Ts) Phenotype Is Caused by a Reduction in the Cellular Level of the argU tRNA for the Rare Codons AGA and AGG." J. Bacteriol. **186**(17): 5899-5905.

http://jb.asm.org/cgi/content/abstract/186/17/5899

The Escherichia coli argU10(Ts) mutation in the argU gene, encoding the minor tRNAArg species for the rare codons AGA and AGG, causes pleiotropic defects, including growth inhibition at high temperatures, as well as the Pin phenotype at 30{degrees}C. In the present study, we first showed that the codon selectivity and the arginine-accepting activity of the argU tRNA are both essential for complementing the temperature-sensitive growth, indicating that this defect is caused at the level of translation. An in vitro analysis of the effects of the argU10(Ts) mutation on tRNA functions revealed that the affinity with elongation factor Tu-GTP of the argU10(Ts) mutant tRNA is impaired at 30 and 43{degrees}C, and this defect is more serious at the higher temperature. The arginine acceptance is also impaired significantly but to similar extents at the two temperatures. An in vivo analysis of aminoacylation levels showed that 30% of the argU10(Ts) tRNA molecules in the mutant cells are actually deacylated at 30{degrees}C, while most of the argU tRNA molecules in the wild-type cells are aminoacylated. Furthermore, the cellular level of this mutant tRNA is one-tenth that of the wild-type argU tRNA. At 43{degrees}C, the cellular level of the argU10(Ts) tRNA is further reduced to a trace amount, while neither the cellular abundance nor the aminoacylation level of the wild-type argU tRNA changes. We concluded that the phenotypic properties of the argU10(Ts) mutant result from these reduced intracellular levels of the tRNA, which are probably caused by the defective interactions with elongation factor Tu and arginyl-tRNA synthetase.

Schmid, A. K., H. A. Howell, et al. (2005). "Global Transcriptional and Proteomic Analysis of the Sig1 Heat Shock Regulon of Deinococcus radiodurans." J. Bacteriol. **187**(10): 3339-3351.

http://jb.asm.org/cgi/content/abstract/187/10/3339

The sig1 gene, predicted to encode an extracytoplasmic function-type heat shock sigma factor of Deinococcus radiodurans, has been shown to play a central role in the positive regulation of the

heat shock operons groESL and dnaKJ. To determine if Sig1 is required for the regulation of additional heat shock genes, we monitored the global transcriptional and proteomic profiles of a D. radiodurans R1 sig1 mutant and wild-type cells in response to elevated temperature stress. Thirty-one gene products were identified that showed heat shock induction in the wild type but not in the sig1 mutant. Quantitative real-time PCR experiments verified the transcriptional requirement of Sig1 for the heat shock induction of the mRNA of five of these genes--dnaK, groES, DR1314, pspA, and hsp20. hsp20 appears to encode a new member of the small heat shock protein superfamily, DR1314 is predicted to encode a hypothetical protein with no recognizable orthologs, and pspA is predicted to encode a protein involved in maintenance of membrane integrity. Deletion mutation analysis demonstrated the importance in heat shock protection of hsp20 and DR1314. The promoters of dnaKJE, groESL, DR1314, pspA, and hsp20 were mapped and, combined with computer-based pattern searches of the upstream regions of the 26 other Sig1 regulon members, these results suggested that Sig1 might recognize both {sigma}70-type and {sigma}W-type promoter consensus sequences. These results expand the D. radiodurans Sig1 heat shock regulon to include 31 potential new members, including not only factors with cytoplasmic functions, such as groES and dnaK, but also those with extracytoplasmic functions, like pspA.

Schneider, K., C. N. Kastner, et al. (2002). "Identification of a Gene Cluster in Klebsiella pneumoniae Which Includes citX, a Gene Required for Biosynthesis of the Citrate Lyase Prosthetic Group." J. Bacteriol. **184**(9): 2439-2446.

http://jb.asm.org/cgi/content/abstract/184/9/2439

The biosynthesis of the 2'-(5"-phosphoribosyl)-3'-dephospho-coenzyme A (CoA) prosthetic group of citrate lyase (EC 4.1.3.6), a key enzyme of citrate fermentation, proceeds via the initial formation of the precursor 2'-(5"-triphosphoribosyl)-3'-dephospho-CoA and subsequent transfer to apo-citrate lyase with removal of pyrophosphate. In Escherichia coli, the two steps are catalyzed by CitG and CitX, respectively, and the corresponding genes are part of the citrate lyase gene cluster, citCDEFXG. In the homologous citCDEFG operon of Klebsiella pneumoniae, citX is missing. A search for K. pneumoniae citX led to the identification of a second genome region involved in citrate fermentation which comprised the citWX genes and the divergent citYZ genes. The citX gene was confirmed to encode holo-citrate lyase synthase, whereas citW was shown to encode a citrate carrier, the third one identified in this species. The citYZ genes were found to encode a two-component system consisting of the sensor kinase CitY and the response regulator CitZ. Remarkably, both proteins showed [>=]40% sequence identity to the citrate-sensing CitA-CitB two-component system, which is essential for the induction of the citrate fermentation genes in K. pneumoniae. A citZ insertion mutant was able to grow anaerobically with citrate, indicating that CitZ is not essential for expression of citrate fermentation genes. CitX synthesis was induced to a basal level under anaerobic conditions, independent of citrate, CitB, and CitZ, and to maximal levels during anaerobic growth with citrate as the sole carbon source. Similar to the other citrate fermentation enzymes, CitX synthesis was apparently subject to catabolite repression.

Sebbane, F., M.-A. Mandrand-Berthelot, et al. (2002). "Genes Encoding Specific Nickel Transport Systems Flank the Chromosomal Urease Locus of Pathogenic Yersiniae." J. Bacteriol. **184**(20): 5706-5713.

http://jb.asm.org/cgi/content/abstract/184/20/5706

The transition metal nickel is an essential cofactor for a number of bacterial enzymes, one of which is urease. Prior to its incorporation into metalloenzyme active sites, nickel must be

imported into the cell. Here, we report identification of two loci corresponding to nickel-specific transport systems in the gram-negative, ureolytic bacterium Yersinia pseudotuberculosis. The loci are located on each side of the chromosomal urease gene cluster ureABCEFGD and have the same orientation as the latter. The yntABCDE locus upstream of the ure genes encodes five predicted products with sequence homology to ATP-binding cassette nickel permeases present in several gram-negative bacteria. The ureH gene, located downstream of ure, encodes a single-component carrier which displays homology to polypeptides of the nickel-cobalt transporter family. Transporters with homology to these two classes are also present (again in proximity to the urease locus) in the other two pathogenic yersiniae, Y. pestis and Y. enterocolitica. An Escherichia coli nikA insertion mutant recovered nickel uptake ability following heterologous complementation with either the ynt or the ureH plasmid-borne gene of Y. pseudotuberculosis, demonstrating that each carrier is necessary and sufficient for nickel transport. Deletion of ynt in Y. pseudotuberculosis almost completely abolished bacterial urease activity, whereas deletion of ureH had no effect. Nevertheless, rates of nickel transport were significantly altered in both ynt and ureH mutants. Furthermore, the ynt ureH double mutant was totally devoid of nickel uptake ability, thus indicating that Ynt and UreH constitute the only routes for nickel entry. Both Ynt and UreH show selectivity for Ni2+ ions. This is the first reported identification of genes coding for both kinds of nickel-specific permeases situated adjacent to the urease gene cluster in the genome of a microorganism.

Sharma, V. K. and R. L. Zuerner (2004). "Role of hha and ler in Transcriptional Regulation of the esp Operon of Enterohemorrhagic Escherichia coli O157:H7." J. Bacteriol. **186**(21): 7290-7301.

http://jb.asm.org/cgi/content/abstract/186/21/7290

The locus of enterocyte effacement (LEE), which includes five major operons (LEE1 through LEE4 and tir), enables enterohemorrhagic Escherichia coli (EHEC) O157:H7 to produce attaching and effacing lesions on host cells. Expression of LEE2, LEE3, and tir is positively regulated by ler, a gene located in LEE1. Transcriptional regulation of the esp operon (LEE4), however, is not well defined. Transposon mutagenesis was used to identify transcriptional regulators of the esp operon by screening for mutants with increased {beta}-galactosidase activity in an EHEC O157:H7 strain harboring an esp:lac transcriptional fusion. All mutants with significant increases in {beta}-galactosidase activity had transposon insertions in hha (hha:Tn). Specific complementation of the hha:Tn mutation with a plasmid-encoded copy of hha reduced {beta}-galactosidase activity to the level expressed in the parental esp:lac strain. Purified Hha, however, bound poorly to the esp promoter, suggesting that Hha might repress the transcription of a positive regulator of esp. Transposon mutagenesis of a {Delta}hha esp:lac strain expressing elevated levels of {beta}-galactosidase resulted in ler mutants with reduced {beta}-galactosidase activity. Purified Hha bound to the ler promoter with a higher affinity, and complementation of a {Delta}hha mutation in a {Delta}hha ler:lac strain repressed {beta}-galactosidase activity to the level expressed in a ler:lac strain. A positive regulatory role of ler in esp expression was demonstrated by specific binding of Ler to the esp promoter, reduced expression of {beta}-galactosidase in {Delta}ler esp:lac strains with and without hha, and severalfold-increased transcription of ler and espA in strains lacking hha. These results indicate that hha-mediated repression of ler causes reduced expression of the esp operon.

Slepenkin, A., L. M. de la Maza, et al. (2005). "Interaction between Components of the Type III Secretion System of Chlamydiaceae." J. Bacteriol. **187**(2): 473-479.

http://jb.asm.org/cgi/content/abstract/187/2/473

Members of the family Chlamydiaceae possess at least 13 genes, distributed throughout the

chromosome, that are homologous with genes of known type III secretion systems (TTS). The aim of this study was to use putative TTS proteins of Chlamydophila pneumoniae, whose equivalents in other bacterial TTS function as chaperones, to identify interactions between chlamydial proteins. Using the BacterioMatch Two-Hybrid Vector system (Stratagene, La Jolla, Calif.), lcrH-2 and sycE, positions 1021 and 0325, respectively, from C. pneumoniae CM-1 were used as "bait" to identify target genes (positions 0324, 0705, 0708, 0808 to 0810, 1016 to 1020, and 1022) in close proximity on the chromosome. Interaction between the products of the lcrH-2 (1021) and lcrE (copN) (0324) genes was detected and confirmed by pull-down experiments and enzyme immunoassays using recombinant LcrH-2 and LcrE. As further confirmation of this interaction, the homologous genes from Chlamydia trachomatis, serovar E, and Chlamydophila psittaci, Texas turkey, were also cloned in the two-hybrid system to determine if LcrH-2 and LcrE would interact with their orthologs in other species. Consistent with their genetic relatedness, LcrH-2 from C. pneumoniae interacted with LcrE produced from the three species of Chlamydiaceae; LcrH-2 from C. psittaci reacted with LcrE from C. pneumoniae but not from C. trachomatis; and C. trachomatis LcrH-2 did not react with LcrE from the other two species. Deletions from the N and C termini of LcrE from C. pneumoniae identified the 50 C-terminal amino acids as essential for the interaction with LcrH-2. Thus, it appears that in the Chlamydiaceae TTS, LcrH-2 interacts with LcrE, and therefore it may serve as a chaperone for this protein.

Sorger, D. and G. Daum (2002). "Synthesis of Triacylglycerols by the Acyl-Coenzyme A:Diacyl-Glycerol Acyltransferase Dga1p in Lipid Particles of the Yeast Saccharomyces cerevisiae." J. Bacteriol. **184**(2): 519-524.

http://jb.asm.org/cgi/content/abstract/184/2/519

The terminal step of triacylglycerol (TAG) formation in the yeast Saccharomyces cerevisiae is catalyzed by the enzyme acyl-CoA:diacylglycerol acyltransferase (DAGAT). In this study we demonstrate that the gene product of YOR245c, Dga1p, catalyzes a major yeast DAGAT activity which is localized to lipid particles. Enzyme measurements employing a newly established assay containing radioactively labeled diacylglycerol (DAG) as a substrate and unlabeled palmitoyl-CoA as a cosubstrate revealed a 70- to 90-fold enrichment of DAGAT in lipid particles over the homogenate but also a 2- to 3-fold enrichment in endoplasmic reticulum fractions. In a dga1 deletion strain, the DAGAT activity in lipid particles is dramatically reduced, whereas the activity in microsomes is affected only to a minor extent. Thus, we propose the existence of DAGAT isoenzymes in the microsomal fraction. Furthermore, we unveiled an acyl-CoA-independent TAG synthase activity in lipid particles which is distinct from Dga1p and the phosphatidylcholine:DAGAT Lro1p. This acyl-CoA-independent TAG synthase utilizes DAG as an acceptor and free fatty acids as cosubstrates and occurs independently of the acyl-CoA synthases Faa1p to Faa4p. Based on lipid analysis of the respective deletion strains, Lro1p and Dga1p are the major contributors to total cellular TAG synthesis, whereas other TAG synthesizing systems appear to be of minor importance. In conclusion, at least three different pathways are involved in the formation of storage TAG in the yeast.

Soupene, E., W. C. van Heeswijk, et al. (2003). "Physiological Studies of Escherichia coli Strain MG1655: Growth Defects and Apparent Cross-Regulation of Gene Expression." J. Bacteriol. **185**(18): 5611-5626.

http://jb.asm.org/cgi/content/abstract/185/18/5611

Escherichia coli strain MG1655 was chosen for sequencing because the few mutations it carries (ilvG rfb-50 rph-1) were considered innocuous. However, it has a number of growth defects.

Internal pyrimidine starvation due to polarity of the rph-1 allele on pyrE was problematic in continuous culture. Moreover, the isolate of MG1655 obtained from the E. coli Genetic Stock Center also carries a large deletion around the fnr (fumarate-nitrate respiration) regulatory gene. Although studies on DNA microarrays revealed apparent cross-regulation of gene expression between galactose and lactose metabolism in the Stock Center isolate of MG1655, this was due to the occurrence of mutations that increased lacY expression and suppressed slow growth on galactose. The explanation for apparent cross-regulation between galactose and N-acetylglucosamine metabolism was similar. By contrast, cross-regulation between lactose and maltose metabolism appeared to be due to generation of internal maltosaccharides in lactose-grown cells and may be physiologically significant. Lactose is of restricted distribution: it is normally found together with maltosaccharides, which are starch degradation products, in the mammalian intestine. Strains designated MG1655 and obtained from other sources differed from the Stock Center isolate and each other in several respects. We confirmed that use of other E. coli strains with MG1655-based DNA microarrays works well, and hence these arrays can be used to study any strain of interest. The responses to nitrogen limitation of two urinary tract isolates and an intestinal commensal strain isolated recently from humans were remarkably similar to those of MG1655.

Taylor, D. L., P. N. Ward, et al. (2003). "Identification of a Differentially Expressed Oligopeptide Binding Protein (OppA2) in Streptococcus uberis by Representational Difference Analysis of cDNA." J. Bacteriol. **185**(17): 5210-5219.

http://jb.asm.org/cgi/content/abstract/185/17/5210

Streptococcus uberis is an increasingly significant cause of intramammary infection in the dairy cow, presently responsible for approximately 33% of all cases of bovine mastitis in the United Kingdom. Following experimentally induced infection of the lactating mammary gland, S. uberis is found predominantly in the luminal areas of secretory alveoli and ductular tissue, indicating that much of the bacterial growth occurs in residual and newly synthesized milk. With the objective of identifying potential virulence determinants in a clinical isolate of S. uberis, we have used representational difference analysis of cDNA to identify genes that show modified expression in milk. We have identified a number of differentially expressed genes that may contribute to the overall pathogenicity of the organism. Of these, a transcript encoding a putative oligopeptide binding protein (OppA) was further characterized. We have found that S. uberis possesses two oppA-like open reading frames, oppA1 and oppA2, which are up-regulated to different degrees following growth in milk. Mutants lacking either oppA1 or oppA2 are viable and have an increased resistance to the toxic peptide derivative aminopterin; however, only mutants lacking oppA1 display a lower rate of growth in milk. In addition, expression of the oppA genes appears to be coordinated by different mechanisms. We conclude that the oppA genes encode oligopeptide binding proteins, possibly displaying different specificities, required for the efficient growth of S. uberis in milk.

Tomas, C. A., K. V. Alsaker, et al. (2003). "DNA Array-Based Transcriptional Analysis of Asporogenous, Nonsolventogenic Clostridium acetobutylicum Strains SKO1 and M5." J. Bacteriol. **185**(15): 4539-4547.

http://jb.asm.org/cgi/content/abstract/185/15/4539

The large-scale transcriptional program of two Clostridium acetobutylicum strains (SKO1 and M5) relative to that of the parent strain (wild type [WT]) was examined by using DNA microarrays. Glass DNA arrays containing a selected set of 1,019 genes (including all 178 pSOL1 genes) covering more than 25% of the whole genome were designed, constructed, and validated for data

reliability. Strain SKO1, with an inactivated spo0A gene, displays an asporogenous, filamentous, and largely deficient solventogenic phenotype. SKO1 displays downregulation of all solvent formation genes, sigF, and carbohydrate metabolism genes (similar to genes expressed as part of the stationary-phase response in Bacillus subtilis) but also several electron transport genes. A major cluster of genes upregulated in SKO1 includes abrB, the genes from the major chemotaxis and motility operons, and glycosylation genes. Strain M5 displays an asporogenous and nonsolventogenic phenotype due to loss of the megaplasmid pSOL1, which contains all genes necessary for solvent formation. Therefore, M5 displays downregulation of all pSOL1 genes expressed in the WT. Notable among other genes expressed more highly in WT than in M5 were sigF, several two-component histidine kinases, spo0A, cheA, cheC, many stress response genes, fts family genes, DNA topoisomerase genes, and central-carbon metabolism genes. Genes expressed more highly in M5 include electron transport genes (but different from those downregulated in SKO1) and several motility and chemotaxis genes. Most of these expression patterns were consistent with phenotypic characteristics. Several of these expression patterns are new or different from what is known in B. subtilis and can be used to test a number of functional-genomic hypotheses.

Umeno, D. and F. H. Arnold (2004). "Evolution of a Pathway to Novel Long-Chain Carotenoids." J. Bacteriol. **186**(5): 1531-1536.

http://jb.asm.org/cgi/content/abstract/186/5/1531

Using methods of laboratory evolution to force the C30 carotenoid synthase CrtM to function as a C40 synthase, followed by further mutagenesis at functionally important amino acid residues, we have discovered that synthase specificity is controlled at the second (rearrangement) step of the two-step reaction. We used this information to engineer CrtM variants that can synthesize previously unknown C45 and C50 carotenoid backbones (mono- and diisopentenylphytoenes) from the appropriate isoprenyldiphosphate precursors. With this ability to produce new backbones in Escherichia coli comes the potential to generate whole series of novel carotenoids by using carotenoid-modifying enzymes, including desaturases, cyclases, hydroxylases, and dioxygenases, from naturally occurring pathways.

Wang, G., S. P. Kennedy, et al. (2004). "Arsenic Resistance in Halobacterium sp. Strain NRC-1 Examined by Using an Improved Gene Knockout System." J. Bacteriol. **186**(10): 3187-3194.

http://jb.asm.org/cgi/content/abstract/186/10/3187

The genome sequence of Halobacterium sp. strain NRC-1 encodes genes homologous to those responsible for conferring resistance to arsenic. These genes occur on both the large extrachromosomal replicon pNRC100 (arsADRC and arsR2M) and on the chromosome (arsB). We studied the role of these ars genes in arsenic resistance genetically by construction of gene knockouts. Deletion of the arsADRC gene cluster in a Halobacterium NRC-1 {Delta}ura3 strain resulted in increased sensitivity to arsenite and antimonite but not arsenate. In contrast, knockout of the chromosomal arsB gene did not show significantly increased sensitivity to arsenite or arsenate. We also found that knockout of the arsM gene produced sensitivity to arsenite, suggesting a second novel mechanism of arsenic resistance involving a putative arsenite(III)-methyltransferase. These results indicate that Halobacterium sp. strain NRC-1 contains an arsenite and antimonite extrusion system with significant differences from bacterial counterparts. Deletion analysis was facilitated by an improved method for gene knockouts/replacements in Halobacterium that relies on both selection and counterselection of ura3 using a uracil dropout medium and 5-fluoroorotic acid. The arsenite and antimonite resistance elements were shown to be regulated, with resistance to arsenic in the wild type inducible by exposure to a sublethal

concentration of the metal. Northern hybridization and reverse transcription-PCR analyses showed that arsA, arsD, arsR, arsM, arsC, and arsB, but not arsR2, are inducible by arsenite and antimonite. We discuss novel aspects of arsenic resistance in this halophilic archaeon and technical improvements in our capability for gene knockouts in the genome.

Willis, S. H., K. M. Kazmierczak, et al. (2002). "N4 RNA Polymerase II, a Heterodimeric RNA Polymerase with Homology to the Single-Subunit Family of RNA Polymerases." J. Bacteriol. **184**(18): 4952-4961.

http://jb.asm.org/cgi/content/abstract/184/18/4952

Bacteriophage N4 middle genes are transcribed by a phage-coded, heterodimeric, rifampin-resistant RNA polymerase, N4 RNA polymerase II (N4 RNAPII). Sequencing and transcriptional analysis revealed that the genes encoding the two subunits comprising N4 RNAPII are translated from a common transcript initiating at the N4 early promoter Pe3. These genes code for proteins of 269 and 404 amino acid residues with sequence similarity to the single-subunit, phage-like RNA polymerases. The genes encoding the N4 RNAPII subunits, as well as a synthetic construct encoding a fusion polypeptide, have been cloned and expressed. Both the individually expressed subunits and the fusion polypeptide reconstitute functional enzymes in vivo and in vitro.

Xu, H., Y. Zhang, et al. (2004). "Isoleucine Biosynthesis in Leptospira interrogans Serotype lai Strain 56601 Proceeds via a Threonine-Independent Pathway." J. Bacteriol. **186**(16): 5400-5409.

http://jb.asm.org/cgi/content/abstract/186/16/5400

Three leuA-like protein-coding sequences were identified in Leptospira interrogans. One of these, the cimA gene, was shown to encode citramalate synthase (EC 4.1.3.-). The other two encoded {alpha}-isopropylmalate synthase (EC 4.1.3.12). Expressed in Escherichia coli, the citramalate synthase was purified and characterized. Although its activity was relatively low, it was strictly specific for pyruvate as the keto acid substrate. Unlike the citramalate synthase of the thermophile Methanococcus jannaschii, the L. interrogans enzyme is temperature sensitive but exhibits a much lower Km (0.04 mM) for pyruvate. The reaction product was characterized as (R)-citramalate, and the proposed {beta}-methyl-D-malate pathway was further confirmed by demonstrating that citraconate was the substrate for the following reaction. This alternative pathway for isoleucine biosynthesis from pyruvate was analyzed both in vitro by assays of leptospiral isopropylmalate isomerase (EC 4.2.1.33) and {beta}-isopropylmalate dehydrogenase (EC 1.1.1.85) in E. coli extracts bearing the corresponding clones and in vivo by complementation of E. coli ilvA, leuC/D, and leuB mutants. Thus, the existence of a leucine-like pathway for isoleucine biosynthesis in L. interrogans under physiological conditions was unequivocally proven. Significant variations in either the enzymatic activities or mRNA levels of the cimA and leuA genes were detected in L. interrogans grown on minimal medium supplemented with different levels of the corresponding amino acids or in cells grown on serum-containing rich medium. The similarity of this metabolic pathway in leptospires and archaea is consistent with the evolutionarily primitive status of the eubacterial spirochetes.

Abbas, T., M. Olivier, et al. (2002). "Differential Activation of p53 by the Various Adducts of Mitomycin C." J. Biol. Chem. **277**(43): 40513-40519.

http://www.jbc.org/cgi/content/abstract/277/43/40513

Mitomycin C (MC) is a cytotoxic chemotherapeutic agent that causes DNA damage in the form of DNA cross-links as well as a variety of DNA monoadducts and is known to induce p53. The various DNA adducts formed upon treatment of mouse mammary tumor cells with MC as well as 10-decarbamoyl MC (DMC) and 2,7-diaminomitosene (2,7-DAM), the major MC metabolite, have been elucidated. The cytotoxicity of DMC parallels closely that of MC in a number of rodent cell lines tested, whereas 2,7-DAM is relatively noncytotoxic. In this study, we investigate the ability of MC, DMC, and 2,7-DAM to activate p53 at equidose concentrations by treating tissue culture cell lines with the three mitomycins. Whereas MC and DMC induced p53 protein levels and increased the levels of p21 and Gadd45 mRNA, 2,7-DAM did not. Furthermore, MC and DMC, but not 2,7-DAM, were able to induce apoptosis efficiently in ML-1 cells. Therefore the 2,7-DAM monoadducts were unable to activate the p53 pathway. Interestingly, DMC was able to initiate apoptosis via a p53-independent pathway whereas MC was not. This is the first finding that adducts of a multiadduct type DNA-damaging agent are differentially recognized by DNA damage sensor pathways.

Abdollahi, A., D. Pisarcik, et al. (2003). "LOT1 (PLAGL1/ZAC1), the Candidate Tumor Suppressor Gene at Chromosome 6q24-25, Is Epigenetically Regulated in Cancer." J. Biol. Chem. **278**(8): 6041-6049.

http://www.jbc.org/cgi/content/abstract/278/8/6041

LOT1 is a zinc-finger nuclear transcription factor, which possesses anti-proliferative effects and is frequently silenced in ovarian and breast cancer cells. The LOT1 gene is localized at chromosome 6q24-25, a chromosomal region maternally imprinted and linked to growth retardation in several organs and progression of disease states such as transient neonatal diabetes mellitus. Toward understanding the molecular mechanism underlying the loss of LOT1 expression in cancer, we have characterized the genomic structure and analyzed its epigenetic regulation. Genome mapping of LOT1 in comparison with the other splice variants, namely ZAC1 and PLAGL1, revealed that its mRNA (~4.7 kb; GenBankTM accession number U72621) is potentially spliced using six exons spanning at least 70 kb of the human genome. 5'-RACE (rapid amplification of cDNA ends) data indicate the presence of at least two transcription start sites. We found that in vitro methylation of the LOT1 promoter causes a significant loss in its ability to drive luciferase transcription. To determine the nature of in vivo methylation of LOT1, we used bisulfite-sequencing strategies on genomic DNA. We show that in the ovarian and breast cancer cell lines and/or tumors the 5'-CpG island of LOT1 is a differentially methylated region. In these cell lines the ratio of methylated to unmethylated CpG dinucleotides in this region ranged from 31 to 99% and the ovarian tumors have relatively higher cytosine methylation than normal tissues. Furthermore, we show that trichostatin A, a specific inhibitor of histone deacetylase, relieves transcriptional silencing of LOT1 mRNA in malignantly transformed cells. It appears that, unlike DNA methylation, histone deacetylation does not target the promoter, and rather it is indirect and may be elicited by a mechanism upstream of the LOT1 regulatory pathway. Taken together, the data suggest that expression of LOT1 is under the control of two epigenetic modifications and that, in the absence of loss of heterozygosity, the biallelic (two-hit) or maximal silencing of LOT1 requires both processes.

Adachi, H. and M. Tsujimoto (2002). "Characterization of the Human Gene Encoding the Scavenger Receptor Expressed by Endothelial Cell and Its Regulation by a Novel Transcription Factor, Endothelial Zinc Finger Protein-2." J. Biol. Chem. **277**(27): 24014-24021.

http://www.jbc.org/cgi/content/abstract/277/27/24014

The scavenger receptor expressed by endothelial cell (SREC), mediates the selective uptake of modified low density lipoprotein (LDL), such as acetylated LDL and oxidized LDL, into endothelial cells. The SREC gene spans 12 kilobase pairs and contains 11 exons. Analysis of full-length cDNA clones of SREC from a peripheral blood leukocyte cDNA library revealed that at least five alternatively spliced cDNAs were present, and two of them encoded soluble forms of SREC. The transcription start site of the SREC gene was mapped, and DNA sequence analysis revealed an Sp1 binding site in its proximal region. Deletion analysis of the 5'-flanking sequence revealed that sequence between base pairs [-]108 and [-]98 was critical for the promoter activity. This region contained half of an inverted repeat (IR) sequence with a triple nucleotide spacer (IR-3). A protected sequence between base pairs [-]268 and +17 was defined by in vitro DNase I footprinting analysis using human umbilical vein endothelial cell (HUVEC) nuclear extract. A novel transcription factor, endothelial zinc finger protein-2 (EZF-2), that binds to the 5'-flanking critical region of the SREC promoter activity was cloned from a HUVEC cDNA library employing a one-hybrid system. Whereas purified recombinant Sp1 alone produced similar protection in in vitro DNase I footprinting analysis, EZF-2 also bound to the 5'-flanking region SREC promoter. Co-transfection of SREC promoter and Sp1 or EZF-2 expression plasmids in HUVEC revealed that EZF-2 but not Sp1 increased SREC promoter activity. On the other hand, the mutation of either the Sp1 motif or IR-3 motif resulted in a decrease in the promoter activity. These results suggest that whereas Sp1 is the major nuclear protein bound to the regulatory region of the promoter, both EZF-2 and Sp1 are responsible for its regulation.

Agorio, A., C. Chalar, et al. (2003). "Alternative mRNAs Arising from Trans-splicing Code for Mitochondrial and Cytosolic Variants of Echinococcus granulosus Thioredoxin Glutathione Reductase." J. Biol. Chem. **278**(15): 12920-12928.

http://www.jbc.org/cgi/content/abstract/278/15/12920

Thioredoxin and glutathione systems are the major thiol-dependent redox systems in animal cells. They transfer via the reversible oxidoreduction of thiols the reducing equivalents of NADPH to numerous substrates and substrate reductases and constitute major defenses against oxidative stress. In this study, we cloned from the helminth parasite Echinococcus granulosus two trans-spliced mRNA variants that encode thioredoxin glutathione reductases (TGR). These variants code for mitochondrial and cytosolic selenocysteine-containing isoforms that possess identical glutaredoxin (Grx) and thioredoxin reductase (TR) domains and differ exclusively in their N termini. Western blot analysis of subcellular fractions with specific anti-TGR antibodies showed that TGR is present in both compartments. The biochemical characterization of the native purified TGR suggests that the Grx and TR domains of the enzyme can function either coupled or independently of each other, because the Grx domain can accept electrons from either TR domains or the glutathione system and the TR domains can transfer electrons to either the fused Grx domain or to E. granulosus thioredoxin.

Ahmad, Z., M. Salim, et al. (2002). "Human Biliverdin Reductase Is a Leucine Zipper-like DNA-binding Protein and Functions in Transcriptional Activation of Heme Oxygenase-1 by Oxidative Stress." J. Biol. Chem. **277**(11): 9226-9232.

http://www.jbc.org/cgi/content/abstract/277/11/9226

Human biliverdin reductase (hBVR) is a serine/threonine kinase that catalyzes reduction of the heme oxygenase (HO) activity product, biliverdin, to bilirubin. A domain of biliverdin reductase (BVR) has primary structural features that resemble leucine zipper proteins. A heptad repeat of five leucines (L1-L5), a basic domain, and a conserved alanine characterize the domain. In hBVR, a lysine replaces L3. The secondary structure model of hBVR predicts an [alpha]-helix-turn-[beta]-sheet for this domain. hBVR translated by the rabbit reticulocyte lysate system appears on a nondenaturing gel as a single band with molecular mass of ~69 kDa. The protein on a denaturing gel separates into two anti-hBVR immunoreactive proteins of ~39.9 + 34.6 kDa. The dimeric form, but not purified hBVR, binds to a 100-mer DNA fragment corresponding to the mouse HO-1 (hsp32) promoter region encompassing two activator protein (AP-1) sites. The specificity of DNA binding is suggested by the following: (a) hBVR does not bind to the same DNA fragment with one or zero AP-1 sites; (b) a 56-bp random DNA with one AP-1 site does not form a complex with hBVR; (c) in vitro translated HO-1 does not interact with the 100-mer DNA fragment with two AP-1 sites; (d) mutation of Lys143, Leu150, or Leu157 blocks both the formation of the ~69-kDa specimens and hBVR DNA complex formation; and (e) purified preparations of hBVR or hHO-1 do not bind to DNA with two AP-1 sites. The potential significance of the AP-1 binding is suggested by the finding that the response of HO-1, in COS cells stably transfected with antisense hBVR, with 66% reduced BVR activity, to superoxide anion ([IMG]img001.gif" ALT="O₂">) formed by menadione is attenuated, whereas induction by heme is not affected. We propose a role for BVR in the signaling cascade for AP-1 complex activation necessary for HO-1 oxidative stress response.

Allaman-Pillet, N., J. Storling, et al. (2003). "Calcium- and Proteasome-dependent Degradation of the JNK Scaffold Protein Islet-brain 1." J. Biol. Chem. **278**(49): 48720-48726.

http://www.jbc.org/cgi/content/abstract/278/49/48720

In models of type 1 diabetes, cytokines induce pancreatic {beta}-cell death by apoptosis. This process seems to be facilitated by a reduction in the amount of the islet-brain 1/JNK interacting protein 1 (IB1/JIP1), a JNK-scaffold with an anti-apoptotic effect. A point mutation S59N at the N terminus of the scaffold, which segregates in diabetic patients, has the functional consequence of sensitizing cells to apoptotic stimuli. Neither the mechanisms leading to IB1/JIP1 down-regulation by cytokines nor the mechanisms leading to the decreased capacity of the S59N mutation to protect cells from apoptosis are understood. Here, we show that IB1/JIP1 stability is modulated by intracellular calcium. The effect of calcium depends upon JNK activation, which primes the scaffold for ubiquitination-mediated degradation via the proteasome machinery. Furthermore, we observe that the S59N mutation decreases IB1/JIP1 stability by sensitizing IB1/JIP1 to calcium- and proteasome-dependent degradation. These data indicate that calcium influx initiated by cytokines mediates ubiquitination and degradation of IB1/JIP1 and may, therefore, provide a link between calcium influx and JNK-mediated apoptosis in pancreatic {beta}-cells.

Arai, H., T. Furuya, et al. (2004). "Neurotoxic Effects of Lipopolysaccharide on Nigral Dopaminergic Neurons Are Mediated by Microglial Activation, Interleukin-1{beta}, and Expression of Caspase-11 in Mice." J. Biol. Chem. **279**(49): 51647-51653.

http://www.jbc.org/cgi/content/abstract/279/49/51647

The endotoxin lipopolysaccharide (LPS), a component of the Gram-negative bacterial cell wall, selectively induces degeneration of substantia nigral (SN) dopaminergic neurons via activation of

microglial cells in rats and mice. Caspase-11 plays a crucial role in LPS-induced septic shock in mice. We examined the mechanism of LPS neurotoxicity on SN dopaminergic neurons in C57BL/6 mice and caspase-11 knockout mice. Mice were stereotaxically injected with LPS into the SN on one side and vehicle into the SN of the other side. Immunohistochemistry, Western blotting analysis, enzyme-linked immunosorbent assay, and reverse transcriptase-PCR were performed to evaluate damage of SN dopaminergic neurons and activation of microglial cells. Intranigral injection of LPS at 1 or 3 {micro}g/{micro}l/site decreased tyrosine hydroxylase-positive neurons and increased microglial cells in the SN compared with the contralateral side injected with vehicle at days 7 and 14 post-injection in C57BL/6 mice. Intranigral injection of LPS at 3 {micro}g/{micro}l/site induced the expression of caspase-11 mRNA in the ventral midbrain at 6, 8, and 12 h post-injection, and the expression of caspase-11-positive cells in the SN at 8 and 12 h post-injection. Moreover, LPS at 3 {micro}g/{micro}l/site increased interleukin-1{beta} content in the ventral midbrain at 12 and 24 h post-injection. LPS failed to elicit these responses in caspase-11 knockout mice. Our results indicate that the neurotoxic effects of LPS on nigral dopaminergic neurons are mediated by microglial activation, interleukin-1{beta}, and caspase-11 expression in mice.

Ayabe, T., H. Wulff, et al. (2002). "Modulation of Mouse Paneth Cell alpha -Defensin Secretion by mIKCa1, a Ca2+-activated, Intermediate Conductance Potassium Channel." J. Biol. Chem. **277**(5): 3793-3800.

http://www.jbc.org/cgi/content/abstract/277/5/3793

Paneth cells in small intestinal crypts secrete microbicidal [alpha]-defensins in response to bacteria and bacterial antigens (Ayabe, T., Satchell, D. P., Wilson, C. L., Parks, W. C., Selsted, M. E., and Ouellette, A. J. (2000) Nat. Immunol. 1, 113-138). We now report that the Ca2+-activated K+ channel mIKCa1 modulates mouse Paneth cell secretion. mIKCa1 cDNA clones identified in a mouse small intestinal crypt library by hybridization to human IKCa1 cDNA probes were isolated, and DNA sequence analysis showed that they were identical to mIKCa1 cDNAs isolated from erythroid cells and liver. The genomic organization was found to be conserved between mouse and human IKCa1 as shown by comparisons of the respective cDNA and genomic sequences. Reverse transcriptase-PCR experiments using nested primers amplified mIKCa1 from the lower half of bisected crypts and from single Paneth cells, but not from the upper half of bisected crypts, villus epithelium, or undifferentiated crypt epithelial cells, suggesting a lineage-specific role for mIKCa1 in mouse small bowel epithelium. The cloned mIKCa1 channel was calcium-activated and was blocked by ten structurally diverse peptide and nonpeptide inhibitors with potencies spanning 9 orders of magnitude and indistinguishable from that of the human homologue. Consistent with channel blockade, charybdotoxin, clotrimazole, and the highly selective IKCa1 inhibitors, TRAM-34 and TRAM-39, inhibited (~50%) Paneth cell secretion stimulated by bacteria or bacterial lipopolysaccharide, measured both as bactericidal activity and secreted cryptdin protein, but the inactive analog, TRAM-7, did not block secretion. These results demonstrate that mIKCa1 is modulator of Paneth cell [alpha]-defensin secretion and disclose an involvement in mucosal defense of the intestinal epithelium against ingested bacterial pathogens.

Barlic, J., D. H. McDermott, et al. (2004). "Interleukin (IL)-15 and IL-2 Reciprocally Regulate Expression of the Chemokine Receptor CX3CR1 through Selective NFAT1- and NFAT2-dependent Mechanisms." J. Biol. Chem. **279**(47): 48520-48534.

http://www.jbc.org/cgi/content/abstract/279/47/48520

We have recently reported that interleukin (IL)-15 and IL-2, which signal through IL-2R{beta}{gamma}, oppositely regulate expression of the proinflammatory chemokine receptor

CX3CR1. Here we delineate molecular mechanisms responsible for this paradox. By using a luciferase reporter plasmid, we identified a 433-bp region spanning the major transcriptional start point of human CX3CR1 that, when expressed in human peripheral blood mononuclear cells (PBMCs), possessed strong constitutive promoter activity. IL-2 and IL-15 treatment increased and abolished this activity, respectively, mimicking their effects on endogenous CX3CR1. IL-2 and IL-15 have been reported to also have opposite effects on the immunoregulatory transcription factor NFAT (nuclear factor of activated T cells), and the 433-bp region contains a {kappa}B-like NFAT site. The effects of IL-15 and IL-2 on both CX3CR1 reporter activity and endogenous CX3CR1 transcription in PBMCs were abolished by the NFAT inhibitors cyclosporin A and VIVIT. Moreover, mutation of the {kappa}B-like NFAT sequence markedly attenuated IL-2 and IL-15 modulation of CX3CR1 promoter-reporter activity in PBMCs. Furthermore, chromatin immunoprecipitation revealed that IL-15 promoted specific recruitment of NFAT1 but not NFAT2 to the CX3CR1 promoter, whereas IL-2 had the converse effect. This appears to be relevant in vivo because mouse CX3CR1 mRNA was expressed in both PBMCs and splenocytes from NFAT1-/- mice injected with recombinant IL-15 but was undetectable in cells from IL-15-injected NFAT1+/+ BALB/c mice; as predicted, IL-2 up-regulated cx3cr1 in both mouse strains to a similar extent. Thus, by pharmacologic, genetic, and biochemical criteria in vitro and in vivo, our results suggest that IL-15 and IL-2 oppositely regulate CX3CR1 gene expression by differentially recruiting NFAT1 and NFAT2 to a {kappa}B-like NFAT site within the CX3CR1 promoter. We propose that expression of CX3CR1 and possibly other immunoregulatory genes may be determined in part by the balance of NFAT1 and NFAT2 activity in leukocytes.

Basu, S. S., M. J. Karbarz, et al. (2002). "Expression Cloning and Characterization of the C28 Acyltransferase of Lipid A Biosynthesis in Rhizobium leguminosarum." J. Biol. Chem. **277**(32): 28959-28971.

http://www.jbc.org/cgi/content/abstract/277/32/28959

An unusual feature of lipid A from plant endosymbionts of the Rhizobiaceae family is the presence of a 27-hydroxyoctacosanoic acid (C28) moiety. An enzyme that incorporates this acyl chain is present in extracts of Rhizobium leguminosarum, Rhizobium etli, and Sinorhizobium meliloti but not Escherichia coli. The enzyme transfers 27-hydroxyoctacosanate from a specialized acyl carrier protein (AcpXL) to the precursor Kdo2 ((3-deoxy-D-manno-octulosonic acid)2)-lipid IVA. We now report the identification of five hybrid cosmids that direct the overexpression of this activity by screening ~4000 lysates of individual colonies of an R. leguminosarum 3841 genomic DNA library in the host strain S. meliloti 1021. In these heterologous constructs, both the C28 acyltransferase and C28-AcpXL are overproduced. Sequencing of a 9-kb insert from cosmid pSSB-1, which is also present in the other cosmids, shows that acpXL and the lipid A acyltransferase gene (lpxXL) are close to each other but not contiguous. Nine other open reading frames around lpxXL were also sequenced. Four of them encode orthologues of fatty acid and/or polyketide biosynthetic enzymes. AcpXL purified from S. meliloti expressing pSSB-1 is fully acylated, mainly with 27-hydroxyoctacosanoate. Expression of lpxXL in E. coli behind a T7 promoter results in overproduction in vitro of the expected R. leguminosarum acyltransferase, which is C28-AcpXL-dependent and utilizes (3-deoxy-D-manno-octulosonic acid)2-lipid IVA as the acceptor. These findings confirm that lpxXL is the structural gene for the C28 acyltransferase. LpxXL is distantly related to the lauroyltransferase (LpxL) of E. coli lipid A biosynthesis, but highly significant LpxXL orthologues are present in Agrobacterium tumefaciens, Brucella melitensis, and all sequenced strains of Rhizobium, consistent with the occurrence of long secondary acyl chains in the lipid A molecules of these organisms.

Beaulieu, N., S. Morin, et al. (2002). "An Essential Role for DNA Methyltransferase DNMT3B in Cancer Cell Survival." J. Biol. Chem. **277**(31): 28176-28181.

http://www.jbc.org/cgi/content/abstract/277/31/28176

Abnormal methylation and associated silencing of tumor suppressor genes is a common feature of many types of cancers. The observation of persistent methylation in human cancer cells lacking the maintenance methyltransferase DNMT1 suggests the involvement of other DNA methyltransferases in gene silencing in cancer. To test this hypothesis, we have evaluated methylation and gene expression in cancer cells specifically depleted of DNMT3A or DNMT3B, de novo methyltransferases that are expressed in adult tissues. Here we have shown that depletion of DNMT3B, but not DNMT3A, induced apoptosis of human cancer cells but not normal cells. DNMT3B depletion reactivated methylation-silenced gene expression but did not induce global or juxtacentromeric satellite demethylation as did specific depletion of DNMT1. Furthermore, the effect of DNMT3B depletion was rescued by exogenous expression of either of the splice variants DNMT3B2 or DNMT3B3 but not DNMT1. These results indicate that DNMT3B has significant site selectivity that is distinct from DNMT1, regulates aberrant gene silencing, and is essential for cancer cell survival.

Beekman, J. M., J. E. Bakema, et al. (2004). "Modulation of Fc{gamma}RI (CD64) Ligand Binding by Blocking Peptides of Periplakin." J. Biol. Chem. **279**(32): 33875-33881.

http://www.jbc.org/cgi/content/abstract/279/32/33875

Fc{gamma}RI requires both the intracellular domain of the {alpha}-chain and associated leukocyte Fc receptor (FcR) {gamma}-chains for its biological function. We recently found the C terminus of periplakin to selectively interact with the cytoplasmic domain of the Fc{gamma}RI {alpha}-chain. It thereby enhances the capacity of Fc{gamma}RI to bind, internalize, and present antigens on MHC class II. Here, we characterized the domains involved in Fc{gamma}RI-periplakin interaction using truncated and alanine-substituted Fc{gamma}RI mutants and randomly mutagenized periplakin. This allowed us to design TAT peptides that selectively interfered with endogenous Fc{gamma}RI-periplakin interactions. The addition of these peptides to Fc{gamma}RI-expressing cells modulated Fc{gamma}RI ligand binding, as assessed by erythrocyte-antibody-rosetting. These data support a dominant-negative role of C-terminal periplakin for Fc{gamma}RI biological activity and implicate periplakin as a novel regulator of Fc{gamma}RI in immune cells.

Benezra, M., N. Chevallier, et al. (2003). "BRCA1 Augments Transcription by the NF-{kappa}B Transcription Factor by Binding to the Rel Domain of the p65/RelA Subunit." J. Biol. Chem. **278**(29): 26333-26341.

http://www.jbc.org/cgi/content/abstract/278/29/26333

BRCA1 is a tumor suppressor gene mutated in cases of hereditary breast and ovarian cancer. BRCA1 protein is involved in apoptosis and growth/tumor suppression. In this study, we present evidence that p65/RelA, one of the two subunits of the transcription factor NF-{kappa}B, binds to the BRCA1 protein. Treatment of 293T cells with the cytokine tumor necrosis factor-{alpha} induces an interaction between endogenous p65/RelA and BRCA1. GST-protein affinity assay experiments reveal that the Rel homology domain of the p65/RelA subunit of NF-{kappa}B interacts with multiple sites within the N-terminal region of BRCA1. Transient transfection of BRCA1 significantly enhances the ability of the tumor necrosis factor-{alpha} or interleukin-1{beta} to activate transcription from the promoters of NF-{kappa}B target genes. Mutation of the NF-{kappa}B-binding sites in the NF-{kappa}B reporter blocks the effect of BRCA1 on transcription. Also the ability of BRCA1 to activate NF-{kappa}B target genes is inhibited by a

super-stable inhibitor of NF-{kappa}B and by the chemical inhibitor SN-50. These data indicate that BRCA1 acts as a co-activator with NF-{kappa}B. In addition, we show that cells infected with an adenovirus expressing BRCA1 up-regulate the endogenous expression of NF-{kappa}B target genes Fas and interferon-{beta}. Together, this information suggests that BRCA1 may play a role in cell life-death decisions following cell stress by modulation of the activity of NF-{kappa}B.

Benlloch, M., A. Ortega, et al. (2005). "Acceleration of Glutathione Efflux and Inhibition of {gamma}-Glutamyltranspeptidase Sensitize Metastatic B16 Melanoma Cells to Endothelium-induced Cytotoxicity." J. Biol. Chem. **280**(8): 6950-6959.

http://www.jbc.org/cgi/content/abstract/280/8/6950

Highly metastatic B16 melanoma (B16M)-F10 cells, as compared with the low metastatic B16M-F1 line, have higher GSH content and preferentially overexpress BCL-2. In addition to its anti-apoptotic properties, BCL-2 inhibits efflux of GSH from B16M-F10 cells and thereby may facilitate metastatic cell resistance against endothelium-induced oxidative/nitrosative stress. Thus, we investigated in B16M-F10 cells which molecular mechanisms channel GSH release and whether their modulation may influence metastatic activity. GSH efflux was abolished in multidrug resistance protein 1 knock-out (MRP-/-1) B16M-F10 transfected with the Bcl-2 gene or in MRP-/-1 B16M-F10 cells incubated with L-methionine, which indicates that GSH release from B16M-F10 cells is channeled through MRP1 and a BCL-2-dependent system (likely related to an L-methionine-sensitive GSH carrier previously detected in hepatocytes). The BCL-2-dependent system was identified as the cystic fibrosis transmembrane conductance regulator, since monoclonal antibodies against this ion channel or H-89 (a protein kinase A-selective inhibitor)-induced inhibition of cystic fibrosis transmembrane conductance regulator gene expression completely blocked the BCL-2-sensitive GSH release. By using a perifusion system that mimics in vivo conditions, we found that GSH depletion in metastatic cells can be achieved by using Bcl-2 antisense oligodeoxynucleotide- and verapamil (an MRP1 activator)-induced acceleration of GSH efflux, in combination with acivicin-induced inhibition of {gamma}-glutamyltranspeptidase (which limits GSH synthesis by preventing cysteine generation from extracellular GSH). When applied under in vivo conditions, this strategy increased tumor cytotoxicity (up to [~]90%) during B16M-F10 cell adhesion to the hepatic sinusoidal endothelium.

Bennin, D. A., A. S. A. Don, et al. (2002). "Cyclin G2 Associates with Protein Phosphatase 2A Catalytic and Regulatory B' Subunits in Active Complexes and Induces Nuclear Aberrations and a G1/S Phase Cell Cycle Arrest." J. Biol. Chem. **277**(30): 27449-27467.

http://www.jbc.org/cgi/content/abstract/277/30/27449

Cyclin G2, together with cyclin G1 and cyclin I, defines a novel cyclin family expressed in terminally differentiated tissues including brain and muscle. Cyclin G2 expression is up-regulated as cells undergo cell cycle arrest or apoptosis in response to inhibitory stimuli independent of p53 (Horne, M., Donaldson, K., Goolsby, G., Tran, D., Mulheisen, M., Hell, J. and Wahl, A. (1997) J. Biol. Chem. 272, 12650-12661). We tested the hypothesis that cyclin G2 may be a negative regulator of cell cycle progression and found that ectopic expression of cyclin G2 induces the formation of aberrant nuclei and cell cycle arrest in HEK293 and Chinese hamster ovary cells. Cyclin G2 is primarily partitioned to a detergent-resistant compartment, suggesting an association with cytoskeletal elements. We determined that cyclin G2 and its homolog cyclin G1 directly interact with the catalytic subunit of protein phosphatase 2A (PP2A). An okadaic acid-sensitive (<2 nM) phosphatase activity coprecipitates with endogenous and ectopic cyclin G2. We found that cyclin G2 also associates with various PP2A B' regulatory subunits, as previously shown for cyclin G1. The PP2A/A subunit is not detectable in cyclin G2-PP2A-B'-C complexes. Notably,

cyclin G2 colocalizes with both PP2A/C and B' subunits in detergent-resistant cellular compartments, suggesting that these complexes form in living cells. The ability of cyclin G2 to inhibit cell cycle progression correlates with its ability to bind PP2A/B' and C subunits. Together, our findings suggest that cyclin G2-PP2A complexes inhibit cell cycle progression.

Besteiro, S., M. Biran, et al. (2002). "Succinate Secreted by Trypanosoma brucei Is Produced by a Novel and Unique Glycosomal Enzyme, NADH-dependent Fumarate Reductase." J. Biol. Chem. **277**(41): 38001-38012.

http://www.jbc.org/cgi/content/abstract/277/41/38001

In all trypanosomatids, including Trypanosoma brucei, glycolysis takes place in peroxisome-like organelles called glycosomes. These are closed compartments wherein the energy and redox (NAD+/NADH) balances need to be maintained. We have characterized a T. brucei gene called FRDg encoding a protein 35% identical to Saccharomyces cerevisiae fumarate reductases. Microsequencing of FRDg purified from glycosome preparations, immunofluorescence, and Western blot analyses clearly identified this enzyme as a glycosomal protein that is only expressed in the procyclic form of T. brucei but is present in all the other trypanosomatids studied, i.e. Trypanosoma congolense, Crithidia fasciculata and Leishmania amazonensis. The specific inactivation of FRDg gene expression by RNA interference showed that FRDg is responsible for the NADH-dependent fumarate reductase activity detected in glycosomal fractions and that at least 60% of the succinate secreted by the T. brucei procyclic form (in the presence of D-glucose as the sole carbon source) is produced in the glycosome by FRDg. We conclude that FRDg plays a key role in the energy metabolism by participating in the maintenance of the glycosomal NAD+/NADH balance. We have also detected a significant pyruvate kinase activity in the cytosol of the T. brucei procyclic cells that was not observed previously. Consequently, we propose a revised model of glucose metabolism in procyclic trypanosomes that may also be valid for all other trypanosomatids except the T. brucei bloodstream form. Interestingly, H. Gest has hypothesized previously (Gest, H. (1980) FEMS Microbiol. Lett. 7, 73-77) that a soluble NADH-dependent fumarate reductase has been present in primitive organisms and evolved into the present day fumarate reductases, which are quinol-dependent. FRDg may have the characteristics of such an ancestral enzyme and is the only NADH-dependent fumarate reductase characterized to date.

Bierl, C., B. Voetsch, et al. (2004). "Determinants of Human Plasma Glutathione Peroxidase (GPx-3) Expression." J. Biol. Chem. **279**(26): 26839-26845.

http://www.jbc.org/cgi/content/abstract/279/26/26839

Plasma glutathione peroxidase (GPx-3) is a selenocysteine-containing protein with antioxidant properties. GPx-3 deficiency has been associated with cardiovascular disease and stroke. The regulation of GPx-3 expression remains largely uncharacterized, however, and we studied its transcriptional and translational determinants in a cultured cell system. In transient transfections of a renal cell line (Caki-2), the published sequence cloned upstream of a luciferase reporter gene produced minimal activity (relative luminescence (RL) = 0.6 {+/-} 0.4). Rapid amplification of cDNA ends was used to identify a novel transcription start site that is located 233 bp downstream (3') of the published site and that produced a >25-fold increase in transcriptional activity (RL = 16.8 {+/-} 1.9; $p < 0.0001$). Analysis of the novel GPx-3 promoter identified Sp-1- and hypoxia-inducible factor-1-binding sites, as well as the redox-sensitive metal response element and antioxidant response element. Hypoxia was identified as a strong transcriptional regulator of GPx-3 expression, in part through the presence of the hypoxia-inducible factor-1-binding site, leading to an almost 3-fold increase in expression levels after 24 h compared with normoxic conditions

(normalized RL = 3.5 {+/-} 0.3 versus 1.2 {+/-} 0.1; $p < 0.001$). We also investigated the role of the translational cofactors tRNASec, SECIS-binding protein-2, and SelD (selenophosphate synthetase D) in GPx-3 protein expression. tRNASec and SelD significantly enhanced GPx-3 expression, whereas SECIS-binding protein-2 showed a trend toward increased expression. These results demonstrate the presence of a novel functional transcription start site for the human GPx-3 gene with a promoter regulated by hypoxia, and identify unique translational determinants of GPx-3 expression.

Billich, A., F. Bornancin, et al. (2003). "Phosphorylation of the Immunomodulatory Drug FTY720 by Sphingosine Kinases." J. Biol. Chem. **278**(48): 47408-47415.

http://www.jbc.org/cgi/content/abstract/278/48/47408

The immunomodulatory drug FTY720 is phosphorylated in vivo, and the resulting FTY720 phosphate as a ligand for sphingosine-1-phosphate receptors is responsible for the unique biological effects of the compound. So far, phosphorylation of FTY720 by murine sphingosine kinase (SPHK) 1a had been documented. We found that, while FTY720 is also phosphorylated by human SPHK1, the human type 2 isoform phosphorylates the drug 30-fold more efficiently, because of a lower Km of FTY720 for SPHK2. Similarly, murine SPHK2 was more efficient than SPHK1a. Among splice variants of the human SPHKs, an N-terminally extended SPHK2 isoform was even more active than SPHK2 itself. Further SPHK superfamily members, namely ceramide kinase and a "SPHK-like" protein, failed to phosphorylate sphingosine and FTY720. Thus, only SPHK1 and 2 appear to be capable of phosphorylating FTY720. Using selective assay conditions, SPHK1 and 2 activities in murine tissues were measured. While activity of SPHK2 toward sphingosine was generally lower than of SPHK1, FTY720 phosphorylation was higher under conditions favoring SPHK2. In human endothelial cells, while activity of SPHK1 toward sphingosine was 2-fold higher than of SPHK2, FTY720 phosphorylation was 7-fold faster under SPHK2 assay conditions. Finally, FTY720 was poorly phosphorylated in human blood as compared with rodent blood, in line with the low activity of SPHK1 and in particular of SPHK2 in human blood. To conclude, both SPHK1 and 2 are capable of phosphorylating FTY720, but SPHK2 is quantitatively more important than SPHK1.

Boraston, A. B., E. Kwan, et al. (2003). "Recognition and Hydrolysis of Noncrystalline Cellulose." J. Biol. Chem. **278**(8): 6120-6127.

http://www.jbc.org/cgi/content/abstract/278/8/6120

Cellulase Cel5A from alkalophilic Bacillus sp. 1139 contains a family 17 carbohydrate-binding module (BspCBM17) and a family 28 CBM (BspCBM28) in tandem. The two modules have significantly similar amino acid sequences, but amino acid residues essential for binding are not conserved. BspCBM28 was obtained as a discrete polypeptide by engineering the cel5A gene. BspCBM17 could not be obtained as a discrete polypeptide, so a family 17 CBM from endoglucanase Cel5A of Clostridium cellulovorans, CcCBM17, was used to compare the binding characteristics of the two families of CBM. Both CcCBM17 and BspCBM28 recognized two classes of binding sites on amorphous cellulose: a high affinity site (Ka ~1 x 106 M[-]1) and a low affinity site (Ka ~2 x 104 M[-]1). They did not compete for binding to the high affinity sites, suggesting that they bound at different sites on the cellulose. A polypeptide, BspCBM17/CBM28, comprising the tandem CBMs from Cel5A, bound to amorphous cellulose with a significantly higher affinity than the sum of the affinities of CcCBM17 and BspCBM28, indicating cooperativity between the linked CBMs. Cel5A mutants were constructed that were defective in one or both of the CBMs. The mutants differed from the wild-type enzyme in the amounts and sizes of the soluble products produced from amorphous cellulose. This suggests that either the CBMs can

modify the action of the catalytic module of Cel5A or that they target the enzyme to areas of the cellulose that differ in susceptibility to hydrolysis.

Bozinovski, S., J. E. Jones, et al. (2002). "Granulocyte/Macrophage-Colony-stimulating Factor (GM-CSF) Regulates Lung Innate Immunity to Lipopolysaccharide through Akt/Erk Activation of NFkappa B and AP-1 in Vivo." J. Biol. Chem. **277**(45): 42808-42814.

http://www.jbc.org/cgi/content/abstract/277/45/42808

The lung innate immune response to lipopolysaccharide (LPS) coordinates cellular inflammation, mediator, and protease release essential for host defense but deleterious in asthma, chronic obstructive pulmonary disease, and cystic fibrosis. In vitro, LPS signals to the transcription factors NF[kappa]B via TLR4, MyD88, and IL-1R-associated kinase (IRAK), to AP-1 by mitogen-activated protein (MAP) kinases, and via an alternate route in IRAK-deficient mice, but the in vivo lung signaling pathway(s) are not understood. We investigated the role of Akt and Erk1/2 as LPS intensely stimulates granulocyte/macrophage-colony-stimulating factor (GM-CSF) release, and neutralizing GM-CSF profoundly suppressed LPS-induced inflammation, suppressed expression and activity of lung proteases, significantly reduced GM-CSF and tumor necrosis factor [alpha] (TNF[alpha]) mRNA expression, and dampened nuclear localization of both NF[kappa]B (p50/65) and AP-1. LPS markedly activated Akt and Erk1/2, but not p38, in a GM-CSF-dependent manner in direct temporal association with NF[kappa]B and AP-1 activation. Pharmacological inhibition of Akt or Erk activation in LPS-treated tracheal explants ex vivo inhibited the release of GM-CSF. These data implicate GM-CSF-dependent activation of Akt in the amplification of this response and demonstrate the role of Erks rather than p38 in lung LPS inflammatory responses. Inhibition of GM-CSF may be of therapeutic benefit in inflammatory diseases in which LPS contributes to lung damage.

Bream, J. H., D. L. Hodge, et al. (2004). "A Distal Region in the Interferon-{gamma} Gene Is a Site of Epigenetic Remodeling and Transcriptional Regulation by Interleukin-2." J. Biol. Chem. **279**(39): 41249-41257.

http://www.jbc.org/cgi/content/abstract/279/39/41249

Interferon-{gamma} (IFN-{gamma}) is a multifunctional cytokine that defines the development of Th1 cells and is critical for host defense against intracellular pathogens. IL-2 is another key immunoregulatory cytokine that is involved in T helper differentiation and is known to induce IFN-{gamma} expression in natural killer (NK) and T cells. Despite concerted efforts to identify the one or more transcriptional control mechanisms by which IL-2 induces IFN-{gamma} mRNA expression, no such genomic regulatory regions have been described. We have identified a DNase I hypersensitivity site [~]3.5-4.0 kb upstream of the transcriptional start site. Using chromatin immunoprecipitation assays we found constitutive histone H3 acetylation in this distal region in primary human NK cells, which is enhanced by IL-2 treatment. This distal region is also preferentially acetylated on histones H3 and H4 in primary Th1 cells as compared with Th2 cells. Within this distal region we found a Stat5-like motif, and in vitro DNA binding assays as well as in vivo chromosomal immunoprecipitation assays showed IL-2-induced binding of both Stat5a and Stat5b to this distal element in the IFNG gene. We examined the function of this Stat5-binding motif by transfecting human peripheral blood mononuclear cells with -3.6 kb of IFNG-luciferase constructs and found that phorbol 12-myristate 13-acetate/ionomycin-induced transcription was augmented by IL-2 treatment. The effect of IL-2 was lost when the Stat5 motif was disrupted. These data led us to conclude that this distal region serves as both a target of chromatin remodeling in the IFNG locus as well as an IL-2-induced transcriptional enhancer that binds Stat5 proteins.

Brown, A. J., S. M. Goldsworthy, et al. (2003). "The Orphan G Protein-coupled Receptors GPR41 and GPR43 Are Activated by Propionate and Other Short Chain Carboxylic Acids." J. Biol. Chem. **278**(13): 11312-11319.

http://www.jbc.org/cgi/content/abstract/278/13/11312

GPR41 and GPR43 are related members of a homologous family of orphan G protein-coupled receptors that are tandemly encoded at a single chromosomal locus in both humans and mice. We identified the acetate anion as an agonist of human GPR43 during routine ligand bank screening in yeast. This activity was confirmed after transient transfection of GPR43 into mammalian cells using Ca2+ mobilization and [35S]guanosine 5'-O-(3-thiotriphosphate) binding assays and by coexpression with GIRK G protein-regulated potassium channels in Xenopus laevis oocytes. Other short chain carboxylic acid anions such as formate, propionate, butyrate, and pentanoate also had agonist activity. GPR41 is related to GPR43 (52% similarity; 43% identity) and was activated by similar ligands but with differing specificity for carbon chain length, with pentanoate being the most potent agonist. A third family member, GPR42, is most likely a recent gene duplication of GPR41 and may be a pseudogene. GPR41 was expressed primarily in adipose tissue, whereas the highest levels of GPR43 were found in immune cells. The identity of the cognate physiological ligands for these receptors is not clear, although propionate is known to occur in vivo at high concentrations under certain pathophysiological conditions.

Budagian, V., E. Bulanova, et al. (2003). "Signaling through P2X7 Receptor in Human T Cells Involves p56lck, MAP Kinases, and Transcription Factors AP-1 and NF-kappa B." J. Biol. Chem. **278**(3): 1549-1560.

http://www.jbc.org/cgi/content/abstract/278/3/1549

ATP-gated ion channel P2X receptors are expressed on the surface of most immune cells and can trigger multiple cellular responses, such as membrane permeabilization, cytokine production, and cell proliferation or apoptosis. Despite broad distribution and pleiotropic activities, signaling pathways downstream of these ionotropic receptors are still poorly understood. Here, we describe intracellular signaling events in Jurkat cells treated with millimolar concentrations of extracellular ATP. Within minutes, ATP treatment resulted in the phosphorylation and activation of p56lck kinase, extracellular signal-regulated kinase (ERK), and c-Jun N-terminal kinase but not p38 kinase. These effects were wholly dependent upon the presence of extracellular Ca2+ ions in the culture medium. Nevertheless, calmodulin antagonist calmidazolium and CaM kinase inhibitor KN-93 both had no effect on the activation of p56lck and ERK, whereas a pretreatment of Jurkat cells with MAP kinase kinase inhibitor P098059 was able to abrogate phosphorylation of ERK. Further, expression of c-Jun and c-Fos proteins and activator protein (AP-1) DNA binding activity were enhanced in a time-dependent manner. In contrast, DNA binding activity of NF-[kappa]B was reduced. ATP failed to stimulate the phosphorylation of ERK and c-Jun N-terminal kinase and activation of AP-1 in the p56lck-deficient isogenic T cell line JCaM1, suggesting a critical role for p56lck kinase in downstream signaling. Regarding the biological significance of the ATP-induced signaling events we show that although extracellular ATP was able to stimulate proliferation of both Jurkat and JCaM1 cells, an increase in interleukin-2 transcription was observed only in Jurkat cells. The nucleotide selectivity and pharmacological profile data supported the evidence that the ATP-induced effects in Jurkat cells were mediated through the P2X7 receptor. Taken together, these results demonstrate the ability of extracellular ATP to activate multiple downstream signaling events in a human T-lymphoblastoid cell line.

Cabral, A., D. F. Fischer, et al. (2003). "Distinct Functional Interactions of Human Skn-1 Isoforms with Ese-1 during Keratinocyte Terminal Differentiation." J. Biol. Chem. **278**(20): 17792-17799.

http://www.jbc.org/cgi/content/abstract/278/20/17792

Among the three major POU proteins expressed in human skin, Oct-1, Tst-1/Oct-6, and Skn-1/Oct-11, only the latter induced SPRR2A, a marker of keratinocyte terminal differentiation. In this study, we have identified three Skn-1 isoforms, which encode proteins with various N termini, generated by alternative promoter usage. These isotypes showed distinct expression patterns in various skin samples, internal squamous epithelia, and cultured human keratinocytes. Skn-1a and Skn-1d1 bound the SPRR2A octamer site with comparable affinity and functioned as transcriptional activators. Skn-1d2 did not affect SPRR2A expression. Skn-1a, the largest protein, functionally cooperated with Ese-1/Elf-3, an epithelial-specific transcription factor, previously implicated in SPRR2A induction. This cooperativity, which depended on an N-terminal pointed-like domain in Skn-1a, was not found for Skn-1d1. Actually, Skn-1d1 counteracted the cooperativity between Skn-1a and Ese-1. Apparently, the human Skn-1 locus encodes multifunctional protein isotypes, subjected to biochemical cross-talk, which are likely to play a major role in the fine-tuning of keratinocyte terminal differentiation.

Cabral, W. A., A. Fertala, et al. (2002). "Procollagen with Skipping of alpha 1(I) Exon 41 Has Lower Binding Affinity for alpha 1(I) C-telopeptide, Impaired in Vitro Fibrillogenesis, and Altered Fibril Morphology." J. Biol. Chem. **277**(6): 4215-4222.

http://www.jbc.org/cgi/content/abstract/277/6/4215

Previous in vitro data on type I collagen self-assembly into fibrils suggested that the amino acid 776-796 region of the [alpha]1(I) chain is crucial for fibril formation because it serves as the recognition site for the telopeptide of a docking collagen monomer. We used a natural collagen mutation with a deletion of amino acids 766-801 to confirm the importance of this region for collagen fibril formation. The proband has type III osteogenesis imperfecta and is heterozygous for a COL1A1 IVS 41 A+4 [right-arrow] C substitution. The intronic mutation causes splicing of exon 41, confirmed by sequencing of normal and shorter reverse transcriptase-PCR products. Reverse transcriptase-PCR using RNA from proband dermal fibroblasts and clonal cell lines showed the mutant cDNA was about 15% of total [alpha]1(I) cDNA. The mutant transcript is translated; structurally abnormal [alpha] chains are demonstrated in the cell layer of proband fibroblasts by SDS-urea-PAGE. The proportion of mutant chains in the secreted procollagen was determined to be 10% by resistance to digestion with MMP-1, since chains lacking exon 41 are missing the vertebral collagenase cleavage site. Secreted proband collagen was used for analysis of kinetics of binding of [alpha]1(I) C-telopeptide using an optical biosensor. Telopeptide had slower association and faster dissociation from proband than from normal collagen. Purified proband pC-collagen was used to study fibril formation. The presence of the mutant molecules decreases the rate of fibril formation. The fibrils formed in the presence of 10-15% mutant molecules have strikingly increased length compared with normal collagen, but are well organized, as demonstrated by D-periodicity. These results suggest that some collagen molecules containing the mutant chain are incorporated into fibrils and that the absence of the telopeptide binding region from even a small portion of the monomers interferes with fibril growth. Both abnormal fibrils and slower remodeling may contribute to the severe phenotype.

Cabral, W. A., E. Makareeva, et al. (2005). "Mutations near amino end of alpha 1(I) collagen cause combined OI/EDS by interference with N-propeptide processing." J. Biol. Chem.: M414698200.

http://www.jbc.org/cgi/content/abstract/M414698200v1

Patients with OI/EDS form a distinct subset of osteogenesis imperfecta patients. In addition to skeletal fragility, they have characteristics of Ehlers-Danlos syndrome. We identified 7 children with types III or IV OI, plus severe large and small joint laxity and early progressive scoliosis. In each child with OI/EDS, we identified a mutation in the first 90 residues of the helical region of [alpha]1(I) collagen. These mutations prevent or delay removal of the procollagen N-propeptide by purified N-proteinase (ADAMTS-2) in vitro and in pericellular assays. The mutant pN-collagen which results is efficiently incorporated into matrix by cultured fibroblasts and osteoblasts and is prominently present in newly incorporated and immaturely crosslinked collagen. Dermal collagen fibrils have significantly reduced cross-sectional diameters, corroborating incorporation of pN-collagen into fibrils in vivo. Differential scanning calorimetry revealed that these mutant collagens are less stable than the corresponding procollagens, which is not seen with other type I collagen helical mutations. These mutations disrupt a distinct folding region of high thermal stability in the first 90 residues at the amino end of type I collagen and alter the secondary structure of the adjacent N-proteinase cleavage site. Thus, these OI/EDS collagen mutations are directly responsible for the bone fragility of OI and indirectly responsible for EDS symptoms, by interference with N-propeptide removal.

Cabral, W. A., M. V. Mertts, et al. (2003). "Type I Collagen Triplet Duplication Mutation in Lethal Osteogenesis Imperfecta Shifts Register of alpha Chains throughout the Helix and Disrupts Incorporation of Mutant Helices into Fibrils and Extracellular Matrix." J. Biol. Chem. **278**(12): 10006-10012.

http://www.jbc.org/cgi/content/abstract/278/12/10006

The majority of collagen mutations causing osteogenesis imperfecta (OI) are glycine substitutions that disrupt formation of the triple helix. A rare type of collagen mutation consists of a duplication or deletion of one or two Gly-X-Y triplets. These mutations shift the register of collagen chains with respect to each other in the helix but do not interrupt the triplet sequence, yet they have severe clinical consequences. We investigated the effect of shifting the register of the collagen helix by a single Gly-X-Y triplet on collagen assembly, stability, and incorporation into fibrils and matrix. These studies utilized a triplet duplication in COL1A1 exon 44 that occurred in the cDNA and gDNA of two siblings with lethal OI. The normal allele encodes three identical Gly-Ala-Hyp triplets at aa 868-876, whereas the mutant allele encodes four. The register shift delays helix formation, causing overmodification. Differential scanning calorimetry yielded a decrease in Tm of 2 {degrees}C for helices with one mutant chain and a 6 {degrees}C decrease in helices with two mutant chains. An in vitro binary co-processing assay of N-proteinase cleavage demonstrated that procollagen with the triplet duplication has slower N-propeptide cleavage than in normal controls or procollagen with pro[alpha]1(I) G832S, G898S, or G997S substitutions, showing that the register shift persists through the entire helix. The register shift disrupts incorporation of mutant collagen into fibrils and matrix. Proband fibrils formed inefficiently in vitro and contained only normal helices and helices with a single mutant chain. Helices with two mutant chains and a significant portion of helices with one mutant chain did not form fibrils. In matrix deposited by proband fibroblasts, mutant chains were abundant in the immaturely cross-linked fraction but constituted a minor fraction of maturely cross-linked chains. The profound effects of shifting the collagen triplet register on chain interactions in the helix and on fibril formation correlate with the severe clinical consequences.

Carrillo, C. D., E. Taboada, et al. (2004). "Genome-wide Expression Analyses of Campylobacter jejuni NCTC11168 Reveals Coordinate Regulation of Motility and Virulence by flhA." J. Biol. Chem. **279**(19): 20327-20338.

http://www.jbc.org/cgi/content/abstract/279/19/20327

We examined two variants of the genome-sequenced strain, Campylobacter jejuni NCTC11168, which show marked differences in their virulence properties including colonization of poultry, invasion of Caco-2 cells, and motility. Transcript profiles obtained from whole genome DNA microarrays and proteome analyses demonstrated that these differences are reflected in late flagellar structural components and in virulence factors including those involved in flagellar glycosylation and cytolethal distending toxin production. We identified putative {sigma}28 and {sigma}54 promoters for many of the affected genes and found that greater differences in expression were observed for {sigma}28-controlled genes. Inactivation of the gene encoding {sigma}28, fliA, resulted in an unexpected increase in transcripts with {sigma}54 promoters, as well as decreased transcription of {sigma}28-regulated genes. This was unlike the transcription profile observed for the attenuated C. jejuni variant, suggesting that the reduced virulence of this organism was not entirely due to impaired function of {sigma}28. However, inactivation of flhA, an important component of the flagellar export apparatus, resulted in expression patterns similar to that of the attenuated variant. These findings indicate that the flagellar regulatory system plays an important role in campylobacter pathogenesis and that flhA is a key element involved in the coordinate regulation of late flagellar genes and of virulence factors in C. jejuni.

Cecchetto, G., S. Amillis, et al. (2004). "The AzgA Purine Transporter of Aspergillus nidulans: CHARACTERIZATION OF A PROTEIN BELONGING TO A NEW PHYLOGENETIC CLUSTER." J. Biol. Chem. **279**(5): 3132-3141.

http://www.jbc.org/cgi/content/abstract/279/5/3132

The azgA gene of Aspergillus nidulans encodes a hypoxanthine-adenine-guanine transporter. It has been cloned by a novel transposon methodology. The null phenotype of azgA was defined by a number of mutations, including a large deletion. In mycelia, the azgA gene is, like other genes of purine catabolism, induced by uric acid and repressed by ammonium. Its transcription depends on the pathway-specific UaY zinc binuclear cluster protein and the broad domain AreA GATA factor. AzgA is not closely related to any other characterized membrane protein, but many close homologues of unknown function are present in fungi, plants, and prokaryotes but not metazoa. Two of three data bases and the phylogeny presented in this article places proteins of this family in a cluster clearly separated (but perhaps phylogenetically related) from the NAT family that includes other eukaryotic and prokaryotic nucleobase transporters. Thus AzgA is the first characterized member of this family or subfamily of membrane proteins.

Chang, W.-T. and A. M. Huang (2004). "{alpha}-Pal/NRF-1 Regulates the Promoter of the Human Integrin-associated Protein/CD47 Gene." J. Biol. Chem. **279**(15): 14542-14550.

http://www.jbc.org/cgi/content/abstract/279/15/14542

Integrin-associated protein (IAP or CD47) is expressed in a variety of tissues, including the nervous system and immune system. To understand how cells control the expression of the IAP gene, we cloned the 5'-proximal region of the human IAP gene and investigated IAP promoter activity by transient transfection. RT-PCR confirmed the expression of IAP transcripts in human neuroblastoma IMR-32 and hepatoma HepG2 cells. Deletion analysis identified a core promoter of the human IAP gene located between nucleotide positions -232 and -12 relative to the translation initiation codon in these two cell lines. Site-directed mutagenesis and gel electrophoretic mobility shift assay identified a {alpha}-Pal/NRF-1 binding element within the IAP core promoter. Supershift assays using the {alpha}-Pal/NRF-1 antiserum confirmed the binding of

this transcription factor on the {alpha}-Pal/NRF-1 site. Overexpression of the DNA binding domain of {alpha}-Pal/NRF-1 in cells enhanced DNA-{alpha}-Pal/NRF-1 binding in vitro. Furthermore, overexpression of full-length {alpha}-Pal/NRF-1 significantly enhanced IAP promoter activity while overexpression of dominant-negative mutant reduced promoter activity both in the cultured human cell lines and primary mouse cortical cells. These results revealed that {alpha}-Pal/NRF-1 is an essential transcription factor in the regulation of human IAP gene expression.

Cheng, D., T. C. Nelson, et al. (2003). "Identification of Acyl Coenzyme A:Monoacylglycerol Acyltransferase 3, an Intestinal Specific Enzyme Implicated in Dietary Fat Absorption." J. Biol. Chem. **278**(16): 13611-13614.

http://www.jbc.org/cgi/content/abstract/278/16/13611

Acyl coenzyme A:monoacylglycerol acyltransferase (MGAT) catalyzes the synthesis of diacylglycerol using 2-monoacylglycerol and fatty acyl coenzyme A. This enzymatic reaction is believed to be an essential and rate-limiting step for the absorption of fat in the small intestine. Although the first MGAT-encoding cDNA, designated MGAT1, has been recently isolated, it is not expressed in the small intestine and hence cannot account for the high intestinal MGAT enzyme activity that is important for the physiology of fat absorption. In the current study, we report the identification of a novel MGAT, designated MGAT3, and present evidence that it fulfills the criteria to be the elusive intestinal MGAT. MGAT3 encodes a ~36-kDa transmembrane protein that is highly homologous to MGAT1 and -2. In humans, expression of MGAT3 is restricted to gastrointestinal tract with the highest level found in the ileum. At the cellular level, recombinant MGAT3 is localized to the endoplasmic reticulum. Recombinant MGAT3 enzyme activity produced in insect Sf9 cells selectively acylates 2-monoacylglycerol with higher efficiency than other stereoisomers. The molecular identification of MGAT3 will facilitate the evaluation of using intestinal MGAT as a potential point of intervention for antiobesity therapies.

Cheng, S., H. Afif, et al. (2004). "Activation of Peroxisome Proliferator-activated Receptor {gamma} Inhibits Interleukin-1{beta}-induced Membrane-associated Prostaglandin E2 Synthase-1 Expression in Human Synovial Fibroblasts by Interfering with Egr-1." J. Biol. Chem. **279**(21): 22057-22065.

http://www.jbc.org/cgi/content/abstract/279/21/22057

Membrane-associated prostaglandin (PG) E2 synthase-1 (mPGES-1) catalyzes the conversion of PGH2 to PGE2, which contributes to many biological processes. Peroxisome proliferator-activated receptor {gamma} (PPAR{gamma}) is a ligand-activated transcription factor and plays an important role in growth, differentiation, and inflammation in different tissues. Here, we examined the effect of PPAR{gamma} ligands on interleukin-1{beta} (IL-1{beta})-induced mPGES-1 expression in human synovial fibroblasts. PPAR{gamma} ligands 15-deoxy-{Delta}12,14 prostaglandin J2 (15d-PGJ2) and the thiazolidinedione troglitazone (TRO), but not PPAR{alpha} ligand Wy14643, dose-dependently suppressed IL-1{beta}-induced PGE2 production, as well as mPGES-1 protein and mRNA expression. 15d-PGJ2 and TRO suppressed IL-1{beta}-induced activation of the mPGES-1 promoter. Overexpression of wild-type PPAR{gamma} further enhanced, whereas overexpression of a dominant negative PPAR{gamma} alleviated, the suppressive effect of both PPAR{gamma} ligands. Furthermore, pretreatment with an antagonist of PPAR{gamma}, GW9662, relieves the suppressive effect of PPAR{gamma} ligands on mPGES-1 protein expression, suggesting that the inhibition of mPGES-1 expression is mediated by PPAR{gamma}. We demonstrated that PPAR{gamma} ligands suppressed Egr-1-mediated induction of the activities of the mPGES-1 promoter and of a synthetic reporter construct containing three tandem repeats of an Egr-1 binding site. The

suppressive effect of PPAR{gamma} ligands was enhanced in the presence of a PPAR{gamma} expression plasmid. Electrophoretic mobility shift and supershift assays for Egr-1 binding sites in the mPGES-1 promoter showed that both 15d-PGJ2 and TRO suppressed IL-1{beta}-induced DNA-binding activity of Egr-1. These data define mPGES-1 and Egr-1 as novel targets of PPAR{gamma} and suggest that inhibition of mPGES-1 gene transcription may be one of the mechanisms by which PPAR{gamma} regulates inflammatory responses.

Chiang, M.-C., Y.-C. Lee, et al. (2005). "cAMP-response Element-binding Protein Contributes to Suppression of the A2A Adenosine Receptor Promoter by Mutant Huntingtin with Expanded Polyglutamine Residues." J. Biol. Chem. **280**(14): 14331-14340.

http://www.jbc.org/cgi/content/abstract/280/14/14331

Huntington's disease is a neurodegenerative disease resulting from a CAG (glutamine) trinucleotide expansion in exon 1 of the Huntingtin (Htt) gene. The role of the striatum-enriched A2A adenosine receptor (A2A-R) in Huntington's disease has attracted much attention lately. In the present study, we found that expression of mutant Htt with expanded poly(Q) significantly reduced the transcript levels of the endogenous A2A-R in PC12 cells and primary striatal neurons. Cotransfection of various promoter constructs of the A2A-R gene and an expression construct of poly(Q)-expanded Htt revealed that the Htt mutant suppressed the core promoter activity of the A2A-R gene. Stimulation of the A2A-R using CGS21680 forskolin, and a constitutively active cAMP-response element-binding protein (CREB) mutant elevated the reduced promoter activity of the A2A-R gene by mutant Htt. Moreover, the effect of CGS was blocked by an A2A-R-selective antagonist (CSC), two inhibitors of protein kinase A, and two dominant negative mutants of (CREB). The protein kinase A/CREB pathway therefore is involved in regulating A2A-R promoter activity. Consistently, an atypical CRE site (TCCAGG) is located in the core promoter region of the A2A-R gene. Electrophoretic gel mobility shift assay and mutational inactivation further demonstrated the functional binding of CREB to the core promoter region and showed that expression of poly(Q)-expanded Htt abolished the binding of CREB to this site. Stimulation of the A2A-R restored the reduced CREB binding caused by the mutant and concurrently reduced mutant Htt aggregation. Collectively, the poly(Q)-expanded mutant Htt suppressed expression of the A2A-R by inhibiting its core promoter at least partially by preventing CREB binding.

Chipuk, J. E., L. V. Stewart, et al. (2002). "Identification and Characterization of A Novel Rat Ov-Serpin Family Member, Trespin." J. Biol. Chem. **277**(29): 26412-26421.

http://www.jbc.org/cgi/content/abstract/277/29/26412

Serpins are responsible for regulating a variety of proteolytic processes through a unique irreversible suicide substrate mechanism. To discover novel genes regulated by transforming growth factor-[beta]1 (TGF-[beta]1), we performed differential display reverse transcriptase-PCR analysis of NRP-152 rat prostatic epithelial cells and cloned a novel rat serpin that is transcriptionally down-regulated by TGF-[beta] and hence named trespin (TGF-[beta]-repressible serine proteinase inhibitor (trespin). Trespin is a 397-amino acid member of the ov-serpin clade with a calculated molecular mass of 45.2 kDa and 72% amino acid sequence homology to human bomapin; however, trespin exhibits different tissue expression, cellular localization, and proteinase specificity compared with bomapin. Trespin mRNA is expressed in many tissues, including brain, heart, kidney, liver, lung, prostate, skin, spleen, and stomach. FLAG-trespin expressed in HEK293 cells is localized predominantly in the cytoplasm and is not constitutively secreted. The presence of an arginine at the P1 position of trespin's reactive site loop suggests that trespin inhibits trypsin-like proteinases. Accordingly, in vitro transcribed and translated trespin

forms detergent-stable and thermostable complexes with plasmin and elastase but not subtilisin A, trypsin, chymotrypsin, thrombin, or papain. Trespin interacts with plasmin at a near 1:1 stoichiometry, and immunopurified mammal-expressed trespin inhibits plasmin in a dose-dependent manner. These data suggest that trespin is a novel and functional member of the rat ov-serpin family.

Collin, M. and V. A. Fischetti (2004). "A Novel Secreted Endoglycosidase from Enterococcus faecalis with Activity on Human Immunoglobulin G and Ribonuclease B." J. Biol. Chem. **279**(21): 22558-22570.

http://www.jbc.org/cgi/content/abstract/279/21/22558

The human pathogen Enterococcus faecalis can degrade the N-linked glycans of human RNase B to acquire nutrients, but no gene or protein has been associated with this activity. We identified an 88-kDa secreted protein, endoglycosidase (Endo) E, which is most likely responsible for this activity. EndoE, encoded by ndoE, consists of an {alpha}-domain with a family 18 glycosyl hydrolase motif and a {beta}-domain similar to family 20 glycosyl hydrolases. Phylogenetic analysis of EndoE indicates that the {alpha}-domain is related to human chitobiases, and the {beta}-domain is related to bacterial and human hexosaminidases. Recombinant expression of full-length EndoE or EndoE{alpha}, site-directed mutagenesis of the catalytic residues, mass spectroscopy, and homology modeling shows that EndoE{alpha} hydrolyzes the glycan on human RNase B, whereas EndoE{beta} hydrolyzes the conserved glycan on IgG. Denaturation experiments indicate that the chitinase activity on RNase B is not dependent on the tertiary structure, although it is on IgG. The ndoE gene and secreted EndoE are present in most E. faecalis but not in Enterococcus faecium isolates. Correspondingly, E. faecalis, but not E. faecium, degrades the glycan on RNase B during growth. Thus, we have identified a secreted enzyme from E. faecalis, EndoE, which by two distinct activities hydrolyzes the glycans on RNase B and IgG. Both activities could be important for the molecular pathogenesis and persistence of E. faecalis during human infections.

Dai, E., H. Guan, et al. (2003). "Serp-1, a Viral Anti-inflammatory Serpin, Regulates Cellular Serine Proteinase and Serpin Responses to Vascular Injury." J. Biol. Chem. **278**(20): 18563-18572.

http://www.jbc.org/cgi/content/abstract/278/20/18563

Complex DNA viruses have tapped into cellular serpin responses that act as key regulatory steps in coagulation and inflammatory cascades. Serp-1 is one such viral serpin that effectively protects virus-infected tissues from host inflammatory responses. When given as purified protein, Serp-1 markedly inhibits vascular monocyte invasion and plaque growth in animal models. We have investigated mechanisms of viral serpin inhibition of vascular inflammatory responses. In vascular injury models, Serp-1 altered early cellular plasminogen activator (tissue plasminogen activator), inhibitor (PAI-1), and receptor (urokinase-type plasminogen activator) expression ($p < 0.01$). Serp-1, but not a reactive center loop mutant, up-regulated PAI-1 serpin expression in human endothelial cells. Treatment of endothelial cells with antibody to urokinase-type plasminogen activator and vitronectin blocked Serp-1-induced changes. Significantly, Serp-1 blocked intimal hyperplasia ($p < 0.0001$) after aortic allograft transplant ($p < 0.0001$) in PAI-1-deficient mice. Serp-1 also blocked plaque growth after aortic isograft transplant and after wire-induced injury ($p < 0.05$) in PAI-1-deficient mice indicating that increase in PAI-1 expression is not required for Serp-1 to block vasculopathy development. Serp-1 did not inhibit plaque growth in uPAR-deficient mice after aortic allograft transplant. We conclude that the poxviral serpin, Serp-1, attenuates vascular inflammatory responses to injury through a pathway mediated by native uPA receptors and vitronectin.

Danielli, A., F. C. Kafatos, et al. (2003). "Cloning and Characterization of Four Anopheles gambiae Serpin Isoforms, Differentially Induced in the Midgut by Plasmodium berghei Invasion." J. Biol. Chem. **278**(6): 4184-4193.

http://www.jbc.org/cgi/content/abstract/278/6/4184

The genomic locus SRPN10 of the malaria vector Anopheles gambiae codes for four alternatively spliced serine protease inhibitors of the serpin superfamily. The four 40- to 42-kDa isoforms differ only at their C terminus, which bears the reactive site loop, and exhibit protein sequence similarity with other insect serpins and mammalian serpins of the ovalbumin family. Inhibition experiments with recombinant purified SRPN10 serpins reveal distinct and specific inhibitory activity of three isoforms toward different proteases. All isoforms are mainly expressed in the midgut but also in pericardial cells and hemocytes of the mosquito. The cellular localization of SRPN10 serpins is nucleocytoplasmic in pericardial cells, in hemocytes and in a hemocyte-like mosquito cell line, but in the gut the proteins are mostly localized in the nucleus. Although the transcript levels of all SRPN10 isoforms are marginally affected by bacterial challenge, the transcripts of two isoforms (KRAL and RCM) are induced in female mosquitoes in response to midgut invasion by Plasmodium berghei ookinetes. The KRAL and RCM SRPN10 isoforms represent new potential markers to study the ookinete midgut invasion process in anopheline mosquitoes.

Dave, V., T. Childs, et al. (2004). "Nuclear Factor of Activated T Cells Regulates Transcription of the Surfactant Protein D Gene (Sftpd) via Direct Interaction with Thyroid Transcription Factor-1 in Lung Epithelial Cells." J. Biol. Chem. **279**(33): 34578-34588.

http://www.jbc.org/cgi/content/abstract/279/33/34578

Surfactant protein D (SP-D) plays critical roles in host defense, surfactant homeostasis, and pulmonary immunomodulation. Here, we identify a role of nuclear factor of activated T cells (NFATs) in regulation of murine SP-D gene (Sftpd) transcription. An NFAT-dependent enhancer modulated by NFATs or calcineurin and sensitive to cyclosporin was identified in the Sftpd promoter. Ionomycin and phorbol 12-myristate 13-acetate further increased the activity of this enhancer, whereas VIVIT, a potent NFAT inhibitor peptide, selectively interfered with the calcineurin-NFAT interaction and abolished enhancer function. Gel supershift and DNase I protection assays identified DNA elements that bind NFAT in the Sftpd promoter. Calcineurin and NFATc3 proteins were detected in the embryonic and adult mouse lung epithelium, and the mRNA expression profiles of the NFATs were similar in immortalized mouse lung epithelial cells and alveolar epithelial type II cells. NFATc3 and TTF-1 activated the Sftpd promoter, synergized transcription, co-immunoprecipitated from mouse lung epithelial cells, and physically interacted in vitro. Components of the calcineurin/NFAT pathway were identified in respiratory epithelial cells of the lung that potentially augment rapid assembly of a multiprotein transcription complex on Sftpd promoter inducing SP-D expression.

Deroo, T., T. Denayer, et al. (2004). "Global Inhibition of Lef1/Tcf-dependent Wnt Signaling at Its Nuclear End Point Abrogates Development in Transgenic Xenopus Embryos." J. Biol. Chem. **279**(49): 50670-50675.

http://www.jbc.org/cgi/content/abstract/279/49/50670

Analysis of canonical Wnt signaling during vertebrate development by means of knock-out or

transgenic approaches is often hampered by functional redundancy as well as pathway bifurcations downstream of the manipulated components. We report the design of an optimized chimera capable of blocking transcriptional activation of Lef1/Tcf-{beta}-catenin target genes, thus enabling intervention with the canonical Wnt pathway at its nuclear end point. This construct was made hormone-inducible, both functionally and transcriptionally, and was transgenically integrated in Xenopus embryos. Down-regulation of target genes was clearly observed upon treatment of these embryos with dexamethasone. In addition, exposure of variously aged transgenic embryos to dexamethasone caused complex phenotypes with many new but also several recognizable features stemming from inhibition of canonical Wnt signaling. At least in some tissues, a significant reduction in cell proliferation and an increase in programmed cell death appeared to underlie these phenotypes. Our inducible transgenic system can serve a broad range of experimental settings designed to unveil new functional aspects of Lef1/Tcf-{beta}-catenin signaling during vertebrate embryogenesis.

Dettwiler, S., C. Aringhieri, et al. (2004). "Distinct Sequence Motifs within the 68-kDa Subunit of Cleavage Factor Im Mediate RNA Binding, Protein-Protein Interactions, and Subcellular Localization." J. Biol. Chem. **279**(34): 35788-35797.

http://www.jbc.org/cgi/content/abstract/279/34/35788

Cleavage factor Im (CF Im) is required for the first step in pre-mRNA 3'-end processing and can be reconstituted in vitro from its heterologously expressed 25- and 68-kDa subunits. The binding of CF Im to the pre-mRNA is one of the earliest steps in the assembly of the cleavage and polyadenylation machinery and facilitates the recruitment of other processing factors. We identified regions in the subunits of CF Im involved in RNA binding, protein-protein interactions, and subcellular localization. CF Im68 has a modular domain organization consisting of an N-terminal RNA recognition motif and a C-terminal alternating charge domain. However, the RNA recognition motif of CF Im68 on its own is not sufficient to bind RNA but is necessary for association with the 25-kDa subunit. RNA binding appears to require a CF Im68/25 heterodimer. Whereas multiple protein interactions with other 3'-end-processing factors are detected with CF Im25, CF Im68 interacts with SRp20, 9G8, and hTra2{beta}, members of the SR family of splicing factors, via its C-terminal alternating charge domain. This domain is also required for targeting CF Im68 to the nucleus. However, CF Im68 does not concentrate in splicing speckles but in foci that partially colocalize with paraspeckles, a subnuclear component in which other proteins involved in transcriptional control and RNA processing have been found.

Di Carlo, A., R. De Mori, et al. (2004). "Hypoxia Inhibits Myogenic Differentiation through Accelerated MyoD Degradation." J. Biol. Chem. **279**(16): 16332-16338.

http://www.jbc.org/cgi/content/abstract/279/16/16332

Cells undergo a variety of biological responses when placed in hypoxic conditions, including alterations in metabolic state and growth rate. Here we investigated the effect of hypoxia on the ability of myogenic cells to differentiate in culture. Exposure of myoblasts to hypoxia strongly inhibited multinucleated myotube formation and the expression of differentiation markers. We showed that hypoxia reversibly inhibited MyoD, Myf5, and myogenin expression. One key step in skeletal muscle differentiation involves the up-regulation of the cell cycle-dependent kinase inhibitors p21 and p27 as well as the product of the retinoblastoma gene (pRb). Myoblasts cultured under hypoxic conditions in differentiation medium failed to up-regulate both p21 and pRb despite the G1 cell cycle arrest, as evidenced by p27 accumulation and pRb hypophosphorylation. Hypoxia-dependent inhibition of differentiation was associated with MyoD degradation by the ubiquitin-proteasome pathway. MyoD overexpression in C2C12 myoblasts

overrode the differentiation block imposed by hypoxic conditions. Thus, hypoxia by inducing MyoD degradation blocked accumulation of early myogenic differentiation markers such as myogenin and p21 and pRb, preventing both permanent cell cycle withdraw and terminal differentiation. Our study revealed a novel anti-differentiation effect exerted by hypoxia in myogenic cells and identified MyoD degradation as a relevant target of hypoxia.

Dinadayala, P., A. Lemassu, et al. (2004). "Revisiting the Structure of the Anti-neoplastic Glucans of Mycobacterium bovis Bacille Calmette-Guerin: STRUCTURAL ANALYSIS OF THE EXTRACELLULAR AND BOILING WATER EXTRACT-DERIVED GLUCANS OF THE VACCINE SUBSTRAINS." J. Biol. Chem. **279**(13): 12369-12378.

http://www.jbc.org/cgi/content/abstract/279/13/12369

The attenuated strain of Mycobacterium bovis Bacille Calmette-Guerin (BCG), used worldwide to prevent tuberculosis and leprosy, is also clinically used as an immunotherapeutic agent against superficial bladder cancer. An anti-tumor polysaccharide has been isolated from the boiling water extract of the Tice substrain of BCG and tentatively characterized as consisting primarily of repeating units of 6-linked-glucosyl residues. Mycobacterium tuberculosis and other mycobacterial species produce a glycogen-like {alpha}-glucan composed of repeating units of 4-linked glucosyl residues substituted at some 6 positions by short oligoglucosyl units that also exhibits an anti-tumor activity. Therefore, the impression prevails that mycobacteria synthesize different types of anti-neoplastic glucans or, alternatively, the BCG substrains are singular in producing a unique type of glucan that may confer to them their immunotherapeutic property. The present study addresses this question through the comparative analysis of {alpha}-glucans purified from the extracellular materials and boiling water extracts of three vaccine substrains. The polysaccharides were purified, and their structural features were established by mono- and two-dimensional NMR spectroscopy and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry of the enzymatic and chemical degradation products of the purified compounds. The glucans isolated by the two methods from the three substrains of BCG were shown to exhibit identical structural features shared with the glycogen-like {alpha}-glucan of M. tuberculosis and other mycobacteria. Incidentally, we observed an occasional release of dextrans from Sephadex columns that may explain the reported occurrence of 6-substituted {alpha}-glucans in mycobacteria.

Dolgachev, V., M. S. Farooqui, et al. (2004). "De Novo Ceramide Accumulation Due to Inhibition of Its Conversion to Complex Sphingolipids in Apoptotic Photosensitized Cells." J. Biol. Chem. **279**(22): 23238-23249.

http://www.jbc.org/cgi/content/abstract/279/22/23238

The oxidative stress induced by photodynamic therapy (PDT) with the photosensitizer phthalocyanine 4 is accompanied by increases in ceramide mass. To assess the regulation of de novo sphingolipid metabolism during PDT-induced apoptosis, Jurkat human T lymphoma and Chinese hamster ovary cells were labeled with [14C]serine, a substrate of serine palmitoyltransferase (SPT), the enzyme catalyzing the initial step in the sphingolipid biosynthesis. A substantial elevation in [14C]ceramide with a concomitant decrease in [14C]sphingomyelin was detected. The labeling of [14C]ceramide was completely abrogated by the SPT inhibitor ISP-1. In addition, ISP-1 partly suppressed PDT-induced apoptosis. Pulse-chase experiments showed that the contribution of sphingomyelin degradation to PDT-initiated increase in de novo ceramide was absent or minor. PDT had no effect on either mRNA amounts of the SPT subunits LCB1 and LCB2, LCB1 protein expression, or SPT activity in Jurkat cells. Moreover in Chinese hamster ovary cells LCB1 protein underwent substantial photodestruction, and SPT activity was

profoundly inhibited after treatment. We next examined whether PDT affects conversion of ceramide to complex sphingolipids. Sphingomyelin synthase, as well as glucosylceramide synthase, was inactivated by PDT in both cell lines in a dose-dependent manner. These results are the first to show that in the absence of SPT up-regulation PDT induces accumulation of de novo ceramide by inhibiting its conversion to complex sphingolipids.

Dorsam, G. and E. J. Goetzl (2002). "Vasoactive Intestinal Peptide Receptor-1 (VPAC-1) Is a Novel Gene Target of the Hemolymphopoietic Transcription Factor Ikaros." J. Biol. Chem. **277**(16): 13488-13493.

http://www.jbc.org/cgi/content/abstract/277/16/13488

Vasoactive intestinal peptide and its G-protein-coupled receptors, VPAC-1 and VPAC-2, are highly expressed in the immune system and modulate diverse T cell functions. The human VPAC-1 5'-flanking region (1.4 kb) contains four high affinity Ikaros (IK) consensus sequences. Ikaros native protein from T cell nuclear extracts and IK-1 and IK-2 recombinant proteins recognized an IK high affinity binding motif in the VPAC-1 promoter in electrophoretic mobility shift assays by a sequence-specific mechanism, and anti-IK antibodies supershifted this complex. Stable NIH-3T3 clones overexpressing IK-1 or IK-2 isoforms were generated to investigate Ikaros regulation of endogenous VPAC-1 expression as assessed by quantifying VPAC-1 mRNA and protein. By traditional and fluorometric-based kinetic reverse transcription-PCR and 125I-labeled vasoactive intestinal peptide binding, both IK-1 and IK-2 suppressed endogenous VPAC-1 expression in NIH-3T3 clones by a range of 50-93%. When a series of nested deletions of the VPAC-1 luciferase reporter construct were transiently transfected into IK-2 clones there was up to a 41% decrease in transcriptional activity compared with vector control. Two major IK-2 binding domains also were identified at [-]1076 to [-]623 bp and at [-]222 to [-]35 bp, respectively. As both Ikaros and its novel target VPAC-1 are highly expressed in T cells, this system may be a dominant determinant of the VPAC-1 expression in immune responses.

Dunty, J. M. and M. D. Schaller (2002). "The N Termini of Focal Adhesion Kinase Family Members Regulate Substrate Phosphorylation, Localization, and Cell Morphology." J. Biol. Chem. **277**(47): 45644-45654.

http://www.jbc.org/cgi/content/abstract/277/47/45644

The focal adhesion kinase (FAK) and cell adhesion kinase [beta] (CAK[beta], PYK2, CADTK, RAFTK) are highly homologous FAK family members, yet clearly have unique roles in the cell. Comparative analyses of FAK and CAK[beta] have revealed intriguing differences in their activities. These differences were investigated further through the characterization of a set of FAK/CAK[beta] chimeric kinases. CAK[beta] exhibited greater catalytic activity than FAK in vitro, providing a molecular basis for differential substrate phosphorylation by FAK and CAK[beta] in vivo. Furthermore, the N terminus may regulate catalytic activity since chimeras containing the FAK N terminus and CAK[beta] catalytic domain exhibited a striking high level of catalytic activity and substrate phosphorylation. Unexpectedly, a modulatory role for the N termini in subcellular localization was also revealed. Chimeras containing the FAK N terminus and CAK[beta] C terminus localized to focal adhesions, whereas chimeras containing the N and C termini of CAK[beta] did not. Finally, prominent changes in cell morphology were induced upon expression of chimeras containing the CAK[beta] N terminus, which were not associated with apoptotic cell death, cell cycle progression delay, or changes in Rho activity. These results demonstrate novel regulatory roles for the N terminus of FAK family kinases.

Elfering, S. L., T. M. Sarkela, et al. (2002). "Biochemistry of Mitochondrial Nitric-oxide Synthase." J. Biol. Chem. **277**(41): 38079-38086.

http://www.jbc.org/cgi/content/abstract/277/41/38079

We reported that the generation of nitric oxide by mitochondria is catalyzed by a constitutive, mitochondrial nitric-oxide synthase (mtNOS). Given that this production may establish the basis for a novel regulatory pathway of energy metabolism, oxygen consumption, and oxygen free radical production, it becomes imperative to identify unequivocally and characterize this enzyme to provide a basis for its regulation. The mitochondrial localization of mtNOS was supported by following the hepatic distribution of mtNOS, immunoblotting submitochondrial fractions, and immunohistochemistry of liver tissues. mtNOS was identified as brain NOS[alpha] by various methods (mass spectrometry of proteolytic fragments, amino acid analysis, molecular weight, pI, and analysis of PCR fragments), excluding the occurrence of a novel isoform or other splice variants. Distribution of mtNOS transcript indicated its occurrence in liver, brain, heart, muscle, kidney, lung, testis, and spleen. In contrast to brain NOS, mtNOS has two post-translational modifications: acylation with myristic acid and phosphorylation at the C terminus. The former modification is a reversible and post-translational process, which may serve for subcellular targeting or membrane anchoring. The latter modification could be linked to enzymatic regulation. These results are discussed in terms of the role that nitric oxide may have in cellular bioenergetics.

Ezoe, S., I. Matsumura, et al. (2005). "GATA Transcription Factors Inhibit Cytokine-dependent Growth and Survival of a Hematopoietic Cell Line through the Inhibition of STAT3 Activity." J. Biol. Chem. **280**(13): 13163-13170.

http://www.jbc.org/cgi/content/abstract/280/13/13163

Although GATA-1 and GATA-2 were shown to be essential for the development of hematopoietic cells by gene targeting experiments, they were also reported to inhibit the growth of hematopoietic cells. Therefore, in this study, we examined the effects of GATA-1 and GATA-2 on cytokine signals. A tamoxifen-inducible form of GATA-1 (GATA-1/ERT) showed a minor inhibitory effect on interleukin-3 (IL-3)-dependent growth of an IL-3-dependent cell line Ba/F3. On the other hand, it drastically inhibited TPO-dependent growth and gp130-mediated growth/survival of Ba/F3. Similarly, an estradiol-inducible form of GATA-2 (GATA-2/ER) disrupted thrombopoietin (TPO)-dependent growth and gp130-mediated growth/survival of Ba/F3. As for this mechanism, we found that both GATA-1 and GATA-2 directly bound to STAT3 both in vitro and in vivo and inhibited its DNA-binding activity in gel shift assays and chromatin immunoprecipitation assays, whereas they hardly affected STAT5 activity. In addition, endogenous GATA-1 was found to interact with STAT3 in normal megakaryocytes, suggesting that GATA-1 may inhibit STAT3 activity in normal hematopoietic cells. Furthermore, we found that GATA-1 suppressed STAT3 activity through its N-zinc finger domain. Together, these results suggest that, besides the roles as transcription factors, GATA family proteins modulate cytokine signals through protein-protein interactions, thereby regulating the growth and survival of hematopoietic cells.

Fujii, I., Y. Yasuoka, et al. (2004). "Hydrolytic Polyketide Shortening by Ayg1p, a Novel Enzyme Involved in Fungal Melanin Biosynthesis." J. Biol. Chem. **279**(43): 44613-44620.

http://www.jbc.org/cgi/content/abstract/279/43/44613

The pentaketide 1,3,6,8-tetrahydroxynaphthalene (T4HN) is a key precursor of 1,8-dihydroxynaphthalene-melanin, an important virulence factor in pathogenic fungi, where T4HN is believed to be the direct product of pentaketide synthases. We showed recently the involvement of a novel protein, Ayg1p, in the formation of T4HN from the heptaketide precursor YWA1 in Aspergillus fumigatus. To investigate the mechanism of its enzymatic function, Ayg1p was purified from an Aspergillus oryzae strain that overexpressed the ayg1 gene. The Ayg1p converted the naphthopyrone YWA1 to T4HN with a release of the acetoacetic acid. Although Ayg1p does not show significant homology with known enzymes, a serine protease-type hydrolytic motif is present in its sequence, and serine-specific inhibitors strongly inhibited the activity. To identify its catalytic residues, site-directed Ayg1p mutants were expressed in Escherichia coli, and their enzyme activities were examined. The single substitution mutations S257A, D352A, and H380A resulted in a complete loss of enzyme activity in Ayg1p. These results indicated that the catalytic triad Asp352-His380-Ser257 constituted the active-site of Ayg1p. From a Dixon plot analysis, 2-acetyl-1,3,6,8-tetrahydroxynaphthalene was found to be a strong mixed-type inhibitor, suggesting the involvement of an acyl-enzyme intermediate. These studies support the mechanism in which the Ser257 at the active site functions as a nucleophile to attack the YWA1 side-chain 1'-carbonyl and cleave the carbon-carbon bond between the naphthalene ring and the side chain. Acetoacetic acid is subsequently released from the Ser257-O-acetoacetylated Ayg1p by hydrolysis. An enzyme with activity similar to Ayg1p in melanin biosynthesis has not been reported in any other organism.

Fukuchi, M., A. Tabuchi, et al. (2004). "Activity-dependent Transcriptional Activation and mRNA Stabilization for Cumulative Expression of Pituitary Adenylate Cyclase-activating Polypeptide mRNA Controlled by Calcium and cAMP Signals in Neurons." J. Biol. Chem. **279**(46): 47856-47865.

http://www.jbc.org/cgi/content/abstract/279/46/47856

Although it has been established that an activity-dependent gene transcription is induced by the calcium (Ca2+) signals in neurons, it is unclear how the specific mRNA moieties are transiently accumulated in response to synaptic transmission which evokes multiple intracellular signals including Ca2+ and cAMP ones. The expression of pituitary adenylate cyclase activating polypeptide (PACAP), a neuropeptide, is controlled by Ca2+ signals evoked via membrane depolarization in neurons, and, in cultured rat cortical neuronal cells, we found that the Ca2+ signal-mediated activation of the PACAP gene promoter was critically controlled by a single cAMP-response element (CRE) located at around -200, to which the CRE-binding protein predominantly bound. The Ca2+ signal-induced expression of PACAP mRNA was enhanced by forskolin, which evokes cAMP signals. In support, the PACAP gene promoter was synergistically enhanced by Ca2+ and cAMP signals through the CRE, accompanying a prolonged activation of extracellular signal-related protein kinase 1/2 and CRE-binding protein. On the other hand, sole administration of forskolin markedly reduced the cellular content of PACAP mRNA, which was restored by the addition of Ca2+ signals. We found that the stability of PACAP mRNA was increased in response to Ca2+ signals but not that of activity-regulated cytoskeleton-associated protein (Arc) mRNA, indicating an activity-dependent stabilization of specific mRNA species in neurons, which can antagonize the regulation mediated by cAMP signals. Thus, the transcriptional activation and mRNA stabilization are coordinately regulated by Ca2+ and cAMP signals for the cumulative expression of PACAP mRNA in neurons.

Furusato, M., N. Sueyoshi, et al. (2002). "Molecular Cloning and Characterization of Sphingolipid Ceramide N-Deacylase from a Marine Bacterium, Shewanella alga G8." J. Biol. Chem. **277**(19): 17300-17307.

http://www.jbc.org/cgi/content/abstract/277/19/17300

Recently, lyso-sphingolipids have been identified as ligands for several orphan G protein-coupled receptors, although the molecular mechanism for their generation has yet to be clarified. Here, we report the molecular cloning of the enzyme, which catalyzes the generation of lyso-sphingolipids from various sphingolipids (sphingolipid ceramide N-deacylase). The 75-kDa enzyme was purified from the marine bacterium, Shewanella alga G8, and its gene was cloned from a G8 genomic library using sequences of the purified enzyme. The cloned enzyme was composed of 992 amino acids, including a signal sequence of 35 residues, and its molecular weight was estimated to be 109,843. Significant sequence similarities were found with an unknown protein of Streptomyces fradiae Y59 and a Lumbricus terrestris lectin but not other known functional proteins. The 106-kDa recombinant enzyme expressed in Escherichia coli hydrolyzed various glycosphingolipids and sphingomyelin, although it seems to be much less active than the native 75-kDa enzyme. In vitro translation using wheat germ extract revealed the activity of a 75-kDa deletion mutant lacking a C terminus to be much stronger than that of the full-length enzyme, suggesting that C-terminal processing is necessary for full activity.

Gardner, A. F., C. M. Joyce, et al. (2004). "Comparative Kinetics of Nucleotide Analog Incorporation by Vent DNA Polymerase." J. Biol. Chem. **279**(12): 11834-11842.

http://www.jbc.org/cgi/content/abstract/279/12/11834

Comparative kinetic and structural analyses of a variety of polymerases have revealed both common and divergent elements of nucleotide discrimination. Although the parameters for dNTP incorporation by the hyperthermophilic archaeal Family B Vent DNA polymerase are similar to those previously derived for Family A and B DNA polymerases, parameters for analog incorporation reveal alternative strategies for discrimination by this enzyme. Discrimination against ribonucleotides was characterized by a decrease in the affinity of NTP binding and a lower rate of phosphoryl transfer, whereas discrimination against ddNTPs was almost exclusively due to a slower rate of phosphodiester bond formation. Unlike Family A DNA polymerases, incorporation of 9-[(2-hydroxyethoxy)methyl]X triphosphates (where X is adenine, cytosine, guanine, or thymine; acyNTPs) by Vent DNA polymerase was enhanced over ddNTPs via a 50-fold increase in phosphoryl transfer rate. Furthermore, a mutant with increased propensity for nucleotide analog incorporation (VentA488L DNA polymerase) had unaltered dNTP incorporation while displaying enhanced nucleotide analog binding affinity and rates of phosphoryl transfer. Based on kinetic data and available structural information from other DNA polymerases, we propose active site models for dNTP, ddNTP, and acyNTP selection by hyperthermophilic archaeal DNA polymerases to rationalize structural and functional differences between polymerases.

Gelebart, P., T. Kovacs, et al. (2002). "Expression of Endomembrane Calcium Pumps in Colon and Gastric Cancer Cells. INDUCTION OF SERCA3 EXPRESSION DURING DIFFERENTIATION." J. Biol. Chem. **277**(29): 26310-26320.

http://www.jbc.org/cgi/content/abstract/277/29/26310

Calcium mobilization from the endoplasmic reticulum (ER) into the cytosol is a key component of several signaling networks controlling tumor cell growth, differentiation, or apoptosis. Sarco/endoplasmic reticulum calcium transport ATPases (SERCA-type calcium pumps), enzymes that accumulate calcium in the ER, play an important role in these phenomena. We report that SERCA3 expression is significantly reduced or lost in colon carcinomas when compared with

normal colonic epithelial cells, which express this enzyme at a high level. To study the involvement of SERCA enzymes in differentiation, in this work differentiation of colon and gastric cancer cell lines was initiated, and the change in the expression of SERCA isoenzymes as well as intracellular calcium levels were investigated. Treatment of the tumor cells with butyrate or other established differentiation inducing agents resulted in a marked and specific induction of the expression of SERCA3, whereas the expression of the ubiquitous SERCA2 enzymes did not change significantly or was reduced. A similar marked increase in SERCA3 expression was found during spontaneous differentiation of post-confluent Caco-2 cells, and this closely correlated with the induction of other known markers of differentiation. Analysis of the expression of the SERCA3 alternative splice isoforms revealed induction of all three known iso-SERCA3 variants (3a, 3b, and 3c). Butyrate treatment of the KATO-III gastric cancer cells led to higher resting cytosolic calcium concentrations and, in accordance with the lower calcium affinity of SERCA3, to diminished ER calcium content. These data taken together indicate a defect in SERCA3 expression in colon cancers as compared with normal colonic epithelium, show that the calcium homeostasis of the endoplasmic reticulum may be remodeled during cellular differentiation, and indicate that SERCA3 constitutes an interesting new differentiation marker that may prove useful for the analysis of the phenotype of gastrointestinal adenocarcinomas.

Gencic, S. and D. A. Grahame (2003). "Nickel in Subunit beta of the Acetyl-CoA Decarbonylase/Synthase Multienzyme Complex in Methanogens. CATALYTIC PROPERTIES AND EVIDENCE FOR A BINUCLEAR Ni-Ni SITE." J. Biol. Chem. **278**(8): 6101-6110.

http://www.jbc.org/cgi/content/abstract/278/8/6101

The acetyl-CoA decarbonylase/synthase (ACDS) complex catalyzes the central reaction of acetyl C-C bond cleavage in methanogens growing on acetate and is also responsible for synthesis of acetyl units during growth on C-1 substrates. The ACDS [beta] subunit contains nickel and an Fe/S center and reacts with acetyl-CoA forming an acetyl-enzyme intermediate presumably directly involved in acetyl C-C bond activation. To investigate the role of nickel in this process two forms of the Methanosarcina thermophila [beta] subunit were overexpressed in anaerobically grown Escherichia coli. Both contained an Fe/S center but lacked nickel and were inactive in acetyl-enzyme formation in redox-dependent acetyltransferase assays. However, high activity developed during incubation with NiCl2. The native and nickel-reconstituted proteins both contained iron and nickel in a 2:1 ratio, with insignificant levels of other metals, including copper. Binding of nickel elicited marked changes in the UV-visible spectrum, with intense charge transfer bands indicating multiple thiolate ligation to nickel. The kinetics of nickel incorporation matched the time course for enzyme activation. Other divalent metal ions could not substitute for nickel in yielding catalytic activity. Acetyl-CoA was formed in reactions with CoA, CO, and methylcobalamin, directly demonstrating C-C bond activation by the [beta] subunit in the absence of other ACDS subunits. Nickel was indispensable in this process too and was needed to form a characteristic EPR-detectable enzyme-carbonyl adduct in reactions with CO. In contrast to enzyme activation, EPR signal formation did not require addition of reducing agent, indicating indirect catalytic involvement of the paramagnetic species. Site-directed mutagenesis indicated that Cys-278 and Cys-280 coordinate nickel, with Cys-189 essential for Fe/S cluster formation. The results are consistent with an Ni2[Fe4S4] arrangement at the active site. A mechanism for C-C bond activation is proposed that includes a specific role for the Fe4S4 center and accounts for the absolute requirement for nickel.

Gomez, M. F., A. S. Stevenson, et al. (2002). "Opposing Actions of Inositol 1,4,5-Trisphosphate and Ryanodine Receptors on Nuclear Factor of Activated T-cells Regulation in Smooth Muscle." J. Biol. Chem. **277**(40): 37756-37764.

http://www.jbc.org/cgi/content/abstract/277/40/37756

The nuclear factor of activated T-cells (NFAT), originally identified in T-cells, has since been shown to play a role in mediating Ca2+-dependent gene transcription in diverse cell types outside of the immune system. We have previously shown that nuclear accumulation of NFATc3 is induced in ileal smooth muscle by platelet-derived growth factor in a manner that depends on Ca2+ influx through L-type, voltage-dependent Ca2+ channels. Here we show that NFATc3 is also the predominant NFAT isoform expressed in cerebral artery smooth muscle and is induced to accumulate in the nucleus by UTP and other Gq/11-coupled receptor agonists. This induction is mediated by calcineurin and is dependent on sarcoplasmic reticulum Ca2+ release through inositol 1,4,5-trisphosphate receptors and extracellular Ca2+ influx through L-type, voltage-dependent Ca2+ channels. Consistent with results obtained in ileal smooth muscle, depolarization-induced Ca2+ influx fails to induce NFAT nuclear accumulation in cerebral arteries. We also provide evidence that Ca2+ release by ryanodine receptors in the form of Ca2+ sparks may exert an inhibitory influence on UTP-induced NFATc3 nuclear accumulation and further suggest that UTP may act, in part, by inhibiting Ca2+ sparks. These results are consistent with a multifactorial regulation of NFAT nuclear accumulation in smooth muscle that is likely to involve several intracellular signaling pathways, including local effects of sarcoplasmic reticulum Ca2+ release and effects attributable to global elevations in intracellular Ca2+.

Graham, D. E., H. Xu, et al. (2002). "Identification of Coenzyme M Biosynthetic Phosphosulfolactate Synthase. A NEW FAMILY OF SULFONATE-BIOSYNTHESIZING ENZYMES." J. Biol. Chem. **277**(16): 13421-13429.

http://www.jbc.org/cgi/content/abstract/277/16/13421

The hyperthermophilic euryarchaeon Methanococcus jannaschii uses coenzyme M (2-mercaptoethanesulfonic acid) as the terminal methyl carrier in methanogenesis. We describe an enzyme from that organism, (2R)-phospho-3-sulfolactate synthase (ComA), that catalyzes the first step in coenzyme M biosynthesis. ComA catalyzed the stereospecific Michael addition of sulfite to phosphoenolpyruvate over a broad range of temperature and pH conditions. Substrate and product analogs moderately inhibited activity. This enzyme has no significant sequence similarity to previously characterized enzymes; however, its Mg2+-dependent enzyme reaction mechanism may be analogous to one proposed for enolase. A diverse group of microbes and plants have homologs of ComA that could have been recruited for sulfolactate or sulfolipid biosyntheses.

Greenwood, I. A., L. J. Miller, et al. (2002). "The Large Conductance Potassium Channel beta -Subunit Can Interact with and Modulate the Functional Properties of a Calcium-activated Chloride Channel, CLCA1." J. Biol. Chem. **277**(25): 22119-22122.

http://www.jbc.org/cgi/content/abstract/277/25/22119

We have recently compared the biophysical and pharmacological properties of native Ca2+-activated Cl[-] currents in murine portal vein with mCLCA1 channels cloned from murine portal vein myocytes (Britton, F. C., Ohya, S., Horowitz, B., and Greenwood, I. A. (2002) J. Physiol. (Lond.) 539, 107-117). These channels shared a similar relative permeability to various anions, but the expressed channel current lacked the marked time dependence of the native current. In addition, the expressed channel showed a lower Ca2+ sensitivity than the native channel. As non-pore-forming regulatory [beta]-subunits alter the kinetics and increase the Ca2+ sensitivity of Ca2+-dependent K+ channels (BK channels) we investigated whether co-expression of [beta]-

subunits with CLCA1 would alter the kinetics/Ca2+ sensitivity of mCLCA1. Internal dialysis of human embryonic kidney cells stably expressing CLCA1 with 500 nM Ca2+ evoked a significantly larger current when the [beta]-subunit KCNMB1 was co-expressed. In a small number of co-transfected cells marked time dependence to the activation kinetics was observed. Interaction studies using the mammalian two-hybrid technique demonstrated a physical association between CLCA1 and KCNMB1 when co-expressed in human embryonic kidney cells. These data suggest that activation of CLCA1 can be modified by accessory subunits.

Gronlund, H., T. Bergman, et al. (2003). "Formation of Disulfide Bonds and Homodimers of the Major Cat Allergen Fel d 1 Equivalent to the Natural Allergen by Expression in Escherichia coli." J. Biol. Chem. **278**(41): 40144-40151.

http://www.jbc.org/cgi/content/abstract/278/41/40144

Dander from the domestic cat (Felis domesticus) is one of the most common causes of IgE-mediated allergy. Attempts to produce tetrameric folded major allergen Fel d 1 by recombinant methods with structural features similar to the natural allergen have been only partially successful. In this study, a recombinant folded Fel d 1 with molecular and biological properties similar to the natural counterpart was produced. A synthetic gene coding for direct fusion of the Fel d 1 chain 2 N-terminally to chain 1 was constructed by overlapping oligonucleotides in PCR. Escherichia coli expression resulted in a non-covalently associated homodimer with an apparent molecular mass of 30 kDa defined by size exclusion chromatography. Furthermore, each 19,177-Da subunit displayed a disulfide pattern identical to that found in the natural Fel d 1, i.e. Cys3(1) Cys73(2), Cys44(1)-Cys48(2), Cys70(1)-Cys7(2), as determined by electrospray mass spectrometry after tryptic digestion. Circular dichroism analysis showed identical folds of natural and recombinant Fel d 1. Furthermore, recombinant Fel d l reacted specifically with serum IgE, inducing expression of CD203c on basophils and lymphoproliferative responses in cat-allergic patients. The results show that the overall fold and immunological properties of the recombinant Fel d 1 are very similar to those of natural Fel d 1. Moreover, the recombinant Fel d 1 construct provides a tool for defining the three-dimensional structure of Fel d 1 and represents a reagent for diagnosis and allergen-specific immunotherapy of cat allergy.

Gross, I., D. J. Morrison, et al. (2003). "The Receptor Tyrosine Kinase Regulator Sprouty1 Is a Target of the Tumor Suppressor WT1 and Important for Kidney Development." J. Biol. Chem. **278**(42): 41420-41430.

http://www.jbc.org/cgi/content/abstract/278/42/41420

WT1 encodes a transcription factor involved in kidney development and tumorigenesis. Using representational difference analysis, we identified a new set of WT1 targets, including a homologue of the Drosophila receptor tyrosine kinase regulator, sprouty. Sprouty1 was up-regulated in cell lines expressing wild-type but not mutant WT1. WT1 bound to the endogenous sprouty1 promoter in vivo and directly regulated sprouty1 through an early growth response gene-1 binding site. Expression of Sprouty1 and WT1 overlapped in the developing metanephric mesenchyme, and Sprouty1, like WT1, plays a key role in the early steps of glomerulus formation. Disruption of Sprouty1 expression in embryonic kidney explants by antisense oligonucleotides reduced condensation of the metanephric mesenchyme, leading to a decreased number of glomeruli. In addition, sprouty1 was expressed in the ureteric tree and antisense-treated ureteric trees had cystic lumens. Therefore, sprouty1 represents a physiologically relevant target gene of WT1 during kidney development.

Gu, B. J., R. Sluyter, et al. (2004). "An Arg307 to Gln Polymorphism within the ATP-binding Site Causes Loss of Function of the Human P2X7 Receptor." J. Biol. Chem. **279**(30): 31287-31295.

http://www.jbc.org/cgi/content/abstract/279/30/31287

The P2X7 receptor is a ligand-gated channel that is highly expressed on mononuclear cells of the immune system and that mediates ATP-induced apoptosis. Wide variations in the function of the P2X receptor have been observed, explained in part by 7loss-of-function polymorphisms that change Glu496 to Ala (E496A) and Ile568 to Asn (I568N). In this study, a third polymorphism, which substitutes an uncharged glutamine for the highly positively charged Arg307 (R307Q), has been found in heterozygous dosage in 12 of 420 subjects studied. P2X7 function was measured by ATP-induced fluxes of Rb+, Ba2+, and ethidium+ into peripheral blood monocytes or various lymphocyte subsets and was either absent or markedly decreased. Transfection experiments showed that P2X7 carrying the R307Q mutation lacked either channel or pore function despite robust protein synthesis and surface expression of the receptor. The monoclonal antibody (clone L4) that binds to the extracellular domain of wild type P2X7 and blocks P2X7 function failed to bind to the R307Q mutant receptor. Differentiation of monocytes to macrophages up-regulated P2X7 function in cells heterozygous for the R307Q to a value 10-40% of that for wild type macrophages. However, macrophages from a subject who was double heterozygous for R307Q/I568N remained totally non-functional for P2X7, and lymphocytes from the same subject also lacked ATP-stimulated phospholipase D activity. These data identify a third loss-of-function polymorphism affecting the human P2X7 receptor, and since the affected Arg307 is homologous to those amino acids essential for ATP binding to P2X1 and P2X2, it is likely that this polymorphism abolishes the binding of ATP to the extracellular domain of P2X7.

He, H., F. Soncin, et al. (2003). "Elevated Expression of Heat Shock Factor (HSF) 2A Stimulates HSF1-induced Transcription during Stress." J. Biol. Chem. **278**(37): 35465-35475.

http://www.jbc.org/cgi/content/abstract/278/37/35465

Heat shock factor 2 (HSF2) belongs to a family of structurally related transcription factors, which share the property of binding to heat shock elements in the promoters of hsp molecular chaperone genes. However, unlike HSF1, which is essential for hsp gene transcription, the cellular functions of HSF2 are not well known. Here we show that human HSF2, although an ineffective activator of the hsp70 promoter in vitro and in vivo in the absence of stress, participates in the activation of the hsp70 promoter by heat shock. HSF2 was not, however, activated by heat shock in cells deficient in functional HSF1, suggesting a requirement for HSF1 in HSF2-mediated transcriptional enhancement. In addition, HSF2 regulation involves differential activity of two isoforms, HSF2A and HSF2B, which arise from alternative splicing of a common hsf2 gene. Under basal conditions, both HSF2 isoforms are ineffective in activating the hsp70 transcription. However, heat shock differentially activates HSF2A in vivo. This phenomenon appears to be physiologically significant, as human myeloprogenitor cells differentiating along the erythroid lineage express HSF2A de novo and undergo a large increase in capacity to activate the hsp70 promoter. Our experiments further show that HSF1 is physically associated with HSF2 in the cell and that such binding is enhanced by heat shock. Our data suggest a mechanism involving the formation of heterocomplexes between HSF1 and HSF2 with enhanced activity to activate the hsp70 promoter when compared with HSF1 or HSF2 homotrimers.

Hellberg, A., J. Poole, et al. (2002). "Molecular Basis of the Globoside-deficient Pk Blood Group

Phenotype. IDENTIFICATION OF FOUR INACTIVATING MUTATIONS IN THE UDP-N-ACETYLGALACTOSAMINE: GLOBOTRIAOSYLCERAMIDE 3-beta -N-ACETYLGALACTOSAMINYLTRANSFERASE GENE." J. Biol. Chem. **277**(33): 29455-29459.

http://www.jbc.org/cgi/content/abstract/277/33/29455

The biochemistry and molecular genetics underlying the related carbohydrate blood group antigens P, Pk, and LKE in the GLOB collection and P1 in the P blood group system are complex and not fully understood. Individuals with the rare but clinically important erythrocyte phenotypes P1k and P2k lack the capability to synthesize P antigen identified as globoside, the cellular receptor for Parvo-B19 virus and some P-fimbriated Escherichia coli. As in the ABO system, naturally occurring antibodies, anti-P of the IgM and IgG class with hemolytic and cytotoxic capacity, are formed. To define the molecular basis of the Pk phenotype we analyzed the full coding region of a candidate gene reported in 1998 as a member of the 3-[beta]-galactosyltransferase family but later shown to possess UDP-N-acetylgalactosamine:globotriaosylceramide 3-[beta]-N-acetylgalactosaminyltransferase or globoside synthase activity. Homozygosity for different nonsense mutations (C202 [right-arrow] T and 538insA) resulting in premature stop codons was found in blood samples from two individuals of the P2k phenotype. Two individuals with P1k and P2k phenotypes were homozygous for missense mutations causing amino acid substitutions (E266A or G271R) in a highly conserved region of the enzymatically active carboxyl-terminal domain in the transferase. We conclude that crucial mutations in the globoside synthase gene cause the Pk phenotype.

Hermoso, M., C. M. Satterwhite, et al. (2002). "ClC-3 Is a Fundamental Molecular Component of Volume-sensitive Outwardly Rectifying Cl- Channels and Volume Regulation in HeLa Cells and Xenopus laevis Oocytes." J. Biol. Chem. **277**(42): 40066-40074.

http://www.jbc.org/cgi/content/abstract/277/42/40066

Volume-sensitive osmolyte and anion channels (VSOACs) are activated upon cell swelling in most vertebrate cells. Native VSOACs are believed to be a major pathway for regulatory volume decrease (RVD) through efflux of chloride and organic osmolytes. ClC-3 has been proposed to encode native VSOACs in Xenopus laevis oocytes and in some mammalian cells, including cardiac and vascular smooth muscle cells. The relationship between the ClC-3 chloride channel, the native volume-sensitive osmolyte and anion channel (VSOAC) currents, and cell volume regulation in HeLa cells and X. laevis oocytes was investigated using ClC-3 antisense. In situ hybridization in HeLa cells, semiquantitative and real-time PCR, and immunoblot studies in HeLa cells and X. laevis oocytes demonstrated the presence of ClC-3 mRNA and protein, respectively. Exposing both cell types to hypotonic solutions induced cell swelling and activated native VSOACs. Transient transfection of HeLa cells with ClC-3 antisense oligonucleotide or X. laevis oocytes injected with antisense cRNA abolished the native ClC-3 mRNA transcript and protein and significantly reduced the density of native VSOACs activated by hypotonically induced cell swelling. In addition, antisense against native ClC-3 significantly impaired the ability of HeLa cells and X. laevis oocytes to regulate their volume. These results suggest that ClC-3 is an important molecular component underlying VSOACs and the RVD process in HeLa cells and X. laevis oocytes.

Hidaka, K., J. J. Caffrey, et al. (2002). "An Adjacent Pair of Human NUDT Genes on Chromosome X Are Preferentially Expressed in Testis and Encode Two New Isoforms of Diphosphoinositol Polyphosphate Phosphohydrolase." J. Biol. Chem. **277**(36): 32730-32738.

http://www.jbc.org/cgi/content/abstract/277/36/32730

Combinatorial expression of the various isoforms of diphosphoinositol synthases and phosphohydrolases determines the rates of phosphorylation/dephosphorylation cycles that have been functionally linked to vesicle trafficking, stress responses, DNA repair, and apoptosis. We now describe two new 19-kDa diphosphoinositol polyphosphate phosphohydrolases (DIPPs), named types 3[alpha] and 3[beta], which possess the canonical Nudix-type catalytic motif flanked on either side by short Gly-rich sequences. The two enzymes differ only in that Pro-89 in the [alpha] form is replaced by Arg-89 in the [beta] form, making the latter ~2-fold more active in vitro. Another Nudix substrate, diadenosine hexaphosphate, was hydrolyzed less efficiently (kcat/Km = 0.2 x 105 M[-]1 s[-]1) compared with diphosphoinositol polyphosphates (kcat/Km = 2-40 x 105 M[-]1 s[-]1). Catalytic activity in vivo was established by individual overexpression of the human (h) DIPP3 isoforms in HEK293 cells, which reduced cellular levels of diphosphoinositol polyphosphates by 40-50%. The hDIPP3 mRNA is preferentially expressed in testis, accompanied by relatively weak expression in the brain, contrasting with hDIPP1 and hDIPP2 which are widely expressed. The hDIPP3 genes (NUDT10 encodes hDIPP3[alpha]; NUDT11 encodes hDIPP3[beta]) are only 152 kbp apart at p11.22 on chromosome X and probably arose by duplication. Transcription of both genes is inactivated on one of the X chromosomes of human females to maintain appropriate gene dosage. The hDIPP3 pair add tissue-specific diversity to the molecular mechanisms regulating diphosphoinositol polyphosphate turnover.

Hijarrubia, M. J., J. F. Aparicio, et al. (2003). "Domain Structure Characterization of the Multifunctional alpha -Aminoadipate Reductase from Penicillium chrysogenum by Limited Proteolysis. ACTIVATION OF alpha -AMINOADIPATE DOES NOT REQUIRE THE PEPTIDYL CARRIER PROTEIN BOX OR THE REDUCTION DOMAIN." J. Biol. Chem. **278**(10): 8250-8256.

http://www.jbc.org/cgi/content/abstract/278/10/8250

The [alpha]-aminoadipate reductase ([alpha]-AAR) of Penicillium chrysogenum, an enzyme that activates the [alpha]-aminoadipic acid by forming an [alpha]-aminoadipyl adenylate and reduces the activated intermediate to [alpha]-aminoadipic semialdehyde, was purified to homogeneity by immunoaffinity techniques, and the kinetics for [alpha]-aminoadipic acid, ATP, and NADPH were determined. Sequencing of the N-terminal end confirmed the 10 first amino acids deduced from the nucleotide sequence. Its domain structure has been investigated using limited proteolysis and active site labeling. Trypsin and elastase were used to cleave the multienzyme, and the location of fragments within the primary structure was established by N-terminal sequence analysis. Initial proteolysis generated two fragments: an N-terminal fragment housing the adenylation and the peptidyl carrier protein (PCP) domains (116 kDa) and a second fragment containing most of the reductive domain (28 kDa). Under harsher conditions the adenylation domain (about 64 kDa) and the PCP domain (30 kDa) become separated. Time-dependent acylation of [alpha]-AAR and of fragments containing the adenylation domain with tritiated [alpha]-aminoadipate occurred in vitro in the absence of NADPH. Addition of NADPH to the labeled [alpha]-AAR released most of the radioactive substrate. A fragment containing the adenylation domain was labeled even in absence of the PCP box. The labeling of this fragment (lacking PCP) was always weaker than that observed in the di-domain (adenylating and PCP) fragment suggesting that the PCP domain plays a role in the stability of the acyl intermediate. Low intensity direct acylation of the PCP box has also been observed. A domain structure of this multienzyme is proposed.

Ho, R. H., B. F. Leake, et al. (2004). "Ethnicity-dependent Polymorphism in Na+-taurocholate Cotransporting Polypeptide (SLC10A1) Reveals a Domain Critical for Bile Acid Substrate Recognition." J. Biol. Chem. **279**(8): 7213-7222.

http://www.jbc.org/cgi/content/abstract/279/8/7213

The key transporter responsible for hepatic uptake of bile acids from portal circulation is Na+-taurocholate cotransporting polypeptide (NTCP, SLC10A1). This transporter is thought to be critical for the maintenance of enterohepatic recirculation of bile acids and hepatocyte function. Therefore, functionally relevant polymorphisms in this transporter would be predicted to have an important impact on bile acid homeostasis/liver function. However, little is known regarding genetic heterogeneity in NTCP. In this study, we demonstrate the presence of multiple single nucleotide polymorphisms in NTCP in populations of European, African, Chinese, and Hispanic Americans. Specifically four nonsynonymous single nucleotide polymorphisms associated with a significant loss of transport function were identified. Cell surface biotinylation experiments indicated that the altered transport activity of T668C (Ile223 [-&gt] Thr), a variant seen only in African Americans, was due at least in part to decreased plasma membrane expression. Similar expression patterns were observed when the variant alleles were expressed in HepG2 cells, and plasma membrane expression was assessed using immunofluorescence confocal microscopy. Interestingly the C800T (Ser267 [-&gt] Phe) variant, seen only in Chinese Americans, exhibited a near complete loss of function for bile acid uptake yet fully normal transport function for the non-bile acid substrate estrone sulfate, suggesting this position may be part of a region in the transporter critical and specific for bile acid substrate recognition. Accordingly, our study indicates functionally important polymorphisms in NTCP exist and that the likelihood of being carriers of such polymorphisms is dependent on ethnicity.

Hoffmann, C., M. Pop, et al. (2004). "The Yersinia pseudotuberculosis Cytotoxic Necrotizing Factor (CNFY) Selectively Activates RhoA." J. Biol. Chem. **279**(16): 16026-16032.

http://www.jbc.org/cgi/content/abstract/279/16/16026

The cytotoxic necrotizing factors (CNF)1 and CNF2 from pathogenic Escherichia coli strains activate RhoA, Rac1, and Cdc42 by deamidation of Gln63 (RhoA) or Gln61 (Rac and Cdc42). Recently, a novel cytotoxic necrotizing factor termed CNFY was identified in Yersinia pseudotuberculosis strains (Lockman, H. A., Gillespie, R. A., Baker, B. D., and Shakhnovich, E. (2002) Infect. Immun. 70, 2708-2714). We amplified the cnfy gene from genomic DNA of Y. pseudotuberculosis, cloned and expressed the recombinant protein, and studied its activity. Recombinant GST-CNFY induced morphological changes in HeLa cells and caused an upward shift of RhoA in SDS-PAGE, as is known for GST-CNF1 and GST-CNF2. Mass spectrometric analysis of GST-CNFY-treated RhoA confirmed deamidation at Glu63. Treatment of RhoA, Rac1, and Cdc42 with GST-CNFY decreased their GTPase activities, indicating that all of these Rho proteins could serve as substrates for GST-CNFY in vitro. In contrast, RhoA, but not Rac or Cdc42, was the substrate of GST-CNFY in culture cells. GST-CNFY caused marked stress fiber formation in HeLa cells after 2 h. In contrast to GST-CNF1, formation of filopodia or lamellipodia was not induced with GST-CNFY. Accordingly, effector pull-down experiments with lysates of toxin-treated cells revealed strong activation of RhoA but no activation of Rac1 or Cdc42 after 6 h of GST-CNFY-treatment. Moreover, in rat hippocampal neurons, GST-CNFY results in the retraction of neurites, indicating RhoA activation. In contrast, no activation of Rac or Cdc42 was found. Altogether, our data suggest that CNFY from Y. pseudotuberculosis is a strong, selective activator of RhoA, which can be used as a powerful tool for constitutive RhoA activation without concomitant activation of Rac1 or Cdc42.

Hoffmann, L., S. Maury, et al. (2003). "Purification, Cloning, and Properties of an Acyltransferase Controlling Shikimate and Quinate Ester Intermediates in Phenylpropanoid Metabolism." J. Biol. Chem. **278**(1): 95-103.

http://www.jbc.org/cgi/content/abstract/278/1/95

A protein hydrolyzing hydroxycinnamoyl-CoA esters has been purified from tobacco stem extracts by a series of high pressure liquid chromatography steps. The determination of its N-terminal amino acid sequence allowed design of primers permitting the corresponding cDNA to be cloned by PCR. Sequence analysis revealed that the tobacco gene belongs to a plant acyltransferase gene family, the members of which have various functions. The tobacco cDNA was expressed in bacterial cells as a recombinant protein fused to glutathione S-transferase. The fusion protein was affinity-purified and cleaved to yield the recombinant enzyme for use in the study of catalytic properties. The enzyme catalyzed the synthesis of shikimate and quinate esters shown recently to be substrates of the cytochrome P450 3-hydroxylase involved in phenylpropanoid biosynthesis. The enzyme has been named hydroxycinnamoyl-CoA: shikimate/quinate hydroxycinnamoyltransferase. We show that p-coumaroyl-CoA and caffeoyl-CoA are the best acyl group donors and that the acyl group is transferred more efficiently to shikimate than to quinate. The enzyme also catalyzed the reverse reaction, i.e. the formation of caffeoyl-CoA from chlorogenate (5-O-caffeoyl quinate ester). Thus, hydroxycinnamoyl-CoA:shikimate/quinate hydroxycinnamoyltransferase appears to control the biosynthesis and turnover of major plant phenolic compounds such as lignin and chlorogenic acid.

Holmborn, K., J. Ledin, et al. (2004). "Heparan Sulfate Synthesized by Mouse Embryonic Stem Cells Deficient in NDST1 and NDST2 Is 6-O-Sulfated but Contains No N-Sulfate Groups." J. Biol. Chem. **279**(41): 42355-42358.

http://www.jbc.org/cgi/content/abstract/279/41/42355

Heparan sulfate structure differs significantly between various cell types and during different developmental stages. The diversity is created during biosynthesis by sulfotransferases, which add sulfate groups to the growing chain, and a C5-epimerase, which converts selected glucuronic acid residues to iduronic acid. All these modifications are believed to depend on initial glucosamine N-sulfation carried out by the enzyme glucosaminyl N-deacetylase/N-sulfotransferase (NDST). Here we report that heparan sulfate synthesized by mouse embryonic stem cells deficient in NDST1 and NDST2 completely lacks N-sulfation but still contains 6-O-sulfate groups, demonstrating that 6-O-sulfation can occur without prior N-sulfation. Reverse transcriptase-PCR analysis indicates that all three identified 6-O-sulfotransferases are expressed by the cells, 6-O-sulfotransferase-1 being the dominating form. The 6-O-sulfated polysaccharide lacking N-sulfate groups also contains N-unsubstituted glucosamine units, raising questions about how these units are generated.

Horibata, Y., K. Sakaguchi, et al. (2004). "Unique Catabolic Pathway of Glycosphingolipids in a Hydrozoan, Hydra magnipapillata, Involving Endoglycoceramidase." J. Biol. Chem. **279**(32): 33379-33389.

http://www.jbc.org/cgi/content/abstract/279/32/33379

Endoglycoceramidase (EGCase; EC 3.2.1.123) is an enzyme capable of cleaving the glycosidic linkage between oligosaccharides and ceramides of various glycosphingolipids. We detected strong EGCase activity in animals belonging to Cnidaria, Mollusca, and Annelida and cloned the enzyme from a hydra, Hydra magnipapillata. The hydra EGCase, consisting of 517 amino acid residues, showed 19.2% and 50.2% identity to the Rhodococcus and jellyfish EGCases, respectively. The recombinant hydra enzyme, expressed in CHOP (Chinese hamster ovary cells expressing polyoma LT antigen) cells, hydrolyzed [14C]GM1a to produce [14C]ceramide with a

pH optimum at 3.0-3.5. Whole mount in situ hybridization and immunocytochemical analysis revealed that EGCase was widely expressed in the endodermal layer, especially in digestive cells. GM1a injected into the gastric cavity was incorporated and then directly catabolized by EGCase to produce GM1a-oligosaccharide and ceramide, which were further degraded by exoglycosidases and ceramidase, respectively. However, hydra exoglycosidases did not hydrolyze GM1a directly. These results indicate that the EGCase is indispensable for the catabolic processing of dietary glycosphingolipids in hydra, demonstrating the unique catabolic pathway for glycosphingolipids in the animal.

Huynh, T. T., V. T. Huynh, et al. (2003). "Gene Knockdown of {gamma}-Glutamylcysteine Synthetase by RNAi in the Parasitic Protozoa Trypanosoma brucei Demonstrates That It Is an Essential Enzyme." J. Biol. Chem. **278**(41): 39794-39800.

http://www.jbc.org/cgi/content/abstract/278/41/39794

The parasitic protozoa Trypanosoma brucei utilizes a novel cofactor (trypanothione, T(SH)2), which is a conjugate of GSH and spermidine, to maintain cellular redox balance. {gamma}-Glutamylcysteine synthetase ({gamma}-GCS) catalyzes the first step in the biosynthesis of GSH. To evaluate the importance of thiol metabolism to the parasite, RNAi methods were used to knock down gene expression of {gamma}-GCS in procyclic T. brucei cells. Induction of {gamma}-GCS RNAi with tetracycline led to cell death within 4-6 days post-induction. Cell death was preceded by the depletion of the {gamma}-GCS protein and RNA and by the loss of the cellular pools of GSH and T(SH)2. The addition of GSH (80 {micro}M) to cell cultures rescued the RNAi cell death phenotype and restored the intracellular thiol pools to wild-type levels. Treatment of cells with buthionine sulfoximine (BSO), an enzyme-activated inhibitor of {gamma}-GCS, also resulted in cell death. However, the toxicity of the inhibitor was not reversed by GSH, suggesting that BSO has more than one cellular target. BSO depletes intracellular thiols to a similar extent as {gamma}-GCS RNAi; however, addition of GSH did not restore the pools of GSH and T(SH)2. These data suggest that BSO also acts to inhibit the transport of GSH or its peptide metabolites into the cell. The ability of BSO to inhibit both synthesis and transport of GSH likely makes it a more effective cytotoxic agent than an inhibitor with a single mode of action. Finally the potential for the T(SH)2 biosynthetic enzymes to be regulated in response to reduced thiol levels was studied. The expression levels of ornithine decarboxylase and of S-adenosylmethionine decarboxylase, two essential enzymes in spermidine biosynthesis, remained constant in induced {gamma}-GCS RNAi cell lines.

Inacio, A., A. L. Silva, et al. (2004). "Nonsense Mutations in Close Proximity to the Initiation Codon Fail to Trigger Full Nonsense-mediated mRNA Decay." J. Biol. Chem. **279**(31): 32170-32180.

http://www.jbc.org/cgi/content/abstract/279/31/32170

Nonsense-mediated mRNA decay (NMD) is a surveillance mechanism that degrades mRNAs containing premature translation termination codons. In mammalian cells, a termination codon is ordinarily recognized as "premature" if it is located greater than 50-54 nucleotides 5' to the final exon-exon junction. We have described a set of naturally occurring human {beta}-globin gene mutations that apparently contradict this rule. The corresponding {beta}-thalassemia genes contain nonsense mutations within exon 1, and yet their encoded mRNAs accumulate to levels approaching wild-type {beta}-globin ({beta}WT) mRNA. In the present report we demonstrate that the stabilities of these mRNAs with nonsense mutations in exon 1 are intermediate between {beta}WT mRNA and {beta}-globin mRNA carrying a prototype NMD-sensitive mutation in exon 2 (codon 39 nonsense; {beta}39). Functional analyses of these mRNAs with 5'-proximal nonsense mutations demonstrate that their relative resistance to NMD does not reflect abnormal RNA

splicing or translation re-initiation and is independent of promoter identity and erythroid specificity. Instead, the proximity of the nonsense codon to the translation initiation AUG constitutes a major determinant of NMD. Positioning a termination mutation at the 5' terminus of the coding region blunts mRNA destabilization, and this effect is dominant to the "50-54 nt boundary rule." These observations impact on current models of NMD.

Inagaki, T., S. Suzuki, et al. (2003). "The Retinoic Acid-responsive Proline-rich Protein Is Identified in Promyeloleukemic HL-60 Cells." J. Biol. Chem. **278**(51): 51685-51692.

http://www.jbc.org/cgi/content/abstract/278/51/51685

To identify new genes that retinoic acid activates, we employed an mRNA differential display technique and screened for genes that are differentially expressed in promyeloleukemic HL-60 cells incubated in the presence of all-trans-retinoic acid (ATRA) compared with the absence of ATRA. We cloned the coding region of a retinoic acid-induced gene from a human thymus library, which was the mRNA encoding the 666-amino acid human homologue of mouse proline-rich protein 76. We have designated it RARP1 (retinoic acid response proline-rich protein 1). Transcription of an [~]2.4-kbp mRNA occurred mainly in organs with immune functions, such as thymus, spleen, and peripheral leukocytes. Cycloheximide blocked the ATRA-induced expression. In megakaryocyte-like human erythroleukemia HEL cells, the amount of RARP1 mRNA was high, but it was low in human T-lymphoblastoid Jurkat cells. A specific antibody against RARP1 recognized a 110-kDa protein, which accumulates after incubation of HL-60 cells with ATRA. In immunohistochemical experiments, strong RARP1 staining was observed in the megakaryocytes of bone marrow and spleen, and heterogeneous stain was seen in thymus. Transcriptional studies showed that RARP1 expression impaired the transactivation through activator protein1 and serum responseelement in all cell lines we checked, whereas it did not affect the transactivation through cAMP-response element in the same cell lines. Further analysis demonstrated that proline-rich regions of RARP1 are the functional regions regulated for suppression of activator protein1 transactivation. These data suggest that ATRA-inducible RARP1 selectively affects signal transduction and may contribute to myeloid and megakaryocytic differentiation.

Ishisaki, A., H. Hayashi, et al. (2003). "Human Umbilical Vein Endothelium-derived Cells Retain Potential to Differentiate into Smooth Muscle-like Cells." J. Biol. Chem. **278**(2): 1303-1309.

http://www.jbc.org/cgi/content/abstract/278/2/1303

Mouse embryonic stem-derived cells were recently shown to differentiate into endothelial and smooth muscle cells. In the present study, we investigated whether human umbilical vein endothelium-derived cells retain the potential to differentiate into smooth muscle cells. Examination of biochemical markers, including basic calponin, SM22[alpha], prostaglandin E synthase, von Willebrand factor, and PECAM-1, as well as cell contractility, showed that whereas endothelium-derived cells cultured with fibroblast growth factor can be characterized as endothelial cells, when deprived of fibroblast growth factor, a significant fraction differentiates into smooth muscle-like cells. Reapplication of fibroblast growth factor reversed this differentiation. Activin A was up-regulated in fibroblast growth factor-deprived, endothelium-derived cells; moreover, the inhibitory effects of exogenous follistatin and overexpressed Smad7 on smooth muscle-like differentiation confirmed that the differentiation was driven by activin A signaling. These findings indicate that when deprived of fibroblast growth factor, human umbilical vein endothelium-derived cells are capable of differentiating into smooth muscle-like cells through activin A-induced, Smad-dependent signaling, and that maintenance of the endothelial cell phenotype and differentiation into smooth muscle-like cells are reciprocally controlled by

fibroblast growth factor-1 and activin A.

Iwasaki, H., K. Chiba, et al. (2002). "Molecular Characterization of the Starfish Inositol 1,4,5-Trisphosphate Receptor and Its Role during Oocyte Maturation and Fertilization." J. Biol. Chem. **277**(4): 2763-2772.

http://www.jbc.org/cgi/content/abstract/277/4/2763

The release of calcium ions (Ca2+) from their intracellular stores is essential for the fertilization of oocytes of various species. The calcium pools can be induced to release Ca2+ via two main types of calcium channel receptor: the inositol 1,4,5-trisphosphate receptor (IP3R) and the ryanodine receptor. Starfish oocytes have often been used to study intracellular calcium mobilization during oocyte maturation and fertilization, but how the intracellular calcium channels contribute to intracellular calcium mobilization has never been understood fully, because these molecules have not been identified and no specific inhibitors of these channels have ever been found. In this study, we utilized a novel IP3R antagonist, the "IP3 sponge," to investigate the role of IP3 during fertilization of the starfish oocyte. The IP3 sponge strongly and specifically competed with endogenous IP3R for binding to IP3. By injecting IP3 sponge into starfish oocyte, the increase in intracellular calcium and formation of the fertilization envelope were both dramatically blocked, although oocyte maturation was not blocked. To investigate the role of IP3R in the starfish oocyte more precisely, we cloned IP3R from the ovary of starfish, and the predicted amino acid sequence indicated that the starfish IP3R has 58-68% identity to mammalian IP3R types 1, 2, and 3. We then raised antibodies that recognize starfish IP3R, and use of the antibodies to perform immunoblot analysis revealed that the level of expression of IP3R remained unchanged throughout oocyte maturation. An immunocytochemical study, however, revealed that the distribution of starfish IP3R changes during oocyte maturation.

Jackers, P., G. Szalai, et al. (2004). "Ets-dependent Regulation of Target Gene Expression during Megakaryopoiesis." J. Biol. Chem. **279**(50): 52183-52190.

http://www.jbc.org/cgi/content/abstract/279/50/52183

Megakaryopoiesis is the process by which hematopoietic stem cells in the bone marrow differentiate into mature megakaryocytes. The expression of megakaryocytic genes during megakaryopoiesis is controlled by specific transcription factors. Fli-1 and GATA-1 transcription factors are required for development of megakaryocytes and promoter analysis has defined in vitro functional binding sites for these factors in several megakaryocytic genes, including GPIIb, GPIX, and C-MPL. Herein, we utilize chromatin immunoprecipitation to examine the presence of Ets-1, Fli-1, and GATA-1 on these promoters in vivo. Fli-1 and Ets-1 occupy the promoters of GPIIb, GPIX, and C-MPL genes in both Meg-01 and CMK11-5 cells. Whereas GPIIb is expressed in both Meg-01 and CMK11-5 cells, GPIX and C-MPL are only expressed in the more differentiated CMK11-5 cells. Thus, in vivo occupancy by an Ets factor is not sufficient to promote transcription of some megakaryocytic genes. GATA-1 and Fli-1 are both expressed in CMK11-5 cells and co-occupy the GPIX and C-MPL promoters. Transcription of all three megakaryocytic genes is correlated with the presence of acetylated histone H3 and phosphorylated RNA polymerase II on their promoters. We also show that exogenous expression of GATA-1 in Meg-01 cells leads to the expression of endogenous c-mpl and gpIX mRNA. Whereas GPIIb, GPIX, and C-MPL are direct target genes for Fli-1, both Fli-1 and GATA-1 are required for formation of an active transcriptional complex on the C-MPL and GPIX promoters in vivo. In contrast, GPIIb expression appears to be independent of GATA-1 in Meg-01 cells.

Jackson-Hayes, L., S. Song, et al. (2003). "A Thyroid Hormone Response Unit Formed between the Promoter and First Intron of the Carnitine Palmitoyltransferase-Ialpha Gene Mediates the Liver-specific Induction by Thyroid Hormone." J. Biol. Chem. **278**(10): 7964-7972.

http://www.jbc.org/cgi/content/abstract/278/10/7964

Carnitine palmitoyltransferase-I (CPT-I) catalyzes the rate-controlling step of fatty acid oxidation. CPT-I converts long-chain fatty acyl-CoAs to acylcarnitines for translocation across the mitochondrial membrane. The mRNA levels and enzyme activity of the liver isoform, CPT-I[alpha], are greatly increased in the liver of hyperthyroid animals. Thyroid hormone (T3) stimulates CPT-I[alpha] transcription far more robustly in the liver than in non-hepatic tissues. We have shown that the thyroid hormone receptor (TR) binds to a thyroid hormone response element (TRE) located in the CPT-I[alpha] promoter. In addition, elements in the first intron participate in the T3 induction of CPT-I[alpha] gene expression, but the CPT-I[alpha] intron alone cannot confer a T3 response. We found that deletion of sequences in the first intron between +653 and +744 decreased the T3 induction of CPT-I[alpha]. Upstream stimulatory factor (USF) and CCAAT enhancer binding proteins (C/EBPs) bind to elements within this region, and these factors are required for the T3 response. The binding of TR and C/EBP to the CPT-I[alpha] gene in vivo was shown by the chromatin immunoprecipitation assay. We determined that TR can physically interact with USF-1, USF-2, and C/EBP[alpha]. Transgenic mice were created that carry CPT-I[alpha]-luciferase transgenes with or without the first intron of the CPT-I[alpha] gene. In these mouse lines, the first intron is required for T3 induction as well as high levels of hepatic expression. Our data indicate that the T3 stimulates CPT-I[alpha] gene expression in the liver through a T3 response unit consisting of the TRE in the promoter and additional factors, C/EBP and USF, bound in the first intron.

Jackson, M., J. W. Baird, et al. (2002). "Cloning and Characterization of Ehox, a Novel Homeobox Gene Essential for Embryonic Stem Cell Differentiation." J. Biol. Chem. **277**(41): 38683-38692.

http://www.jbc.org/cgi/content/abstract/277/41/38683

We report here the identification and characterization of a novel paired-like homeobox-containing gene (Ehox). This gene, identified in embryonic stem (ES) cells, is differentially expressed during in vitro ES cell differentiation. We have assessed Ehox function using the ES cell in vitro differentiation system. This has involved molecular and biological analyses of the effects of sense or antisense Ehox expression (using episomal vectors) on ES cell differentiation. Analysis of antisense Ehox-expressing ES cells indicates that they are unable to express marker genes associated with hematopoietic, endothelial, or cardiac differentiation following removal of leukemia inhibitory factor. In contrast, overexpression of Ehox using the sense construct accelerated the appearance of these differentiation markers. ES cell self-renewal and differentiation assays reveal that inhibition of Ehox activity results in the maintenance of a stem cell phenotype in limiting concentrations of leukemia inhibitory factor and the almost complete impairment of the cardiomyocyte differentiation capacity of these cells. We therefore conclude that Ehox is a novel homeobox-containing gene that is essential for the earliest stages of murine ES cell differentiation.

Jeanson, L. and J.-F. Mouscadet (2002). "Ku Represses the HIV-1 Transcription. IDENTIFICATION OF A PUTATIVE Ku BINDING SITE HOMOLOGOUS TO THE MOUSE MAMMARY TUMOR VIRUS NRE1 SEQUENCE IN THE HIV-1 LONG TERMINAL REPEAT." J. Biol. Chem. **277**(7): 4918-

4924.

http://www.jbc.org/cgi/content/abstract/277/7/4918

Ku has been implicated in nuclear processes, including DNA break repair, transcription, V(D)J recombination, and telomere maintenance. Its mode of action involves two distinct mechanisms: one in which a nonspecific binding occurs to DNA ends and a second that involves a specific binding to negative regulatory elements involved in transcription repression. Such elements were identified in mouse mammary tumor virus and human T cell leukemia virus retroviruses. The purpose of this study was to investigate a role for Ku in the regulation of human immunodeficiency virus (HIV)-1 transcription. First, HIV-1 LTR activity was studied in CHO-K1 cells and in CH0-derived xrs-6 cells, which are devoid of Ku80. LTR-driven expression of a reporter gene was significantly increased in xrs-6 cells. This enhancement was suppressed after re-expression of Ku80. Second, transcription of HIV-1 was followed in U1 human cells that were depleted in Ku by using a Ku80 antisense RNA. Ku depletion led to a increase of both HIV-1 mRNA synthesis and viral production compared with the parent cells. These results demonstrate that Ku acts as a transcriptional repressor of HIV-1 expression. Finally, a putative Ku-specific binding site was identified within the negative regulatory region of the HIV-1 long terminal repeat, which may account for this repression of transcription.

Jenkins, R. H., G. J. Thomas, et al. (2004). "Myofibroblastic Differentiation Leads to Hyaluronan Accumulation through Reduced Hyaluronan Turnover." J. Biol. Chem. **279**(40): 41453-41460.

http://www.jbc.org/cgi/content/abstract/279/40/41453

During the initiation and progression of fibrosis there is extensive differentiation of cells to a myofibroblastic phenotype. Because the synthesis of hyaluronan (HA) was recently linked to oncogenic epithelial-mesenchymal transformation, the present study investigated whether increased HA synthesis was also associated with myofibroblastic differentiation. HA synthesis and size were measured by incorporation of [3H]glucosamine, ion exchange, and size exclusion chromatography. Hyaluronan synthase (HAS) or hyaluronidase (HYAL) mRNA levels were assessed by reverse transcription-PCR. HYAL was detected by immunoblotting and the degradation of [3H]HA. Between 2- and 3-fold more HA appeared in the conditioned medium and became associated with the cells upon myofibroblastic differentiation. Inhibition of HAS and examination of HAS mRNA expression demonstrated that this was not the result of increased synthesis of HA or the induction of HAS 2. After differentiation, however, myofibroblasts metabolized exogenously supplied [3H]HA at a slower rate than fibroblasts and expressed lower levels of both HYAL 1 and HYAL 2 mRNA. Immunoblotting revealed more HYAL 1 and 2 in the myofibroblast conditioned medium. After acidification, however, there was no difference in HA degradation. This suggests that much of the released HYAL is inactive and that the observed differences in HA degradation are caused by cell-associated rather than secreted activity. This was confirmed by immunohistochemical staining for HYAL 1 and HYAL 2. This finding indicates the potential importance of the HYAL enzymes in controlling fibrotic progression and contrasts HA synthesis as a mediator of oncogenic transformation with that of HA degradation controlling fibrogenic differentiation.

Jhandier, M. N., E. A. Kruglov, et al. (2005). "Portal fibroblasts regulate the proliferation of bile duct epithelia via expression of NTPDase2." J. Biol. Chem.: M412371200.

http://www.jbc.org/cgi/content/abstract/M412371200v1

Bile duct epithelia are the target of a number "cholangiopathies" characterized by disordered bile ductular proliferation. While mechanisms for bile ductular proliferation are unknown, recent evidence suggests that extracellular nucleotides regulate cell proliferation via activation of P2Y receptors. Portal fibroblasts may regulate bile duct epithelial P2Y receptors via expression of the ecto-nucleotidase NTPDase2. Thus, we tested the hypothesis that portal fibroblasts regulate bile duct epithelial proliferation via expression of NTPDase2. We generated a novel co-culture model of Mz-ChA-1 human cholangiocarcinoma cells and primary portal fibroblasts. Cell proliferation was measured by bromodeoxyuridine uptake. NTPDase2 expression was assessed by immunofluorescence and quantitative real-time RT-PCR. NTPDase2 expression in portal fibroblasts was blocked using siRNA. NTPDase2 overexpression in portal myofibroblasts isolated from bile duct ligated rats was achieved by cDNA transfection. Co-culture of Mz-ChA-1 cells with PF decreased their proliferation to 26% of control. Similar decreases in Mz-ChA-1 proliferation were induced by the soluble ecto-nucleotidase apyrase and the P2 receptor inhibitor suramin. The proliferation of Mz-ChA-1 cells returned to baseline when NTPDase2 expression in portal fibroblasts was inhibited using NTPDase2-specific siRNA. Untransfected portal myofibroblasts lacking NTPDase2 had no effect on Mz-ChA-1 proliferation, yet portal myofibroblasts transfected with NTPDase2 cDNA inhibited Mz-ChA-1 proliferation. We conclude that, portal fibroblasts inhibit bile ductular proliferation via expression of NTPDase2 and blockade of P2Y activation. Loss of NTPDase2 may mediate the bile ductular proliferation typical of obstructive cholestasis. This novel crosstalk signaling pathway may mediate pathologic alterations in bile ductular proliferation in other cholangiopathic conditions.

Jiang, H., R. S. Peterson, et al. (2002). "A Requirement for the CD44 Cytoplasmic Domain for Hyaluronan Binding, Pericellular Matrix Assembly, and Receptor-mediated Endocytosis in COS-7 Cells." J. Biol. Chem. **277**(12): 10531-10538.

http://www.jbc.org/cgi/content/abstract/277/12/10531

CD44-negative COS-7 cells were transfected with expression constructs for CD44H (the predominant CD44 isoform), CD44E (epithelial isoform), or truncation mutant derivatives lacking the carboxyl-terminal 67 amino acids of the cytoplasmic domain, CD44H[Delta]67 and CD44E[Delta]67. The truncation mutant CD44H[Delta]67 is identical to a naturally occurring alternatively spliced "short tail" CD44 isoform (CD44st), which incorporates exon 19 in place of exon 20. CD44st lacks intracellular signaling motifs as well as protein domains necessary for interaction with cytoskeletal components. Transfection of COS-7 cells with each construct yielded equivalent levels of mRNA expression, whereas no CD44 expression was observed in parental, nontransfected COS-7 cells. Western analysis and immunostaining of COS-7 transfectants confirmed CD44 protein expression of the truncation mutant derivatives. COS-7 cells transfected with CD44H or CD44E gained the capacity to bind fluorescein-conjugated HA (fl-HA) and assemble HA-dependent pericellular matrices in the presence of exogenously added HA and proteoglycan. In addition, the CD44H- and CD44E-transfected cells were able to internalize surface-bound fl-HA. COS-7 cells transfected with the vector alone or with either of the mutant CD44 isoforms, CD44H[Delta]67 or CD44E[Delta]67, did not exhibit the capacity to assemble pericellular matrices or to bind and internalize the fl-HA. Cotransfection of CD44[Delta]67 mutants together with CD44H reduced the size of the HA-dependent pericellular matrices. Transfection of bovine articular chondrocytes with CD44[Delta]67 also inhibited pericellular matrix assembly. Collectively, these results indicate an obligatory requirement for the CD44 receptor cytoplasmic domain for ligand (HA) binding, formation and retention of the pericellular matrix, as well as CD44-mediated endocytosis of HA. In addition, the results suggest a potential regulatory role for the differentially expressed alternatively spliced short tail CD44 isoform.

Johnson, D. E. and C. C. Richardson (2003). "A Covalent Linkage between the Gene 5 DNA Polymerase

of Bacteriophage T7 and Escherichia coli Thioredoxin, the Processivity Factor: FATE OF THIOREDOXIN DURING DNA SYNTHESIS." J. Biol. Chem. **278**(26): 23762-23772.

http://www.jbc.org/cgi/content/abstract/278/26/23762

Gene 5 protein (gp5) of bacteriophage T7 is a non-processive DNA polymerase, which acquires high processivity by binding to Escherichia coli thioredoxin. The gene 5 protein-thioredoxin complex (gp5/trx) polymerizes thousands of nucleotides before dissociating from a primer-template. We have engineered a disulfide linkage between the gene 5 protein and thioredoxin within the binding surface of the two proteins. The polymerase activity of the covalently linked complex (gp5-S-S-trx) is similar to that of gp5/trx on poly(dA)/oligo(dT). However, gp5-S-S-trx has only one third the polymerase activity of gp5/trx on single-stranded M13 DNA. gp5-S-S-trx has difficulty polymerizing nucleotides through sites of secondary structure on M13 DNA and stalls at these sites, resulting in lower processivity. However, gp5-S-S-trx has an identical processivity and rate of elongation when E. coli single-stranded DNA-binding protein (SSB protein) is used to remove secondary structure from M13 DNA. Upon completing synthesis on a DNA template lacking secondary structure, both complexes recycle intact, without dissociation of the processivity factor, to initiate synthesis on a new DNA template. However, a complex stalled at secondary structure becomes unstable, and both subunits dissociate from each other as the polymerase prematurely releases from M13 DNA.

Joosten, M., M. Blazquez-Domingo, et al. (2004). "Translational Control of Putative Protooncogene Nm23-M2 by Cytokines via Phosphoinositide 3-Kinase Signaling." J. Biol. Chem. **279**(37): 38169-38176.

http://www.jbc.org/cgi/content/abstract/279/37/38169

The expansion and differentiation of hematopoietic progenitors is regulated by cytokine and growth factor signaling. To examine how signal transduction controls the gene expression program required for progenitor expansion, we screened ATLAS filters with polysome-associated mRNA derived from erythroid progenitors stimulated with erythropoietin and/or stem cell factor. The putative proto-oncogene nucleoside diphosphate kinase B (ndpk-B or nm23-M2) was identified as an erythropoietin and stem cell factor target gene. Factor-induced expression of nm23-M2 was regulated specifically at the level of polysome association by a phosphoinositide 3-kinase-dependent mechanism. Identification of the transcription initiation site revealed that nm23-M2 mRNA starts with a terminal oligopyrimidine sequence, which is known to render mRNA translation dependent on mitogenic factors. Recently, the nm23-M2 locus was identified as a common leukemia retrovirus integration site, suggesting that it plays a role in leukemia development. The expression of Nm23 from a retroviral vector in the absence of its 5'-untranslated region caused constitutive polysome association of nm23-M2. Polysome-association and protein expression of endogenous nm23-M2 declined during differentiation of erythroid progenitors, suggesting a role for Nm23-M2 in progenitor expansion. Taken together, nm23-m2 exemplifies that cytokine-dependent control of translation initiation is an important mechanism of gene expression regulation.

Jurado, L. A., S. Song, et al. (2002). "Conserved Amino Acids within CCAAT Enhancer-binding Proteins (C/EBPalpha and beta) Regulate Phosphoenolpyruvate Carboxykinase (PEPCK) Gene Expression." J. Biol. Chem. **277**(31): 27606-27612.

http://www.jbc.org/cgi/content/abstract/277/31/27606

Thyroid hormone and cAMP stimulate transcription of the gene for phosphoenolpyruvate carboxykinase (PEPCK). CCAAT enhancer-binding proteins (C/EBP[alpha] and [beta]) are involved in multiple aspects of the nutritional, developmental and hormonal regulation of PEPCK gene expression. Previously, we have identified a thyroid hormone response element in the PEPCK promoter and demonstrated that C/EBP proteins bound to the P3(I) site are participants in the induction of PEPCK gene expression by thyroid hormone and cAMP. Here, we identify several peptide regions within the transactivation domain of C/EBP[alpha] that enhance the ability of T3 to stimulate gene transcription. We also demonstrate that several conserved amino acids in the transactivation domain of C/EBP[alpha] and C/EBP[beta] are required for the stimulation of basal gene expression and identify amino acids within C/EBP[beta] that participate in the cAMP induction of the PEPCK gene. Finally, we show that the CREB-binding protein (CBP) enhanced the induction of PEPCK gene transcription by thyroid hormone and that CBP is associated with the PEPCK gene in vivo. Our results indicate that both C/EBP proteins and CBP participate in the regulation of PEPCK gene transcription by thyroid hormone.

Kaneto, H., T.-a. Matsuoka, et al. (2005). "A Crucial Role of MafA as a Novel Therapeutic Target for Diabetes." J. Biol. Chem. **280**(15): 15047-15052.

http://www.jbc.org/cgi/content/abstract/280/15/15047

MafA, a recently isolated pancreatic {beta}-cell-specific transcription factor, is a potent activator of insulin gene transcription. In this study, we show that MafA overexpression, together with PDX-1 (pancreatic and duodenal homeobox factor-1) and NeuroD, markedly increases insulin gene expression in the liver. Consequently, substantial amounts of insulin protein were induced by such combination. Furthermore, in streptozotocin-induced diabetic mice, MafA overexpression in the liver, together with PDX-1 and NeuroD, dramatically ameliorated glucose tolerance, while combination of PDX-1 and NeuroD was much less effective. These results suggest a crucial role of MafA as a novel therapeutic target for diabetes.

Kaneto, H., A. Sharma, et al. (2002). "Induction of c-Myc Expression Suppresses Insulin Gene Transcription by Inhibiting NeuroD/BETA2-mediated Transcriptional Activation." J. Biol. Chem. **277**(15): 12998-13006.

http://www.jbc.org/cgi/content/abstract/277/15/12998

Insulin biosynthesis and secretion are critical for pancreatic [beta]-cell function, but both are impaired under diabetic conditions. We have found that hyperglycemia induces the expression of the basic helix-loop-helix transcription factor c-Myc in islets in several different diabetic models. To examine the possible implication of c-Myc in [beta]-cell dysfunction, c-Myc was overexpressed in isolated rat islets using adenovirus. Adenovirus-mediated c-Myc overexpression suppressed both insulin gene transcription and glucose-stimulated insulin secretion. Insulin protein content, determined by immunostaining, was markedly decreased in c-Myc-overexpressing cells. In gel-shift assays c-Myc bound to the E-box in the insulin gene promoter region. Furthermore, in [beta]TC1, MIN6, and HIT-T15 cells and primary rat islets, wild type insulin gene promoter activity was dramatically decreased by c-Myc overexpression, whereas the activity of an E-box mutated insulin promoter was not affected. In HeLa and HepG2 cells c-Myc exerted a suppressive effect on the insulin promoter activity only in the presence of NeuroD/BETA2 but not PDX-1. Both c-Myc and NeuroD can bind the E-box element in the insulin promoter, but unlike NeuroD, the c-Myc transactivation domain lacked the ability to activate insulin gene expression. Additionally p300, a co-activator of NeuroD, did not function as a co-activator of c-Myc. In conclusion, increased expression of c-Myc in [beta]-cells suppresses the insulin gene transcription by inhibiting NeuroD-mediated transcriptional activation. This mechanism may explain some of the [beta]-cell

dysfunction found in diabetes.

Kaneto, H., K. Suzuma, et al. (2002). "Involvement of Protein Kinase C beta 2 in c-myc Induction by High Glucose in Pancreatic beta -Cells." J. Biol. Chem. **277**(5): 3680-3685.

http://www.jbc.org/cgi/content/abstract/277/5/3680

The expression of the basic helix-loop-helix transcription factor c-Myc is induced in pancreatic islets of several different diabetic model animals and is possibly involved in suppression of the insulin gene transcription. In this study, we found that activity of protein kinase C is increased by high glucose, preceding the induction of c-myc expression and that PKC [beta]2 specifically regulates c-myc expression in pancreatic [beta]-cells. Since PKC [alpha], [beta]2, [delta], [epsilon], and [zeta] were expressed in rat pancreatic islets, we prepared each wild type (WT) and dominant negative type (DN) PKC isoform ([alpha], [beta]2, [delta], [epsilon], and [zeta])-expressing adenovirus to examine the effect of each PKC isoform on c-myc expression. In isolated rat pancreatic islets, adenovirus-mediated overexpression of WT PKC [beta]2, but not other PKC isoforms, markedly increased c-myc expression. Moreover, c-myc induction by high glucose was suppressed by adenovirus-mediated overexpression of DN PKC [beta]2 but not by other DN PKC isoforms. Finally, adenovirus-mediated overexpression of WT PKC [beta]2, but not of other PKC isoforms, leads to suppression of the insulin gene transcription in pancreatic islets. These results suggest that at least some of the reduction of insulin gene transcription found in the diabetic state is mediated by PKC [beta]2 regulation of c-myc expression.

Kaneto, H., G. Xu, et al. (2002). "Involvement of c-Jun N-terminal Kinase in Oxidative Stress-mediated Suppression of Insulin Gene Expression." J. Biol. Chem. **277**(33): 30010-30018.

http://www.jbc.org/cgi/content/abstract/277/33/30010

Oxidative stress, which is found in pancreatic [beta]-cells in the diabetic state, suppresses insulin gene transcription and secretion, but the signaling pathways involved in the [beta]-cell dysfunction induced by oxidative stress remain unknown. In this study, subjecting rat islets to oxidative stress activates JNK, p38 MAPK, and protein kinase C, preceding the decrease of insulin gene expression. Adenovirus-mediated overexpression of dominant-negative type (DN) JNK, but not the p38 MAPK inhibitor SB203580 nor the protein kinase C inhibitor GF109203X, protected insulin gene expression and secretion from oxidative stress. Moreover, wild type JNK overexpression suppressed both insulin gene expression and secretion. These results were correlated with changes in the binding of the important transcription factor PDX-1 to the insulin promoter; adenoviral overexpression of DN-JNK preserved PDX-1 DNA binding activity in the face of oxidative stress, whereas wild type JNK overexpression decreased PDX-1 DNA binding activity. Furthermore, to examine whether suppression of the JNK pathway can protect [beta]-cells from the toxic effects of hyperglycemia, rat islets were infected with DN-JNK expressing adenovirus or control adenovirus and transplanted under renal capsules of streptozotocin-induced diabetic nude mice. In mice receiving DN-JNK overexpressing islets, insulin gene expression in islet grafts was preserved, and hyperglycemia was ameliorated compared with control mice. In conclusion, activation of JNK is involved in the reduction of insulin gene expression by oxidative stress, and suppression of the JNK pathway protects [beta]-cells from oxidative stress.

Kang, H.-Y., K.-E. Huang, et al. (2002). "Differential Modulation of Androgen Receptor-mediated

Transactivation by Smad3 and Tumor Suppressor Smad4." J. Biol. Chem. **277**(46): 43749-43756.

http://www.jbc.org/cgi/content/abstract/277/46/43749

Smad proteins have been demonstrated to be key components in the transforming growth factor [beta] signaling cascade. Here we demonstrate that Smad4, together with Smad3, can interact with the androgen receptor (AR) in the DNA-binding and ligand-binding domains, which may result in the modulation of 5[alpha]-dihydrotestosterone-induced AR transactivation. Interestingly, in the prostate PC3 and LNCaP cells, addition of Smad3 can enhance AR transactivation, and co-transfection of Smad3 and Smad4 can then repress AR transactivation in various androgen response element-promoter reporter assays as well as Northern blot and reverse transcription-PCR quantitation assays with prostate-specific antigen mRNA expression. In contrast, in the SW480{middle dot}C7 cells, lacking endogenous functional Smad4, the influence of Smad3 on AR transactivation is dependent on the various androgen response element-promoters. The influence of Smad3/Smad4 on the AR transactivation may involve the acetylation since the treatment of trichostatin A or sodium butyrate can reverse Smad3/Smad4-repressed AR transactivation and Smad3/Smad4 complex can also decrease the acetylation level of AR. Together, these results suggest that the interactions between AR, Smad3, and Smad4 may result in the differential regulation of the AR transactivation, which further strengthens their roles in the prostate cancer progression.

Kang, Z., A. Pirskanen, et al. (2002). "Involvement of Proteasome in the Dynamic Assembly of the Androgen Receptor Transcription Complex." J. Biol. Chem. **277**(50): 48366-48371.

http://www.jbc.org/cgi/content/abstract/277/50/48366

We have used the chromatin immunoprecipitation technique to analyze the formation of the androgen receptor (AR) transcription complex onto prostate-specific antigen (PSA) and kallikrein 2 promoters in LNCaP cells. Our results show that loading of holo-AR and recruitment of RNA polymerase II to the promoters occur transiently. The cyclic nature of AR transcription complex assembly is also illustrated by transient association of coactivators GRIP1 and CREB-binding protein and acetylated histone H3 with the PSA promoter. Treatment of cells with the pure antiandrogen bicalutamide also elicits occupancy of the promoter by AR. In contrast to the agonist-liganded AR, bicalutamide-bound receptor is not capable of recruiting polymerase II, GRIP1, or CREB-binding protein, indicating that the conformation of AR bound to anti-androgen is not competent to assemble transcription complexes. Proteasome is involved in the regulation of AR-dependent transcription, as a proteasome inhibitor, MG-132, prevents the release of the receptor from the PSA promoter, and it also blocks the androgen-induced PSA mRNA accumulation. Furthermore, occupancy of the PSA promoter by the 19 S proteasome subcomplex parallels that by AR. Collectively, formation of the AR transcription complex, encompassing AR, polymerase II, and coactivators, on a regulated promoter is a cyclic process involving proteasome function.

Karbarz, M. J., S. R. Kalb, et al. (2003). "Expression Cloning and Biochemical Characterization of a Rhizobium leguminosarum Lipid A 1-Phosphatase." J. Biol. Chem. **278**(41): 39269-39279.

http://www.jbc.org/cgi/content/abstract/278/41/39269

Lipid A of Rhizobium leguminosarum, a nitrogen-fixing plant endosymbiont, displays several significant structural differences when compared with Escherichia coli. An especially striking feature of R. leguminosarum lipid A is that it lacks both the 1- and 4'-phosphate groups. Distinct

lipid A phosphatases that attack either the 1 or the 4' positions have previously been identified in extracts of R. leguminosarum and Rhizobium etli but not Sinorhizobium meliloti or E. coli. Here we describe the identification of a hybrid cosmid (pMJK-1) containing a 25-kb R. leguminosarum 3841 DNA insert that directs the overexpression of the lipid A 1-phosphatase. Transfer of pMJK-1 into S. meliloti 1021 results in heterologous expression of 1-phosphatase activity, which is normally absent in extracts of strain 1021, and confers resistance to polymyxin. Sequencing of a 7-kb DNA fragment derived from the insert of pMJK-1 revealed the presence of a lipid phosphatase ortholog (designated LpxE). Expression of lpxE in E. coli behind the T7lac promoter results in the appearance of robust 1-phosphatase activity, which is normally absent in E. coli membranes. Matrix-assisted laser-desorption/time of flight and radiochemical analysis of the product generated in vitro from the model substrate lipid IVA confirms the selective removal of the 1-phosphate group. These findings show that lpxE is the structural gene for the 1-phosphatase. The availability of lpxE may facilitate the re-engineering of lipid A structures in diverse Gram-negative bacteria and allow assessment of the role of the 1-phosphatase in R. leguminosarum symbiosis with plants. Possible orthologs of LpxE are present in some intracellular human pathogens, including Francisella tularensis, Brucella melitensis, and Legionella pneumophila.

Karchner, S. I., D. G. Franks, et al. (2002). "Regulatory Interactions among Three Members of the Vertebrate Aryl Hydrocarbon Receptor Family: AHR Repressor, AHR1, and AHR2." J. Biol. Chem. **277**(9): 6949-6959.

http://www.jbc.org/cgi/content/abstract/277/9/6949

The effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and related compounds occur via the aryl hydrocarbon receptor (AHR), a member of the basic helix-loop-helix-Per-ARNT-Sim homology (bHLH-PAS) protein superfamily. A single AHR gene has been identified in mammals, whereas many fish species, including the Atlantic killifish (Fundulus heteroclitus) possess two distinct AHR genes (AHR1 and a novel form, AHR2). A mouse bHLH-PAS protein closely related to AHR and designated AHR repressor (AHRR) is induced by 3-methylcholanthrene and represses the transcriptional activity of the AHR. To determine whether AHRR is the mammalian ortholog of fish AHR2 and to investigate the mechanisms by which AHRR regulates AHR function, we cloned an AHRR ortholog in F. heteroclitus with high sequence identity to the mouse and human AHRRs. Killifish AHRR encodes a 680-residue protein with a predicted molecular mass of 75.2 kDa. We show that in vitro expressed AHRR proteins from human, mouse, and killifish all fail to bind [3H]TCDD or [3H][beta]-naphthoflavone. In transient transfection experiments using a luciferase reporter gene under control of AHR response elements, killifish AHRR inhibited the TCDD-dependent transactivation function of both AHR1 and AHR2. AHRR mRNA is widely expressed in killifish tissues and is inducible by TCDD or polychlorinated biphenyls, but its expression is not altered in a population of fish exhibiting genetic resistance to these compounds. The F. heteroclitus AHRR promoter contains three putative AHR response elements. Both AHR1 and AHR2 activated transcription of luciferase driven by the AHRR promoter, and AHRR could repress its own promoter. Thus, AHRR is an evolutionarily conserved, TCDD-inducible repressor of AHR1 and AHR2 function. Phylogenetic analysis shows that AHRR, AHR1, and AHR2 are distinct genes, members of an AHR gene family; these three vertebrate AHR-like genes descended from a single invertebrate AHR.

Kawaguchi, T., K. Osatomi, et al. (2002). "Mechanism for Fatty Acid "Sparing" Effect on Glucose-induced Transcription. REGULATION OF CARBOHYDRATE-RESPONSIVE ELEMENT-BINDING PROTEIN BY AMP-ACTIVATED PROTEIN KINASE." J. Biol. Chem. **277**(6): 3829-3835.

http://www.jbc.org/cgi/content/abstract/277/6/3829

Carbohydrate-responsive element-binding protein (ChREBP) is a new transcription factor that binds to the carbohydrate-responsive element of the L-type pyruvate kinase gene (L-PK). The aim of this study was to investigate the mechanism by which feeding high fat diets results in decreased activity of ChREBP in the liver (Yamashita, H., Takenoshita, M., Sakurai, M., Bruick, R. K., Henzel, W. J., Shillinglaw, W., Arnot, D., and Uyeda, K. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 9116-9121). We cloned the rat liver ChREBP gene for use throughout this study. Acetate, octanoate, and palmitate inhibited the glucose-induced activation of L-PK transcription in ChREBP-overexpressed hepatocytes. In these hepatocytes, the cytosolic AMP concentration increased 30-fold and AMP-activated protein kinase activity was activated 2-fold. Similarly to the fatty acids, 5-amino-4-imidazolecarboxamide ribotide, a specific activator of AMP-activated protein kinase (AMPK) also inhibited the L-PK transcription activity in ChREBP-overexpressed hepatocytes. Using as a substrate a truncated ChREBP consisting of the C-terminal region, we demonstrated that phosphorylation by AMPK resulted in inactivation of the DNA binding activity. AMPK specifically phosphorylated Ser568 of ChREBP. A S568A mutant of the ChREBP gene showed tight DNA binding and lost its fatty acid sensitivity, whereas a S568D mutant showed weak DNA binding and inhibited L-PK transcription activity even in the absence of fatty acid. These results strongly suggested that the fatty acid inhibition of glucose-induced L-PK transcription resulted from AMPK phosphorylation of ChREBP at Ser568, which inactivated the DNA binding activity. AMPK was activated by the increased AMP that was generated by the fatty acid activation.

Kessler, N., H. Schuhmann, et al. (2004). "The Linear Pentadecapeptide Gramicidin Is Assembled by Four Multimodular Nonribosomal Peptide Synthetases That Comprise 16 Modules with 56 Catalytic Domains." J. Biol. Chem. **279**(9): 7413-7419.

http://www.jbc.org/cgi/content/abstract/279/9/7413

Linear gramicidin is a membrane channel forming pentadecapeptide that is produced via the nonribosomal pathway. It consists of 15 hydrophobic amino acids with alternating L- and D-configuration forming a {beta}-helix-like structure. It has an N-formylated valine and a C-terminal ethanolamine. Here we report cloning and sequencing of the entire biosynthetic gene cluster as well as initial biochemical analysis of a new reductase domain. The biosynthetic gene cluster was identified on two nonoverlapping fosmids and a 13-kilobase pair (kbp) interbridge fragment covering a region of 74 kbp. Four very large open reading frames, lgrA, lgrB, lgrC, and lgrD with 6.8, 15.5, 23.3, and 15.3 kbp, were identified and shown to encode nonribosomal peptide synthetases with two, four, six, and four modules, respectively. Within the 16 modules identified, seven epimerization domains in alternating positions were detected as well as a putative formylation domain fused to the first module LgrA and a putative reductase domain attached to the C-terminal module of LgrD. Analysis of the substrate specificity by phylogenetic studies using the residues of the substrate-binding pockets of all 16 adenylation domains revealed a good agreement of the substrate amino acids predicted with the sequence of linear gramicidin. Additional biochemical analysis of the three adenylation domains of modules 1, 2, and 3 confirmed the colinearity of this nonribosomal peptide synthetase assembly line. Module 16 was predicted to activate glycine, which would then, being the C-terminal residue of the peptide chain, be reduced by the adjacent reductase domain to give ethanolamine, thereby releasing the final product N-formyl-pentadecapeptide-ethanolamine. However, initial biochemical analysis of this reductase showed only a one-step reduction yielding the corresponding aldehyde in vitro.

Kiefer, H. L. B., T. M. Hanley, et al. (2004). "Retinoic Acid Inhibition of Chromatin Remodeling at the Human Immunodeficiency Virus Type 1 Promoter: UNCOUPLING OF HISTONE ACETYLATION AND CHROMATIN REMODELING." J. Biol. Chem. **279**(42): 43604-43613.

http://www.jbc.org/cgi/content/abstract/279/42/43604

All-trans retinoic acid (RA) represses HIV-1 transcription and replication in cultured monocytic cells and in primary monocyte-derived macrophages. Here we examine the role of histone acetylation and chromatin remodeling in RA-mediated repression. RA pretreatment of latently infected U1 promonocytes inhibits HIV-1 expression in response to the histone deacetylase (HDAC) inhibitor, trichostatin A (TSA). TSA is thought to activate HIV-1 transcription by inducing histone hyperacetylation within a regulatory nucleosome, nuc-1, positioned immediately downstream from the transcription start site. Acetylation of nuc-1 is thought to be a critical step in activation that precedes nuc-1 remodeling and, subsequently, transcriptional initiation. Here we demonstrate that TSA treatment induces H3 and H4 hyperacetylation and nuc-1 remodeling. Although RA pretreatment inhibits nuc-1 remodeling and HIV-1 transcription, it has no effect on histone acetylation. This suggests that acetylation and remodeling are not obligatorily coupled. We also show that growth of U1 cells in retinoid-deficient medium induces nuc-1 remodeling and HIV-1 expression but does not induce histone hyperacetylation. These findings suggest that remodeling, not histone hyperacetylation, is the limiting step in transcriptional activation in these cells. Together, these data suggest that RA signaling maintains the chromatin structure of the HIV-1 promoter in a transcriptionally non-permissive state that may contribute to the establishment of latency in monocyte/macrophages.

Kim, S., C. Domon-Dell, et al. (2004). "Down-regulation of the Tumor Suppressor PTEN by the Tumor Necrosis Factor-{alpha}/Nuclear Factor-{kappa}B (NF-{kappa}B)-inducing Kinase/NF-{kappa}B Pathway Is Linked to a Default I{kappa}B-{alpha} Autoregulatory Loop." J. Biol. Chem. **279**(6): 4285-4291.

http://www.jbc.org/cgi/content/abstract/279/6/4285

The PTEN (phosphatase and tensin homolog deleted on chromosome ten) tumor suppressor gene affects multiple cellular processes including cell growth, proliferation, and cell migration by antagonizing phosphatidylinositol 3-kinase (PI3K). However, mechanisms by which PTEN expression is regulated have not been studied extensively. Similar to PTEN, tumor necrosis factor-{alpha} (TNF-{alpha}) affects a wide spectrum of diseases including inflammatory processes and cancer by acting as a mediator of apoptosis, inflammation, and immunity. In this study, we show that treatment of cancer cell lines with TNF-{alpha} decreases PTEN expression. In addition, overexpression of TNF-{alpha} downstream signaling targets, nuclear factor-{kappa}B (NF-{kappa}B)-inducing kinase (NIK) and p65 nuclear factor NF-{kappa}B, lowers PTEN expression, suggesting that TNF-{alpha}-induced down-regulation of PTEN is mediated through a TNF-{alpha}/NIK/NF-{kappa}B pathway. Down-regulation of PTEN by NIK/NF-{kappa}B results in activation of the PI3K/Akt pathway and augmentation of TNF-{alpha}-induced PI3K/Akt stimulation. Importantly, we demonstrate that this effect is associated with a lack of an inhibitor of {kappa}B (I{kappa}B)-{alpha} autoregulatory loop. Moreover, these findings suggest the interaction between PI3K/Akt and NF-{kappa}B via transcriptional regulation of PTEN and offer one possible explanation for increased tumorigenesis in systems in which NF-{kappa}B is chronically activated. In such a tumor system, these findings suggest a positive feedback loop whereby Akt activation of NF-{kappa}B further stimulates Akt via down-regulation of the PI3K inhibitor PTEN.

Ko, Y.-G., K. Nishino, et al. (2005). "Stage-by-Stage Change in DNA Methylation Status of Dnmt1 Locus during Mouse Early Development." J. Biol. Chem. **280**(10): 9627-9634.

http://www.jbc.org/cgi/content/abstract/280/10/9627

Methylation of DNA is involved in tissue-specific gene control, and establishment of DNA methylation pattern in the genome is thought to be essential for embryonic development. Three isoforms of Dnmt1 (DNA methyltransferase 1) transcripts, Dnmt1s, Dnmt1o, and Dnmt1p, are produced by alternative usage of multiple first exons. Dnmt1s is expressed in somatic cells. Dnmt1p is found only in pachytene spermatocytes, whereas Dnmt1o is specific to oocytes and preimplantation embryos. Here we determined that there is a tissue-dependent differentially methylated region (T-DMR) in the 5' region of Dnmt1o but not in that of the Dnmt1s/1p. The methylation status of the Dnmt1o T-DMR was distinctively different in the oocyte from that in the sperm and adult somatic tissues and changed at each stage from fertilization to blastocyst stage, suggesting that active methylation and demethylation occur during preimplantation development. The T-DMR was highly methylated in somatic cells and embryonic stem cells. Analysis using Dnmt-deficient embryonic stem cell lines revealed that Dnmt1, Dnmt3a, and Dnmt3b are each partially responsible for maintenance of methylation of Dnmt1o T-DMR. In particular, there are compensatory and cooperative roles between Dnmt3a and Dnmt3b. Thus, the regulatory region of Dnmt1o, but not of Dnmt1s/1p, appeared to be a target of DNA methylation. The present study also suggested that the DNA methylation status of the gene region dynamically changes during embryogenesis independently of the change in the bulk DNA methylation status.

Kominato, Y., Y. Hata, et al. (2002). "Alternative Promoter Identified between a Hypermethylated Upstream Region of Repetitive Elements and a CpG Island in Human ABO Histo-blood Group Genes." J. Biol. Chem. **277**(40): 37936-37948.

http://www.jbc.org/cgi/content/abstract/277/40/37936

We have studied the expression of human histo-blood group ABO genes during erythroid differentiation, using an ex vivo culture of AC133[-]CD34+ cells obtained from peripheral blood. 5'-Rapid amplification of cDNA ends analysis of RNA from those cells revealed a novel transcription start site, which appeared to mark an alternative starting exon (1a) comprising 27 bp at the 5'-end of a CpG island in ABO genes. Results from reverse transcription-PCR specific to exon 1a indicated that the cells of both erythroid and epithelial lineages utilize this exon as the transcription starting exon. Transient transfection experiments showed that the region just upstream from the transcription start site possesses promoter activity in a cell type-specific manner when placed 5' adjacent to the reporter luciferase gene. Results from bisulfite genomic sequencing and reverse transcription-PCR analysis indicated that hypermethylation of the distal promoter region correlated with the absence of transcripts containing exon 1a, whereas hypermethylation in the interspersed repeats 5' adjacent to the distal promoter was commonly observed in all of the cell lines examined. These results suggest that a functional alternative promoter is located between the hypermethylated region of repetitive elements and the CpG island in the ABO genes.

Kondapalli, J., A. S. Flozak, et al. (2004). "Laminar Shear Stress Differentially Modulates Gene Expression of p120 Catenin, Kaiso Transcription Factor, and Vascular Endothelial Cadherin in Human Coronary Artery Endothelial Cells." J. Biol. Chem. **279**(12): 11417-11424.

http://www.jbc.org/cgi/content/abstract/279/12/11417

We demonstrated previously that laminar shear stress (LSS) enhances human coronary artery endothelial cell (HCAEC) wound closure via a vascular endothelial cadherin (VE-cadherin)-dependent mechanism. VE-cadherin can interact with p120 catenin (p120ctn) to mediate cell locomotion and proliferation. In this study, we hypothesized that p120ctn and an interacting protein, Kaiso, a transcriptional factor with which p120ctn may interact, would be expressed differentially at the wound border and away from the wound border in HCAEC exposed to LSS.

One of the major goals in this study was to assess the differential gene expression of p120ctn, Kaiso, and VE-cadherin in HCAEC at specific locations along the wound border to further our understanding of the molecular mechanisms involved in wound closure. We combined the technique of laser capture microdissection with quantitative real time PCR to compare p120ctn, Kaiso, and VE-cadherin mRNA expression in HCAEC at and away from the wound border under LSS. Total RNA was isolated from 200-1,000 laser-captured HCAEC and reverse transcribed into cDNA. Detection of p120ctn, Kaiso, and VE-cadherin mRNA was carried out using quantitative real time PCR. Normalization of cDNA templates was achieved by glyceraldehyde-3-phosphate dehydrogenase (GAPDH) quantification. Quantitative real time PCR analysis revealed p120ctn:GAPDH ratios, Kaiso: GAPDH ratios, and VE-cadherin:GAPDH ratios, relative to static control for each set, of 0.99-4.18 (mean {+/-} S.E., 1.94 {+/-} 0.404), 1.0-5.24 (2.11 {+/-} 0.51), and 0.99-1.42 (1.09 {+/-} 0.09) after 3 h of LSS, respectively. With these techniques, we found that p120ctn and Kaiso transcripts were increased in laser-captured HCAEC at the wound border compared with HCAEC away from the wound border. In addition, differential expression of p120ctn and Kaiso mRNA was observed in HCAEC depending on how LSS was applied in relation to the wounding process. These techniques may have wide applicability for studying wound healing because gene expression of key adhesion molecules in HCAEC may now be determined from select regions of the endothelial wound border.

Koo, I. C. and R. S. Stephens (2003). "A Developmentally Regulated Two-component Signal Transduction System in Chlamydia." J. Biol. Chem. **278**(19): 17314-17319.

http://www.jbc.org/cgi/content/abstract/278/19/17314

Two-component systems allow bacteria to adapt to changing environmental conditions and may induce developmental changes necessary for survival. Chlamydia trachomatis alternates between two distinct developmental forms, each optimized for survival in a separate niche. Transcriptional regulation of development is not understood. The C. trachomatis genome sequence revealed a single pair of genes (ctcB-ctcC) predicted to encode proteins with sequence conservation to bacterial two-component systems. Sequence analysis revealed that the sensor kinase, CtcB, possessed an energy-sensing PAS domain and phosphorylation site. The response regulator, CtcC, had homology to [sigma]54 activators, possessing conserved receiver and ATPase domains and phosphorylation site, but lacked the C-terminal DNA-binding domain. ctcB and ctcC were expressed late in the developmental cycle, and both proteins were detected in EB lysates. Recombinant CtcB and CtcC were purified from denatured Escherichia coli inclusion bodies and refolded. CtcC was found to aggregate as dimers and tetramers in solution. In vitro phosphorylation assays showed that CtcB autophosphorylated in the presence of Mg2+, Mn2+, and Fe2+ and transferred the phosphoryl group in the presence of CtcC. Collectively, these results show that CtcB and CtcC function as a two-component system and are likely responsible for transcriptional regulation by [sigma]54 holoenzyme during late-stage chlamydial development.

Kruger, R. P., H. C. Winter, et al. (2002). "Cloning, Expression, and Characterization of the Galalpha 1,3Gal High Affinity Lectin from the Mushroom Marasmius oreades." J. Biol. Chem. **277**(17): 15002-15005.

http://www.jbc.org/cgi/content/abstract/277/17/15002

The purification and unique carbohydrate binding properties, including blood group B-specific agglutination and preferential binding to Gal[alpha]1,3Gal-containing sugar epitopes, of the Marasmius oreades agglutinin (MOA) are reported in an accompanying paper (Winter, H. C., Mostafapour, K., and Goldstein, I. J. (2002) J. Biol. Chem. 277, 14996-15001). Here we describe the cloning, characterization, and expression of MOA. MOA was digested with trypsin and

endoproteinase Asp-N, and the peptide fragments were purified by high performance liquid chromatography. Amino acid sequence data were obtained for eight peptides. Using oligonucleotides deduced from the peptide sequences for a reverse transcriptase-PCR, a 41-base pair cDNA was obtained. The 41-base pair fragment allowed the generation a full-length cDNA using 5' and 3' rapid amplification of cDNA ends. MOA cDNA encodes a protein of 293 amino acids that contains a ricin domain. These carbohydrate binding domains were first described in subunits of bacterial toxins and are also commonly found in polysaccharide-degrading enzymes. Whereas these proteins are known to display a variety of sugar binding specificities, none to date are known to share MOA's high affinity for Gal[alpha]1,3Gal and Gal[alpha]1,3Gal[beta]1,4GlcNAc. Recombinantly expressed and purified MOA retains the specificity and affinity observed with the native protein. This study provides the basis for analyzing the underlying cause for the unusual binding specificity of MOA.

Kubota, H., R. W. Storms, et al. (2002). "Variant Forms of alpha -Fetoprotein Transcripts Expressed in Human Hematopoietic Progenitors. IMPLICATIONS FOR THEIR DEVELOPMENTAL POTENTIAL TOWARDS ENDODERM." J. Biol. Chem. **277**(31): 27629-27635.

http://www.jbc.org/cgi/content/abstract/277/31/27629

Hematopoietic stem cells have been identified as multipotent cells that give rise to all adult hematopoietic lineages. Although the hematopoietic lineage is derived from the mesodermal germ layer in the embryo, recent data suggest that bone marrow cells with an antigenic profile consistent with that of hematopoietic stem cells can also differentiate to cell types of the endodermal lineages, such as hepatocytes. However, the molecular mechanisms associated with these events are entirely unknown. For decades, [alpha]-fetoprotein (AFP) has been used as a differentiation marker for endodermal cells, because it was thought that the transcription of AFP mRNA is tightly regulated in a developmental and tissue-specific process. In this report we describe two new variant forms of AFP transcripts in human hematopoietic progenitors that are not expressed in mature cells. The variant AFP (vAFP) cDNA sequences isolated from a multipotent hematopoietic cell line, K562, revealed that the vAFP differed from the authentic transcript, consisting of 15 exons, by replacing exon 1 of AFP with one or two exons located in the 5'-untranslated region of the AFP gene. In addition to the K562 cell line, vAFP transcripts were detected in normal bone marrow, thymus, and brain but were not detected in normal spleen, intestine, liver, or the hepatocellular carcinoma cell line, HepG2. This suggests expression in normal hematopoietic progenitors. This hypothesis was confirmed by the finding that CD34+Lin[-] hematopoietic progenitor cells purified from cord blood by flow cytometric sorting also expressed the variant transcripts. These results suggest that some hematopoietic progenitors are in a state that permits them to express certain types of transcripts that have been considered unique to endoderm.

Kumar, S., L. Gupta, et al. (2004). "Inducible Peroxidases Mediate Nitration of Anopheles Midgut Cells Undergoing Apoptosis in Response to Plasmodium Invasion." J. Biol. Chem. **279**(51): 53475-53482.

http://www.jbc.org/cgi/content/abstract/279/51/53475

Plasmodium berghei invasion of Anopheles stephensi midgut cells causes severe damage, induces expression of nitric-oxide synthase, and leads to apoptosis. The present study indicates that invasion results in tyrosine nitration, catalyzed as a two-step reaction in which nitric-oxide synthase induction is followed by increased peroxidase activity. Ookinete invasion induced localized expression of peroxidase enzymes, which catalyzed protein nitration in vitro in the presence of nitrite and H2O2. Histochemical stainings revealed that when a parasite migrates

laterally and invades more than one cell, the pattern of induced peroxidase activity is similar to that observed for tyrosine nitration. In Anopheles gambiae, ookinete invasion elicited similar responses; it induced expression of 5 of the 16 peroxidase genes predicted by the genome sequence and decreased mRNA levels of one of them. One of these inducible peroxidases has a C-terminal oxidase domain homologous to the catalytic moiety of phagocyte NADPH oxidase and could provide high local levels of superoxide anion ([IMG]f1.gif" BORDER="0">), that when dismutated would generate the local increase in H2O2 required for nitration. Chemically induced apoptosis of midgut cells also activated expression of four ookinete-induced peroxidase genes, suggesting their involvement in general apoptotic responses. The two-step nitration reaction provides a mechanism to precisely localize and circumscribe the toxic products generated by defense reactions involving nitration. The present study furthers our understanding of the biochemistry of midgut defense reactions to parasite invasion and how these may influence the efficiency of malaria transmission by anopheline mosquitoes.

Lau, P., S. J. Nixon, et al. (2004). "ROR{alpha} Regulates the Expression of Genes Involved in Lipid Homeostasis in Skeletal Muscle Cells: CAVEOLIN-3 AND CPT-1 ARE DIRECT TARGETS OF ROR." J. Biol. Chem. **279**(35): 36828-36840.

http://www.jbc.org/cgi/content/abstract/279/35/36828

The staggerer mice carry a deletion in the ROR{alpha} gene and have a prolonged humoral response, overproduce inflammatory cytokines, and are immunodeficient. Furthermore, the staggerer mice display lowered plasma apoA-I/-II, decreased plasma high density lipoprotein cholesterol and triglycerides, and develop hypo-{alpha}-lipoproteinemia and atherosclerosis. However, relatively little is known about ROR{alpha} in the context of target tissues, target genes, and lipid homeostasis. For example, ROR{alpha} is abundantly expressed in skeletal muscle, a major mass peripheral tissue that accounts for [~]40% of total body weight and 50% of energy expenditure. This lean tissue is a primary site of glucose disposal and fatty acid oxidation. Consequently, muscle has a significant role in insulin sensitivity, obesity, and the blood-lipid profile. In particular, the role of ROR{alpha} in skeletal muscle metabolism has not been investigated, and the contribution of skeletal muscle to the ROR-/- phenotype has not been resolved. We utilize ectopic dominant negative ROR{alpha} expression in skeletal muscle cells to understand the regulatory role of RORs in this major mass peripheral tissue. Exogenous dominant negative ROR{alpha} expression in skeletal muscle cells represses the endogenous levels of ROR{alpha} and -{gamma} mRNAs and ROR-dependent gene expression. Moreover, we observed attenuated expression of many genes involved in lipid homeostasis. Furthermore, we show that the muscle carnitine palmitoyltransferase-1 and caveolin-3 promoters are directly regulated by ROR and coactivated by p300 and PGC-1. This study implicates RORs in the control of lipid homeostasis in skeletal muscle. In conclusion, we speculate that ROR agonists would increase fatty acid catabolism in muscle and suggest selective activators of ROR may have therapeutic utility in the treatment of obesity and atherosclerosis.

Laybutt, D. R., M. Glandt, et al. (2003). "Critical Reduction in beta -Cell Mass Results in Two Distinct Outcomes over Time. ADAPTATION WITH IMPAIRED GLUCOSE TOLERANCE OR DECOMPENSATED DIABETES." J. Biol. Chem. **278**(5): 2997-3005.

http://www.jbc.org/cgi/content/abstract/278/5/2997

We have proposed that hyperglycemia-induced dedifferentiation of [beta]-cells is a critical factor for the loss of insulin secretory function in diabetes. Here we examined the effects of the duration of hyperglycemia on gene expression in islets of partially pancreatectomized (Px) rats. Islets were isolated, and mRNA was extracted from rats 4 and 14 weeks after Px or sham Px surgery. Px rats

developed different degrees of hyperglycemia; low hyperglycemia was assigned to Px rats with fed blood glucose levels less than 150 mg/dl, and high hyperglycemia was assigned above 150 mg/dl. [beta]-Cell hypertrophy was present at both 4 and 14 weeks. At the same time points, high hyperglycemia rats showed a global alteration in gene expression with decreased mRNA for insulin, IAPP, islet-associated transcription factors (pancreatic and duodenal homeobox-1, BETA2/NeuroD, Nkx6.1, and hepatocyte nuclear factor 1[alpha]), [beta]-cell metabolic enzymes (glucose transporter 2, glucokinase, mitochondrial glycerol phosphate dehydrogenase, and pyruvate carboxylase), and ion channels/pumps (Kir6.2, VDCC[beta], and sarcoplasmic reticulum Ca2+-ATPase 3). Conversely, genes normally suppressed in [beta]-cells, such as lactate dehydrogenase-A, hexokinase I, glucose-6-phosphatase, stress genes (heme oxygenase-1, A20, and Fas), and the transcription factor c-Myc, were markedly increased. In contrast, gene expression in low hyperglycemia rats was only minimally changed at 4 weeks but significantly changed at 14 weeks, indicating that even low levels of hyperglycemia induce [beta]-cell dedifferentiation over time. In addition, whereas 2 weeks of correction of hyperglycemia completely reverses the changes in gene expression of Px rats at 4 weeks, the changes at 14 weeks were only partially reversed, indicating that the phenotype becomes resistant to reversal in the long term. In conclusion, chronic hyperglycemia induces a progressive loss of [beta]-cell phenotype with decreased expression of [beta]-cell-associated genes and increased expression of normally suppressed genes, these changes being present with even minimal levels of hyperglycemia. Thus, both the severity and duration of hyperglycemia appear to contribute to the deterioration of the [beta]-cell phenotype found in diabetes.

Laybutt, D. R., A. Sharma, et al. (2002). "Genetic Regulation of Metabolic Pathways in beta -Cells Disrupted by Hyperglycemia." J. Biol. Chem. **277**(13): 10912-10921.

http://www.jbc.org/cgi/content/abstract/277/13/10912

In models of type 2 diabetes the expression of [beta]-cell genes is altered, but these changes have not fully explained the impairment in [beta]-cell function. We hypothesized that changes in [beta]-cell phenotype and global alterations in both carbohydrate and lipid pathways are likely to contribute to secretory abnormalities. Therefore, expression of genes involved in carbohydrate and lipid metabolism were analyzed in islets 4 weeks after 85-95% partial pancreatectomy (Px) when [beta]-cells have impaired glucose-induced insulin secretion and ATP synthesis. Px rats after 1 week developed mild to severe hyperglycemia that was stable for the next 3 weeks, whereas neither plasma triglyceride, non-esterified fatty acid, or islet triglyceride levels were altered. Expression of peroxisome proliferator-activated receptors (PPARs), with several target genes, were reciprocally regulated; PPAR[alpha] was markedly reduced even at low level hyperglycemia, whereas PPAR[gamma] was progressively increased with increasing hyperglycemia. Uncoupling protein 2 (UCP-2) was increased as were other genes barely expressed in sham islets including lactate dehydrogenase-A (LDH-A), lactate (monocarboxylate) transporters, glucose-6-phosphatase, fructose-1,6-bisphosphatase, 12-lipoxygenase, and cyclooxygenase 2. On the other hand, the expression of [beta]-cell-associated genes, insulin, and GLUT2 were decreased. Treating Px rats with phlorizin normalized hyperglycemia without effecting plasma fatty acids and reversed the changes in gene expression implicating the importance of hyperglycemia per se in the loss of [beta]-cell phenotype. In addition, parallel changes were observed in [beta]-cell-enriched tissue dissected by laser capture microdissection from the central core of islets. In conclusion, chronic hyperglycemia leads to a critical loss of [beta]-cell differentiation with altered expression of genes involved in multiple metabolic pathways diversionary to normal [beta]-cell glucose metabolism. This global maladaptation in gene expression at the time of increased secretory demand may contribute to the [beta]-cell dysfunction found in diabetes.

Leavey, P. J., C. Gonzalez-Aller, et al. (2002). "A 29-kDa Protein Associated with p67phox Expresses Both Peroxiredoxin and Phospholipase A2 Activity and Enhances Superoxide Anion Production by a Cell-free System of NADPH Oxidase Activity." J. Biol. Chem. **277**(47): 45181-45187.

http://www.jbc.org/cgi/content/abstract/277/47/45181

Production of toxic oxygen metabolites provides a mechanism for microbicidal activity of the neutrophil. The NADPH oxidase enzyme system initiates the production of oxygen metabolites by reducing oxygen to form superoxide anion (O[IMG]img001.gif" ALT="<UP>₂</UP>">). With stimulation of the respiratory burst, cytosolic oxidase components, p47phox, p67phox, and Rac, translocate to the phagolysomal and plasma membranes where they form a complex with cytochrome b558 and express enzyme activity. A 29-kDa neutrophil protein (p29) was identified by co-immunoprecipitation with p67phox. N-terminal sequence analysis of p29 revealed homology to an open reading frame gene described in a myeloid leukemia cell line. A cDNA for p29 identical to the open reading frame protein was amplified from RNA of neutrophils. Significant interaction between p29 and p67phox was demonstrated using a yeast two-hybrid system. A recombinant (rh) p29 was expressed in Sf9 cells resulting in a protein with an apparent molecular weight of 34,000. The rh-p29 showed immunoreactivity with the original rabbit antiserum that detected p47phox and p67phox. In addition, rh-p29 exhibited PLA2 activity, which was Ca2+ independent, optimal at low pH, and preferential for phosphatidylcholine substrates. The recombinant protein protected glutathione synthetase and directly inactivated H2O2. By activity and sequence homology, rh-p29 can be classified as a peroxiredoxin. Finally, O[IMG]img001.gif" ALT="<UP>₂</UP>"> production by plasma membrane and recombinant cytosolic oxidase components in the SDS-activated, cell-free NADPH oxidase system were enhanced by rh-p29. This effect was not inhibited by PLA2 inhibitors. Thus, p29 is a novel protein that associates with p67 and has peroxiredoxin activity. This protein has a potential role in protecting the NADPH oxidase by inactivating H2O2 or altering signaling pathways affected by H2O2.

Lee, C.-G., T. W. Reichman, et al. (2004). "MLE Functions as a Transcriptional Regulator of the roX2 Gene." J. Biol. Chem. **279**(46): 47740-47745.

http://www.jbc.org/cgi/content/abstract/279/46/47740

Dosage compensation is a process that equalizes transcription activity between the sexes. In Drosophila, two non-coding RNA, roX1 and roX2, and at least six protein regulators, MSL-1, MSL-2, MSL-3, MLE, MOF, and JIL-1, have been identified as essential for dosage compensation. Although there is accumulating evidence of the intricate functional and physical interactions between protein and RNA regulators, little is known about how roX RNA expression and function are modulated in coordination with protein regulators. In this report, we have found that a relatively short (about 350 bp) upstream genomic region of the roX2 gene, Prox2, harbors an activity that drives transcription of the downstream gene. Our study has shown that MLE can stimulate the transcription activity of Prox2 and that MLE associates with Prox2 through direct interaction with a newly identified 54-bp repeat, Prox. Our observations suggest a novel mechanism by which roX2 RNA is regulated at the transcriptional level.

Lee, H.-W., D.-H. Ahn, et al. (2002). "Phorbol 12-Myristate 13-Acetate Up-regulates the Transcription of MUC2 Intestinal Mucin via Ras, ERK, and NF-kappa B." J. Biol. Chem. **277**(36): 32624-32631.

http://www.jbc.org/cgi/content/abstract/277/36/32624

MUC2 is a secretory mucin normally expressed by goblet cells of the intestinal epithelium. It is overexpressed in mucinous type colorectal cancers but down-regulated in colorectal adenocarcinoma. Phorbol 12-myristate 13-acetate (PMA) treatment of colon cancer cell lines increases MUC2 expression, so we have undertaken a detailed analysis of the effects of PMA on the promoter activity of the 5'-flanking region of the MUC2 gene using stably and transiently transfected promoter reporter vectors. Protein kinase C inhibitors (bisindolylmaleimide, calphostin C) and inhibitors of mitogen-activated protein/extracellular signal regulated kinase kinase (MEK) (PD98059 and U0126) suppressed up-regulation of MUC2. Src tyrosine kinase inhibitor PP2, a protein kinase A inhibitor (KT5720), and a p38 inhibitor (SB 203580) did not affect transcription. Western blotting and reverse transcription-PCR analysis confirmed these results. In addition, co-transfections with mutants of Ras, Raf, and MEK showed that the induction of MUC2 promoter activity by PMA required these three signaling proteins. Our results demonstrate that PMA activates protein kinase C, stimulating MAP kinase through a Ras- and Raf-dependent mechanism. An important role for nuclear factor [kappa]B (NF-[kappa]B) was also demonstrated using the inhibitor caffeic acid phenethyl ester and electrophoretic mobility shift assays. Such identification of pathways involved in MUC2 up-regulation by PMA in the HM3 colon cancer cell line may serve as a model for the effects of cytokines and growth factors, which regulate MUC2 expression during the progression of colorectal cancer.

Leite, M. F., K. Hirata, et al. (2002). "Molecular Basis for Pacemaker Cells in Epithelia." J. Biol. Chem. **277**(18): 16313-16323.

http://www.jbc.org/cgi/content/abstract/277/18/16313

Intercellular signaling is highly coordinated in excitable tissues such as heart, but the organization of intercellular signaling in epithelia is less clear. We examined Ca2+ signaling in hepatoma cells expressing the hepatocyte gap junction protein connexin32 (cx32) or the cardiac gap junction protein cx43, plus a fluorescently tagged V1a vasopressin receptor (V1aR). Release of inositol 1,4,5-trisphosphate (InsP3) in wild type cells increased Ca2+ in the injected cell but not in neighboring cells, while the Ca2+ signal spread to neighbors when gap junctions were expressed. Photorelease of caged Ca2+ rather than InsP3 resulted in a small increase in Ca2+ that did not spread to neighbors with or without gap junctions. However, photorelease of Ca2+ in cells stimulated with low concentrations of vasopressin resulted in a much larger increase in Ca2+, which spread to neighbors via gap junctions. Cells expressing tagged V1aR similarly had increased sensitivity to vasopressin, and could signal to neighbors via gap junctions. Higher concentrations of vasopressin elicited Ca2+ signals in all cells. In cx32 or cx43 but not in wild type cells, this signaling was synchronized and began in cells expressing the tagged V1aR. Thus, intercellular Ca2+ signals in epithelia are organized by three factors: 1) InsP3 must be generated in each cell to support a Ca2+ signal in that cell; 2) gap junctions are necessary to synchronize Ca2+ signals among cells; and 3) cells with relatively increased expression of hormone receptor will initiate Ca2+ signals and thus serve as pacemakers for their neighbors. Together, these factors may allow epithelia to act in an integrated, organ-level fashion rather than as a collection of isolated cells.

Lemercier, G., B. Espiau, et al. (2004). "A Pyrophosphatase Regulating Polyphosphate Metabolism in Acidocalcisomes Is Essential for Trypanosoma brucei Virulence in Mice." J. Biol. Chem. **279**(5): 3420-3425.

http://www.jbc.org/cgi/content/abstract/279/5/3420

We report the functional characterization of a soluble pyrophosphatase (TbVSP1), which localizes to acidocalcisomes, a vesicular acidic compartment of Trypanosoma brucei. Depending on the

pH and the cofactors Mg2+ or Zn2+, both present in the compartment, the enzyme hydrolyzes either inorganic pyrophosphate (PPi) (kcat = 385 s-1) or tripolyP (polyP3) and polyphosphate (polyP) of 28 residues (polyP28) with kcat values of 52 and 3.5 s-1, respectively. An unusual N-terminal domain of 160 amino acids, containing a putative calcium EF-hand-binding domain, is involved in protein oligomerization. Using double-stranded RNA interference methodology, we produced an inducible bloodstream form (BF) deficient in the TbVSP1 protein (BFiVSP1). The long-chain polyP levels of these mutants were reduced by 60%. Their phenotypes revealed a deficient polyP metabolism, as indicated by their defective response to phosphate starvation and hyposmotic stress. BFiVSP1 did not cause acute virulent infection in mice, demonstrating that TbVSP1 is essential for growth of bloodstream forms in the mammalian host.

Levitin, F., A. Baruch, et al. (2005). "A Novel Protein Derived from the MUC1 Gene by Alternative Splicing and Frameshifting." J. Biol. Chem. **280**(11): 10655-10663.

http://www.jbc.org/cgi/content/abstract/280/11/10655

Genes that have been designated the name "MUC" code for proteins comprising mucin domains. These proteins may be involved in barrier and protective functions. The first such gene to be characterized and sequenced is the MUC1 gene. Here we report a novel small protein derived from the MUC1 gene by alternative splicing that does not contain the hallmark of mucin proteins, the mucin domain. This protein termed MUC1/ZD retains the same N-terminal MUC1 sequences as all of the other known MUC1 protein isoforms. The common N-terminal sequences comprise the signal peptide and a subsequent stretch of 30 amino acids. In contrast, the MUC1/ZD C-terminal 43 amino acids are novel and result from a reading frameshift engendered by a splicing event that forms MUC1/ZD. The expression of MUC1/ZD at the protein level in human tissues is demonstrated by Western blotting, immunohistochemistry, immunoprecipitation, and an ELISA. Utilization was made of affinity-purified MUC1/ZD-specific polyclonal antibodies as well as two different monoclonal antibodies that are monospecific for the MUC1/ZD protein. The MUC1/ZD protein is expressed in tissues as an oligomeric complex composed of monomers linked by disulfide bonds contributed by MUC1/ZD cysteine residues. MUC1/ZD protein expression did not parallel that of the tandem-repeat array-containing MUC1 protein. Results presented here demonstrate for the first time the expression of a novel MUC1 protein isoform MUC1/ZD, which is generated by an alternative splicing event that both deletes the tandem-repeat array and leads to a C-terminal reading frameshift.

Li, B., N. M. Nowak, et al. (2005). "Random Mutagenesis of the M3 Muscarinic Acetylcholine Receptor Expressed in Yeast: IDENTIFICATION OF SECOND-SITE MUTATIONS THAT RESTORE FUNCTION TO A COUPLING-DEFICIENT MUTANT M3 RECEPTOR." J. Biol. Chem. **280**(7): 5664-5675.

http://www.jbc.org/cgi/content/abstract/280/7/5664

The M3 muscarinic receptor is a prototypical member of the class A family of G protein-coupled receptors (GPCRs). To gain insight into the structural mechanisms governing agonist-mediated M3 receptor activation, we recently developed a genetically modified yeast strain (Saccharomyces cerevisiae) which allows the efficient screening of large libraries of mutant M3 receptors to identify mutant receptors with altered/novel functional properties. Class A GPCRs contain a highly conserved Asp residue located in transmembrane domain II (TM II; corresponding to Asp-113 in the rat M3 muscarinic receptor) which is of fundamental importance for receptor activation. As observed previously with other GPCRs analyzed in mammalian expression systems, the D113N point mutation abolished agonist-induced receptor/G protein coupling in yeast. We then subjected the D113N mutant M3 receptor to PCR-based random

mutagenesis followed by a yeast genetic screen to recover point mutations that can restore G protein coupling to the D113N mutant receptor. A large scale screening effort led to the identification of three such second-site suppressor mutations, R165W, R165M, and Y250D. When expressed in the wild-type receptor background, these three point mutations did not lead to an increase in basal activity and reduced the efficiency of receptor/G protein coupling. Similar results were obtained when the various mutant receptors were expressed and analyzed in transfected mammalian cells (COS-7 cells). Interestingly, like Asp-113, Arg-165 and Tyr-250, which are located at the cytoplasmic ends of TM III and TM V, respectively, are also highly conserved among class A GPCRs. Our data suggest a conformational link between the highly conserved Asp-113, Arg-165, and Tyr-250 residues which is critical for receptor activation.

Li, Q., A. K.-K. Ching, et al. (2004). "A Death Receptor-associated Anti-apoptotic Protein, BRE, Inhibits Mitochondrial Apoptotic Pathway." J. Biol. Chem. **279**(50): 52106-52116.

http://www.jbc.org/cgi/content/abstract/279/50/52106

BRE, brain and reproductive organ-expressed protein, was found previously to bind the intracellular juxtamembrane domain of a ubiquitous death receptor, tumor necrosis factor receptor 1 (TNF-R1), and to down-regulate TNF-{alpha}-induced activation of NF-{kappa}B. Here we show that BRE also binds to another death receptor, Fas, and upon overexpression conferred resistance to apoptosis induced by TNF-{alpha}, anti-Fas agonist antibody, cycloheximide, and a variety of stress-related stimuli. However, down-regulation of the endogenous BRE by small interfering RNA increased apoptosis to TNF-{alpha}, but nottoetoposide, indicating that the physiological antiapoptotic role of this protein is specific to death receptor-mediated apoptosis. We further demonstrate that BRE mediates antiapoptosis by inhibiting the mitochondrial apoptotic machinery but without translocation to the mitochondria or nucleus or down-regulation of the cellular level of truncated Bid. Dissociation of BRE rapidly from TNF-R1, but not from Fas, upon receptor ligation suggests that this protein interacts with the death inducing signaling complex during apoptotic induction. Increased association of BREwith phosphorylated, sumoylated, and ubiquitinated proteins after death receptor stimulation was also detected. We conclude that in contrast to the truncated Bid that integrates mitochondrial apoptosis to death receptor-triggered apoptotic cascade, BRE inhibits the integration. We propose that BRE inhibits, by ubiquitination-like activity, components in or proximal to the death-inducing signaling complexes that are necessary for activation of the mitochondria.

Lim, I. A., D. D. Hall, et al. (2002). "Selectivity and Promiscuity of the First and Second PDZ Domains of PSD-95 and Synapse-associated Protein 102." J. Biol. Chem. **277**(24): 21697-21711.

http://www.jbc.org/cgi/content/abstract/277/24/21697

PDZ domains typically interact with the very carboxyl terminus of their binding partners. Type 1 PDZ domains usually require valine, leucine, or isoleucine at the very COOH-terminal (P0) position, and serine or threonine 2 residues upstream at P[-]2. We quantitatively defined the contributions of carboxyl-terminal residues to binding selectivity of the prototypic interactions of the PDZ domains of postsynaptic density protein 95 (PSD-95) and its homolog synapse-associated protein 90 (SAP102) with the NR2b subunit of the N-methyl-D-aspartate-type glutamate receptor. Our studies indicate that all of the last five residues of NR2b contribute to the binding selectivity. Prominent were a requirement for glutamate or glutamine at P[-]3 and for valine at P0 for high affinity binding and a preference for threonine over serine at P[-]2, in the context of the last 11 residues of the NR2b COOH terminus. This analysis predicts a COOH-terminal (E/Q)(S/T)XV consensus sequence for the strongest binding to the first two PDZ domains of PSD-95 and SAP102. A search of the human genome sequences for proteins with a

COOH-terminal (E/Q)(S/T)XV motif yielded 50 proteins, many of which have not been previously identified as PSD-95 or SAP102 binding partners. Two of these proteins, brain-specific angiogenesis inhibitor 1 and protein kinase C[alpha], co-immunoprecipitated with PSD-95 and SAP102 from rat brain extracts.

Lin, S., Q. Shi, et al. (2002). "A Novel S-Adenosyl-L-methionine:Arsenic(III) Methyltransferase from Rat Liver Cytosol." J. Biol. Chem. **277**(13): 10795-10803.

http://www.jbc.org/cgi/content/abstract/277/13/10795

S-Adenosyl-L-methionine (AdoMet):arsenic(III) methyltransferase, purified from liver cytosol of adult male Fischer 344 rats, catalyzes transfer of a methyl group from AdoMet to trivalent arsenicals producing methylated and dimethylated arsenicals. The kinetics of production of methylated arsenicals in reaction mixtures containing enzyme, AdoMet, dithiothreitol, glutathione (GSH), and arsenite are consistent with a scheme in which monomethylated arsenical produced from arsenite is the substrate for a second methylation reaction that yields dimethylated arsenical. The mRNA for this protein predicts a 369-amino acid residue protein (molecular mass 41056) that contains common methyltransferase sequence motifs. Its sequence is similar to Cyt19, a putative methyltransferase, expressed in human and mouse tissues. Reverse transcription-polymerase chain reaction detects S-adenosyl-L-methionine:arsenic(III) methyltransferase mRNA in rat tissues and in HepG2 cells, a human cell line that methylates arsenite and methylarsonous acid. S-Adenosyl-L-methionine:arsenic(III) methyltransferase mRNA is not detected in UROtsa cells, an immortalized human urothelial cell line that does not methylate arsenite. Because methylation of arsenic is a critical feature of its metabolism, characterization of this enzyme will improve our understanding of this metalloid's metabolism and its actions as a toxin and a carcinogen.

Lindvall, H., P. Nevsten, et al. (2004). "A Novel Hormone-sensitive Lipase Isoform Expressed in Pancreatic {beta}-Cells." J. Biol. Chem. **279**(5): 3828-3836.

http://www.jbc.org/cgi/content/abstract/279/5/3828

Hormone-sensitive lipase (HSL) is a key enzyme in fatty acid mobilization in many cell types. Two isoforms of HSL are known to date, namely HSLadi (84 kDa in rat) and HSLtes (130 kDa in rat). These are encoded by the same gene, with exons 1-9 encoding the parts that are common to both and an additional 5'-exon encoding the additional amino acids in HSLtes. HSL of various tissues, among these the islet of Langerhans, is larger than HSLadi, but not as large as HSLtes, indicating that there may be other 5'-coding exons. Here we describe the molecular basis for a novel 89-kDa HSL isoform that is expressed in {beta}-cells, adipocytes, adrenal glands, and ovaries in the rat and that is encoded by exons 1-9 and exon A, which is spliced to exon 1 and thereby introducing an upstream start codon. The additional 5'-base pairs encode a 43-amino acid peptide, which is highly positively charged. Conglomerates of HSL molecules are in close association with the secretory granules of the {beta}-cell, as determined by immunoelectron microscopy with antibodies targeting two separate regions of HSL. We have also determined that the human genomic sequence upstream of exon A has promoter activity in INS-1 cells as well as glucose sensing capability, mediating an increase in expression at high glucose concentration. The minimal promoter is present within 170 bp from the transcriptional start site and maximal glucose responsiveness is conferred by sequence within 850 bp from the transcriptional start site.

Lodhi, K. M., M. H. Ozdener, et al. (2003). "The Upstream Open Reading Frame Mediates Constitutive

Effects on Translation of Cytochrome P-450c27 from the Seventh In-frame AUG Codon in Rat Liver." J. Biol. Chem. **278**(42): 40647-40657.

http://www.jbc.org/cgi/content/abstract/278/42/40647

The 2.3-kb mRNA that codes for cytochrome P-450c27 (CYP27) has an unexpectedly long 5'-untranslated region (UTR) that holds six AUGs, leading to several upstream open reading frames (uORFs). The initiation of translation from the seventh AUG forms a putative 55-kDa precursor, which is processed in mitochondria to form a 52-kDa mature protein. The first three AUGs form fully overlapping uORF1, uORF2, and uORF3 that are in-frame with the seventh AUG and next two form fully overlapping uORF4 and uORF5 that are out-of-frame with the seventh AUG. Although not recognized by the scanning ribosomes under normal conditions, the sixth in-frame AUG forms a putative 57-kDa extension of the main open reading frame. The purpose of this study was to identify the elements in the 5'-UTR that direct CYP27 mRNA translation exclusively from the seventh AUG. Expression of 5' deletion mutants in COS cells reveal that the intact 5'-UTR not only directs the initiation of translation from the seventh AUG but also acts as a negative regulator. A 2-kb deletion mutant that lacks uORF1 initiates translation equally from the sixth and the seventh AUGs, forming both 57- and 55-kDa precursor proteins with a 2-fold increase in rate of translation. However, induction in translation does not affect the levels of the mature 52-kDa form in mitochondria but causes accumulation of the precursor form in cytosol not seen in COS cells transfected with wild-type cDNA. Mutation of the stop codon that terminates uORF1 completely shifts the initiation of translation from the seventh to the first AUG, forming a 67-kDa precursor that is processed into a 52-kDa mature protein in mitochondria. Confirmation of the bicistronic nature of CYP27 mRNA by epitope mapping of uORF1 suggests that translation of CYP27 mRNA from the seventh AUG is directed and regulated by uORF1 expression.

Longo, K. A., J. A. Kennell, et al. (2002). "Wnt Signaling Protects 3T3-L1 Preadipocytes from Apoptosis through Induction of Insulin-like Growth Factors." J. Biol. Chem. **277**(41): 38239-38244.

http://www.jbc.org/cgi/content/abstract/277/41/38239

Ectopic expression of Wnt-1 in 3T3-L1 preadipocytes stabilizes [beta]-catenin, activates TCF-dependent gene transcription, and blocks adipogenesis. Here we report that upon serum withdrawal, Wnt-1 causes 3T3-L1 cells to resist apoptosis through a mechanism that is partially dependent on phosphatidylinositol 3-kinase. Although activation of Wnt signaling by inhibition of GSK-3 activity or ectopic expression of dominant stable [beta]-catenin blocks apoptosis, inhibition of Wnt signaling through expression of dominant negative TCF-4 increases apoptosis. Wnt-1 stimulates 3T3-L1 preadipocytes to secrete factors that increase PKB/Akt phosphorylation at levels comparable with treatment with 10% serum. With DNA microarrays, we identified several secreted antiapoptotic genes that are induced by Wnt-1, notably insulin-like growth factor I (IGF-I) and IGF-II. Consistent with IGFs mediating the antiapoptotic effects of Wnt-1 in preadipocytes, conditioned medium from Wnt-1 expressing 3T3-L1 cells was unable to promote protein kinase B phosphorylation after the addition of recombinant IGFBP-4. Thus, we demonstrated that Wnt-1 induces expression of antiapoptotic genes in 3T3-L1 preadipocytes such as IGF-I and IGF-II, which allows these cells to resist apoptosis in response to serum deprivation.

Lu, K. P. and K. S. Ramos (2003). "Redox Regulation of a Novel L1Md-A2 Retrotransposon in Vascular Smooth Muscle Cells." J. Biol. Chem. **278**(30): 28201-28209.

http://www.jbc.org/cgi/content/abstract/278/30/28201

Activation and reintegration of retrotransposons into the genome is linked to several diseases in human and rodents, but mechanisms of gene activation remain largely unknown. Here we identify a novel gene of L1Md-A2 lineage in vascular smooth muscle cells and show that environmental hydrocarbons enhance gene expression and activate monomer-driven transcription via a redox-sensitive mechanism. Site-directed mutagenesis and progressive deletion analyses identified two antioxidant/electrophile response-like elements (5'-GTGACTCGAGC-3') within the A2/3 and A3 region. These elements mediated activation, with the A3 monomer playing an essential role in transactivation. This signaling pathway may contribute to gene instability during the course of atherogenesis.

Lu, S., Y. Yao, et al. (2002). "Overexpression of Apolipoprotein A-IV Enhances Lipid Transport in Newborn Swine Intestinal Epithelial Cells." J. Biol. Chem. **277**(35): 31929-31937.

http://www.jbc.org/cgi/content/abstract/277/35/31929

Apolipoprotein A-IV (apoA-IV) has myriad functions, including roles as a post-prandial satiety factor and lipid antioxidant. ApoA-IV is expressed in mammalian small intestine and is up-regulated in response to lipid absorption. In newborn swine jejunum, a high fat diet acutely induces a 7-fold increase in apoA-IV expression. To determine whether apoA-IV plays a role in the transport of absorbed lipid, swine apoA-IV was overexpressed in a newborn swine enterocyte cell line, IPEC-1, followed by analysis of the expression of genes related to lipoprotein assembly and lipid transport, as well as quantitation of lipid synthesis and secretion. A full-length swine apoA-IV cDNA was cloned, sequenced, and inserted into a Vp and Rep gene-deficient adeno-associated viral vector, containing the cytomegalovirus immediate early promoter/enhancer and neomycin resistance gene, and was used to transfect IPEC-1 cells. Control cells were transfected with the same vector minus the apoA-IV insert. Using neomycin selection, apoA-IV-overexpressing (+AIV) and control ([-]AIV) clones were isolated for further study. Both undifferentiated ([-]D) and differentiated (+D) +AIV cells expressed 40- to 50-fold higher levels of apoA-IV mRNA and both intracellular and secreted apoA-IV protein compared with [-]AIV cells. Expression of other genes was not affected by apoA-IV overexpression in a manner that would contribute to enhanced lipid secretion. +D +AIV cells secreted 4.9-fold more labeled triacylglycerol (TG), 4.6-fold more labeled cholesteryl ester (CE), and 2-fold more labeled phospholipid (PL) as lipoproteins, mostly in the chylomicron/very low density lipoprotein (VLDL) density range. ApoA-IV overexpression in IPEC-1 cells enhances basolateral TG, CE, and PL secretion in chylomicron/VLDL particles. This enhancement is not associated with up-regulation of other genes involved in lipid transport. ApoA-IV may play a role in facilitating enterocyte lipid transport, particularly in the neonate receiving a diet of high fat breast milk.

Ma, X., J. Hu, et al. (2002). "Mutational Analysis of Human Thioredoxin Reductase 1. EFFECTS ON p53-MEDIATED GENE EXPRESSION AND INTERFERON AND RETINOIC ACID-INDUCED CELL DEATH." J. Biol. Chem. **277**(25): 22460-22468.

http://www.jbc.org/cgi/content/abstract/277/25/22460

The interferon (IFN)-[beta] and all-trans-retinoic acid combination suppresses tumor growth by inducing apoptosis in several tumor cell lines. A genetic technique permitted the isolation of human thioredoxin reductase (TR) as a critical regulator of IFN/all-trans-retinoic acid-induced cell death. Our recent studies have shown that TR1:thioredoxin 1-regulated cell death is effected in part through the activation of p53-dependent responses. To understand its death regulatory function, we have performed a mutational analysis of TR. Human TR1 has three major structural domains, the FAD binding domain, the NADPH binding domain, and an interface domain (ID). Here, we show that the deletion of the C-terminal interface domain results in a constitutive

activation of TR-dependent death responses and promotes p53-dependent gene expression. TR mutant without the ID still retains its dependence on thioredoxin for promoting these responses. Thus, our data suggest that TR-ID acts as a regulatory domain.

Malhotra, J. D., M. C. Koopmann, et al. (2002). "Structural Requirements for Interaction of Sodium Channel beta 1 Subunits with Ankyrin." J. Biol. Chem. **277**(29): 26681-26688.

http://www.jbc.org/cgi/content/abstract/277/29/26681

Sodium channel [beta] subunits modulate channel kinetic properties and cell surface expression levels and function as cell adhesion molecules. [beta]1 and [beta]2 participate in homophilic cell adhesion resulting in ankyrin recruitment to cell contact sites. We hypothesized that a tyrosine residue in the cytoplasmic domain of [beta]1 may be important for ankyrin recruitment and tested our hypothesis using [beta]1 mutants replacing Tyr181 with alanine ([beta]1Y181A), phenylalanine ([beta]1Y181F), or glutamate ([beta]1Y181E), or a truncated construct deleting all residues beyond Tyr181 ([beta]1L182STOP). Ankyrin recruitment was observed in [beta]1L182STOP, showing that residues Ile166-Tyr181 contain the major ankyrin recruiting activity of [beta]1. Ankyrin recruitment was abolished in [beta]1Y181E, suggesting that tyrosine phosphorylation of [beta]1 may inhibit [beta]1-ankyrin interactions. AnkyrinG and [beta]1 associate in rat brain membranes and in transfected cells expressing [beta]1 and ankyrinG in the absence of sodium channel [alpha] subunits. [beta]1 subunits are recognized by anti-phosphotyrosine antibodies following treatment of these cell lines with fibroblast growth factor. [beta]1 and ankryinG association is not detectable in cells following treatment with fibroblast growth factor. AnkyrinG and [beta]1Y181E do not associate even in the absence of fibroblast growth factor treatment. [beta]1 subunit-mediated cell adhesion and ankyrin recruitment may contribute to sodium channel placement at nodes of Ranvier. The phosphorylation state of [beta]1Y181 may be a critical regulatory step in these developmental processes.

Mansouri, A., L. D. Ridgway, et al. (2003). "Sustained Activation of JNK/p38 MAPK Pathways in Response to Cisplatin Leads to Fas Ligand Induction and Cell Death in Ovarian Carcinoma Cells." J. Biol. Chem. **278**(21): 19245-19256.

http://www.jbc.org/cgi/content/abstract/278/21/19245

The efficacy of cisplatin in cancer chemotherapy is limited by the development of resistance. Although the molecular mechanisms involved in chemoresistance are poorly understood, cellular response to cisplatin is known to involve activation of MAPK and other signal transduction pathways. An understanding of early signal transduction events in the response to cisplatin could be valuable for improving the efficacy of cancer therapy. We compared cisplatin-induced activation of three MAPKs, JNK, p38, and ERK, in a cisplatin-sensitive human ovarian carcinoma cell line (2008) and its resistant subclone (2008C13). The JNK and p38 pathways were activated differentially in response to cisplatin, with the cisplatin-sensitive cells showing prolonged activation (8-12 h) and the cisplatin-resistant cells showing only transient activation (1-3 h) of JNK and p38. In the sensitive cells, inhibition of cisplatin-induced JNK and p38 activation blocked cisplatin-induced apoptosis; persistent activation of JNK resulted in hyperphosphorylation of the c-Jun transcription factor, which in turn stimulated the transcription of an immediate downstream target, the death inducer Fas ligand (FasL). Sequestration of FasL by incubation with a neutralizing anti-FasL antibody inhibited cisplatin-induced apoptosis. In contrast, chemoresistance in 2008C13 cells was associated with failure to up-regulate FasL. Moreover, in these cells, selective stimulation of the JNK/p38 MAPK pathways by adenovirus-mediated delivery of recombinant MKK7 or MKK3 led to sensitization to apoptosis through reactivating FasL expression. Thus, the JNK > c-Jun > FasL > Fas pathway plays an important role in

mediating cisplatin-induced apoptosis in ovarian cancer cells, and the duration of JNK activation is critical in determining whether cells survive or undergo apoptosis.

Marchesini, N., W. Osta, et al. (2004). "Role for Mammalian Neutral Sphingomyelinase 2 in Confluence-induced Growth Arrest of MCF7 Cells." J. Biol. Chem. **279**(24): 25101-25111.

http://www.jbc.org/cgi/content/abstract/279/24/25101

Recently, we reported that neutral sphingomyelinase 2 (nSMase2) functions as a bona fide neutral sphingomyelinase and that overexpression of nSMase2 in MCF7 breast cancer cells caused a decrease in cell growth (Marchesini, N., Luberto, C., and Hannun, Y. A. (2003) J. Biol. Chem. 278, 13775-13783). In this study, the role of endogenous nSMase2 in regulating growth arrest was investigated. The results show that endogenous nSMase2 mRNA was up-regulated [~]5-fold when MCF7 cells became growth-arrested at confluence, and total neutral SMase activity was increased by 119 {+/-} 41% with respect to control. Cell cycle analysis showed that up-regulation of endogenous nSMase2 correlated with G0/G1 cell cycle arrest and an increase in total ceramide levels (2.4-fold). Analysis of ceramide species showed that confluence caused selective increases in very long chain ceramide C24:1 (370 {+/-} 54%) and C24:0 (266 {+/-} 81%) during arrest. The role of endogenous nSMase2 in growth regulation and ceramide metabolism was investigated using short interfering RNA (siRNA)-mediated loss-of-function analysis. Down-regulation of nSMase2 with specific siRNA increased the cell population of cells in S phase of the cell cycle by 59 {+/-} 14% and selectively reverted the effects of growth arrest on the increase in levels of very long chain ceramides. Mechanistically, confluence arrest also induced hypophosphorylation of the retinoblastoma protein (6-fold) and induction of p21WAF1 (3-fold). Down-regulation of nSMase2 with siRNA largely prevented the dephosphorylation of the retinoblastoma protein and the induction of p21WAF1, providing a link between the action of nSMase2 and key regulators of cell cycle progression. Moreover, studies on nSMase2 localization in MCF7 cells showed that nSMase2 distributed throughout the cells in subconfluent, proliferating cultures. In contrast, nSMase2 became nearly exclusively located at the plasma membrane in confluent, contact-inhibited cells. Hence, we demonstrate for the first time that nSMase2 functions as a growth suppressor in MCF7 cells, linking confluence to the G0/G1 cell cycle check point.

Martin, V., R. Bredoux, et al. (2002). "Three Novel Sarco/endoplasmic Reticulum Ca2+-ATPase (SERCA) 3 Isoforms. EXPRESSION, REGULATION, AND FUNCTION OF THE MEMBERS OF THE SERCA3 FAMILY." J. Biol. Chem. **277**(27): 24442-24452.

http://www.jbc.org/cgi/content/abstract/277/27/24442

Sarco/endoplasmic reticulum Ca2+-ATPases (SERCAs) pump Ca2+ into the endoplasmic reticulum. Recently, three human SERCA3 (h3a-c) proteins and a previously unknown rat SERCA3 (r3b/c) mRNA have been described. Here, we (i) document two novel human SERCA3 splice variants h3d and h3e, (ii) provide data for the expression and mechanisms regulating the expression of all known SERCA3 variants (r3a, r3b/c, and h3a-e), and (iii) show functional characteristics of the SERCA3 isoforms. h3d and h3e are issued from the insertion of an additional penultimate exon 22 resulting in different carboxyl termini for these variants. Distinct distribution patterns of the SERCA3 gene products were observed in a series of cell lines of hematopoietic, epithelial, embryonic origin, and several cancerous types, as well as in panels of rat and human tissues. Hypertension and protein kinase C, calcineurin, or retinoic acid receptor signaling pathways were found to differently control rat and human splice variant expression, respectively. Stable overexpression of each variant was performed in human embryonic kidney 293 cells, and the SERCA3 isoforms were fully characterized. All SERCA3 isoforms were found

to pump Ca2+ with similar affinities. However, they modulated the cytosolic Ca2+ concentration ([Ca2+]c) and the endoplasmic reticulum Ca2+ content ([Ca2+]er) in different manners. A newly generated polyclonal antibody and a pan-SERCA3 antibody proved the endogenous expression of the three novel SERCA3 proteins, h3d, h3e, and r3b/c. All these data suggest that the SERCA3 gene products have a more widespread role in cellular Ca2+ signaling than previously appreciated.

Massimi, I., E. Park, et al. (2002). "Identification of a Novel Maturation Mechanism and Restricted Substrate Specificity for the SspB Cysteine Protease of Staphylococcus aureus." J. Biol. Chem. **277**(44): 41770-41777.

http://www.jbc.org/cgi/content/abstract/277/44/41770

The SspB cysteine protease of Staphylococcus aureus is expressed in an operon, flanked by the sspA serine protease, and sspC, encoding a 12.9-kDa protein of unknown function. SspB was expressed as a 40-kDa prepropeptide pSspB, which did not undergo autocatalytic maturation. Activity of pSspB was reduced compared with 22-kDa mature SspB, but it was equivalent to mature SspB after incubation with SspA, which specifically removed the pSspB N-terminal propeptide. SspC abrogated the activity of pSspB when incubated in a 1:1 complex but had no effect on SspA or papain. Activity of the pSspB{middle dot}SspC complex was restored when incubated with SspA, and SspC was cleaved by SspA but not pSspB. Thus, SspC maintains pSspB as an inert zymogen, and SspA is required for removal of the propeptide and inactivation of SspC. Like the papain protease family, SspB cleaved substrates with a hydrophobic amino acid at P2 but had a strong preference for arginine at P1. It did not cleave casein, serum albumin, IgG, or IgA, but it promoted detachment of cultured keratinocytes and cleaved fibronectin and fibrinogen at sites recognized by urokinase plasminogen activator and plasmin, respectively. It also processed high molecular weight kininogen in a manner resembling plasma kallikrein. Thus, SspB exhibits a novel maturation mechanism and mimics the specificity of plasma serine proteases.

Mateo, J., S. Kreda, et al. (2003). "Requirement of Cys399 for Processing of the Human Ecto-ATPase (NTPDase2) and Its Implications for Determination of the Activities of Splice Variants of the Enzyme." J. Biol. Chem. **278**(41): 39960-39968.

http://www.jbc.org/cgi/content/abstract/278/41/39960

Ecto-ATPase (CD39L1) corresponds to the type 2 enzyme of the ecto-nucleoside triphosphate diphosphohydrolase family (E-NTPDase). We have isolated from human ECV304 cells three cDNAs with high homology with members of the E-NTPDase family that encode predicted proteins of 495, 472, and 450 amino acids. Sequencing of a genomic DNA clone confirmed that these three sequences correspond to splice variants of the human ecto-ATPase (NTPDase2{alpha},-2{beta}, and -2{gamma}). Although all three enzyme forms were expressed heterologously to similar levels in Chinese hamster ovary cells clone K-1 (CHO-K1) cells, only the 495-amino acid protein (NTPDase2{alpha} exhibited ecto-ATPase activity. Immunolocalization studies demonstrated that NTPDase2{alpha} is fully processed and trafficked to the plasma membrane, whereas the NTPDase2{beta} and -2{gamma} splice variants were retained in not fully glycosylated forms in the endoplasmic reticulum. The potential roles of two highly conserved residues, Cys399 and Asn443, in the activity and cellular trafficking of the ecto-ATPase were examined. Mutation of Cys399, which is absent in NTPDase2{beta} and -2{gamma}, produced a protein completely devoid of nucleotidase activity, while mutation of Asn443 to Asp resulted in substantial loss of activity. Neither the Cys399 nor Asn443 mutants were fully glycosylated, and both were retained in the endoplasmic reticulum. These results indicate that the lack of ecto-

nucleotidase activity exhibited by NTPDase2{beta} and -2{gamma} and the C399S mutant, as well as the large reduction of activity in the N443D mutant are due to alterations in the folding/maturation of these proteins.

Maxwell, M. A., M. E. Cleasby, et al. (2005). "Nur77 Regulates Lipolysis in Skeletal Muscle Cells: EVIDENCE FOR CROSS-TALK BETWEEN THE {beta}-ADRENERGIC AND AN ORPHAN NUCLEAR HORMONE RECEPTOR PATHWAY." J. Biol. Chem. **280**(13): 12573-12584.

http://www.jbc.org/cgi/content/abstract/280/13/12573

Skeletal muscle is a major mass peripheral tissue that accounts for [~]40% of total body weight and 50% of energy expenditure and is a primary site of glucose disposal and fatty acid oxidation. Consequently, muscle has a significant role in insulin sensitivity, obesity, and the blood-lipid profile. Excessive caloric intake is sensed by the brain and induces {beta}-adrenergic receptor ({beta}-AR)-mediated adaptive thermogenesis. {beta}-AR null mice develop severe obesity on a high fat diet. However, the target gene(s), target tissues(s), and molecular mechanism involved remain obscure. We observed that 30-60 min of {beta}-AR agonist (isoprenaline) treatment of C2C12 skeletal muscle cells strikingly activated (>100-fold) the expression of the mRNA encoding the nuclear hormone receptor, Nur77. In contrast, the expression of other nuclear receptors that regulate lipid and carbohydrate metabolism was not induced. Stable transfection of Nur77-specific small interfering RNAs (siNur77) into skeletal muscle cells repressed endogenous Nur77 mRNA expression. Moreover, we observed attenuation of gene and protein expression associated with the regulation of energy expenditure and lipid homeostasis, for example AMP-activated protein kinase {gamma}3, UCP3, CD36, adiponectin receptor 2, GLUT4, and caveolin-3. Attenuation of Nur77 expression resulted in decreased lipolysis. Finally, in concordance with the cell culture model, injection and electrotransfer of siNur77 into mouse tibialis cranialis muscle resulted in the repression of UCP3 mRNA expression. This study demonstrates regulatory cross-talk between the nuclear hormone receptor and {beta}-AR signaling pathways. Moreover, it suggests Nur77 modulates the expression of genes that are key regulators of skeletal muscle lipid and energy homeostasis. In conclusion, we speculate that Nur77 agonists would stimulate lipolysis and increase energy expenditure in skeletal muscle and suggest selective activators of Nur77 may have therapeutic utility in the treatment of obesity.

McDermott, N. B., D. F. Gordon, et al. (2002). "Isolation and Functional Analysis of the Mouse RXRgamma 1 Gene Promoter in Anterior Pituitary Cells." J. Biol. Chem. **277**(39): 36839-36844.

http://www.jbc.org/cgi/content/abstract/277/39/36839

The retinoid X receptor (RXR) isoform RXR[gamma] has limited tissue expression, including brain, skeletal muscle, and anterior pituitary gland. Within the anterior pituitary gland, RXR[gamma] expression is limited primarily to the thyrotropes. In this report, we have isolated ~3 kb of 5'-flanking DNA of the mouse RXR[gamma]1 gene. We have identified the major transcription start site in the thyrotrope-derived TtT-97 cells. Transient transfection studies show that a 1.4-kb promoter fragment has full promoter activity in TtT-97 cells. This promoter has much less activity in thyrotrope-derived [alpha]TSH cells, pituitary-derived GH3 somatomammotropes, and non-pituitary CV-1 cells. None of these cell lines has detectable RXR[gamma]1 mRNA. A previous report has identified a non-consensus direct repeat (DR-1) element in the RXR[gamma]2 gene promoter region that mediates stimulation of promoter activity by 9-cis-retinoic acid (9-cis-RA). Inspection of the RXR[gamma]1 promoter region revealed a non-consensus DR-1 element at [-]232 bp from the transcription start site. Interestingly, RXR[gamma]1 promoter activity was suppressed 50% by 9-cis-RA in the TtT-97 thyrotropes. Further experiments in non-pituitary cells showed that suppression of RXR[gamma]1 promoter

activity was RXR-dependent. Mutagenesis of the DR-1 element abrogated suppression of promoter activity by 9-cis-RA, suggesting that this negative regulation requires both RXR and this specific DR-1 element. In summary, we have isolated the mouse RXR[gamma]1 gene promoter region and identified the major start site in thyrotropes. Promoter activity is uniquely suppressed by 9-cis-RA through a DR-1 element. Isolation and characterization of the mouse RXR[gamma]1 promoter region provides a tool for further investigation focusing on thyrotrope-specific gene expression as well as negative regulation of genes by retinoic acid.

Merckx, A., K. Le Roch, et al. (2003). "Identification and Initial Characterization of Three Novel Cyclin-related Proteins of the Human Malaria Parasite Plasmodium falciparum." J. Biol. Chem. **278**(41): 39839-39850.

http://www.jbc.org/cgi/content/abstract/278/41/39839

The molecular mechanisms regulating cell proliferation and development during the life cycle of malaria parasites remain to be elucidated. The peculiarities of the cell cycle organization during Plasmodium falciparum schizogony suggest that the modalities of cell cycle control in this organism may differ from those in other eukaryotes. Indeed, existing data concerning Plasmodium cell cycle regulators such as cyclin-dependent kinases reveal structural and functional properties that are divergent from those of their homologues in other systems. The work presented here lies in the context of the exploitation of the recently available P. falciparum genome sequence toward the characterization of putative cell cycle regulators. We describe the in silico identification of three open reading frames encoding proteins with maximal homology to various members of the cyclin family and demonstrate that the corresponding polypeptides are expressed in the erythrocytic stages of the infection. We present evidence that these proteins possess cyclin activity by demonstrating either their association with histone H1 kinase activity in parasite extracts or their ability to activate PfPK5, a P. falciparum cyclin-dependent kinase homologue, in vitro. Furthermore, we show that RINGO, a protein with no sequence homology to cyclins but that is nevertheless a strong activator of mammalian CDK1/2, is also a strong activator of PfPK5 in vitro. This raises the possibility that "cryptic" cell cycle regulators may be found among the 50% of the open reading frames in the P. falciparum genome that display no homology to any known proteins.

Mesquita, P., N. Jonckheere, et al. (2003). "Human MUC2 Mucin Gene Is Transcriptionally Regulated by Cdx Homeodomain Proteins in Gastrointestinal Carcinoma Cell Lines." J. Biol. Chem. **278**(51): 51549-51556.

http://www.jbc.org/cgi/content/abstract/278/51/51549

In intestinal metaplasia and 30% of gastric carcinomas, MUC2 intestinal mucin and the intestine-specific transcription factors Cdx-1 and Cdx-2 are aberrantly expressed. The involvement of Cdx-1 and Cdx-2 in the intestinal development and their role in transcription of several intestinal genes support the hypothesis that Cdx-1 and/or Cdx-2 play important roles in the aberrant intestinal differentiation program of intestinal metaplasia and gastric carcinoma. To clarify the mechanisms of transcriptional regulation of the MUC2 mucin gene in gastric cells, pGL3 deletion constructs covering 2.6 kb of the human MUC2 promoter were used in transient transfection assays, enabling us to identify a relevant region for MUC2 transcription in all gastric cell lines. To evaluate the role of Cdx-1 and Cdx-2 in MUC2 transcription we performed co-transfection experiments with expression vectors encoding Cdx-1 and Cdx-2. In two of the four gastric carcinoma cell lines and in all colon carcinoma cell lines we observed transactivation of the MUC2 promoter by Cdx-2. Using gel shift assays we identified two Cdx-2 binding sites at -177/-171 and -191/-187. Only simultaneous mutation of the two sites resulted in inhibition of Cdx-2-mediated transactivation of

MUC2 promoter, implying that both Cdx-2 sites are active. Finally, stable expression of Cdx-2 in a gastric cell line initially not expressing Cdx-2, led to induction of MUC2 expression. In conclusion, this work demonstrates that Cdx-2 activates the expression of MUC2 mucin gene in gastric cells, inducing an intestinal transdifferentiation phenotype that parallels what is observed both in intestinal metaplasia and some gastric carcinomas.

Mills, J. A., K. Motichka, et al. (2004). "Inactivation of the Mycobacterial Rhamnosyltransferase, Which Is Needed for the Formation of the Arabinogalactan-Peptidoglycan Linker, Leads to Irreversible Loss of Viability." J. Biol. Chem. **279**(42): 43540-43546.

http://www.jbc.org/cgi/content/abstract/279/42/43540

Temperature-sensitive mutant 2-20/32 of Mycobacterium smegmatis mc2155 was isolated and genetically complemented with a Mycobacterium tuberculosis H37Rv DNA fragment that contained a single open reading frame. This open reading frame is designated Rv3265c in the M. tuberculosis H37Rv genome. Rv3265c shows homology to the Escherichia coli gene wbbL, which encodes a dTDP-Rha:{alpha}-D-GlcNAc-pyrophosphate polyprenol, {alpha}-3-L-rhamnosyltransferase. In E. coli this enzyme is involved in O-antigen synthesis, but in mycobacteria it is required for the rhamnosyl-containing linker unit responsible for the attachment of the cell wall polymer mycolyl-arabinogalactan to the peptidoglycan. The M. tuberculosis wbbL homologue, encoded by Rv3265c, was shown to be capable of restoring an E. coli K12 strain containing an insertionally inactivated wbbL to O-antigen positive. Likewise, the E. coli wbbL gene allowed 2-20/32 to grow at higher non-permissive temperatures. The rhamnosyltransferase activity of M. tuberculosis WbbL was demonstrated in 2-20/32 as was the loss of this transferase activity in 2-20/32 at elevated temperatures. The wbbL of the temperature-sensitive mutant contained a single-base change that converted what was a proline in mc2155 to a serine residue. Exposure of 2-20/32 to higher non-permissive temperatures resulted in bacteria that could not be recovered at the lower permissive temperatures.

Min, S. H., R. C. M. Simmen, et al. (2002). "Altered Levels of Growth-related and Novel Gene Transcripts in Reproductive and Other Tissues of Female Mice Overexpressing Spermidine/Spermine N1-Acetyltransferase (SSAT)." J. Biol. Chem. **277**(5): 3647-3657.

http://www.jbc.org/cgi/content/abstract/277/5/3647

Overexpression of SSAT (polyamine catabolic enzyme) in female mice results in impaired ovarian folliculogenesis and uterine hypoplasia. To identify the molecular basis for this, the gene expression profiles in uterus and ovary and for comparison, liver and kidney, from non-transgenic (NT) and SSAT transgenic (ST) mice were compared. The mRNA abundance for lipoprotein lipase and glyceraldehyde-3-phosphate dehydrogenase was elevated in all four ST (>NT) tissues. The translation initiation factor-3 subunit 5 mRNA, and transcripts related to endogenous murine leukemia provirus (MLV-related) and murine retrovirus-related sequences (MuRRS) were decreased in ST tissues. A novel calmodulin-related mRNA was strongly induced in ST liver and kidney. SSAT overexpression was associated with increased levels of IGF-binding protein-2 (IGFBP-2) in the uterus and ovary, and a reduction in IGFBP-3 mRNA levels in the uterus. Exogenous spermidine and spermine elevated endogenous IGFBP-2 and SSAT mRNA abundance, whereas, putrescine stimulated IGFBP-2 mRNA abundance and transfected IGFBP-2 gene promoter activity in human (Hec-1-A) uterine cells. Sp1 and BTEB1 mRNAs that encode transcription factors for the IGFBP-2 gene also were induced in some ST tissues. The data suggest that SSAT and polyamines are important for the control of molecular pathways underlying reproductive tract tissue growth, phenotype, and function.

Mishra, D. P. and C. Shaha (2005). "Estrogen-induced Spermatogenic Cell Apoptosis Occurs via the Mitochondrial Pathway: ROLE OF SUPEROXIDE AND NITRIC OXIDE." J. Biol. Chem. **280**(7): 6181-6196.

http://www.jbc.org/cgi/content/abstract/280/7/6181

The detrimental effects of estrogen on testicular function provide a conceptual basis to examine the speculative link between increased exposure to estrogens and spermatogenic cell death. Using an in vitro model, we provide an understanding of the events leading to estrogen-induced apoptosis in cells of spermatogenic lineage. Early events associated with estrogen exposure were up-regulation of FasL and increased generation of H2O2, superoxide, and nitric oxide. The ability of anti-FasL antibodies to prevent several downstream biochemical changes and cell death induced by 17{beta}-estradiol substantiates the involvement of the cell death receptor pathway. Evidence for the amplification of the death-inducing signals through mitochondria was obtained from the transient mitochondrial hyperpolarization observed after estradiol exposure resulting in cytochrome c release. A combination of nitric oxide and superoxide but not H2O2 was responsible for the mitochondrial hyperpolarization. Mn(III) tetrakis(4-benzoic acid)porphyrin chloride, an intracellular peroxynitrite scavenger, was able to reduce mitochondrial hyperpolarization and cell death. Although nitric oxide augmentation occurred through an increase in the expression of inducible nitric-oxide synthase, superoxide up-regulation was a product of estradiol metabolism. All of the above changes were mediated through an estrogen receptor-based mechanism because tamoxifen, the estrogen receptor modulator, was able to rescue the cells from estrogen-induced alterations. This study establishes the importance of the independent capability of cells of the spermatogenic lineage to respond to estrogens and most importantly suggests that low dose estrogens can potentially cause severe spermatogenic cellular dysfunction leading to impaired fertility even without interference of the hypothalamo-hypophyseal axis.

Mitoma, J., B. Petryniak, et al. (2003). "Extended Core 1 and Core 2 Branched O-Glycans Differentially Modulate Sialyl Lewis x-type L-selectin Ligand Activity." J. Biol. Chem. **278**(11): 9953-9961.

http://www.jbc.org/cgi/content/abstract/278/11/9953

It has been established that sialyl Lewis x in core 2 branched O-glycans serves as an E- and P-selectin ligand. Recently, it was discovered that 6-sulfosialyl Lewis x in extended core 1 O-glycans, NeuNAc[alpha]2[right-arrow]3Gal[beta]1[right-arrow]4(Fuc[alpha]1[right-arrow]3(sulfo[right-arrow]6))GlcNAc[beta]1[right-arrow] 3Gal[beta]1[right-arrow]3GalNAc[alpha]1[right-arrow]Ser/Thr, functions as an L-selectin ligand in high endothelial venules. Extended core 1 O-glycans can be synthesized when a core 1 extension enzyme is present. In this study, we first show that [beta]1,3-N-acetylglucosaminyltransferase-3 ([beta]3GlcNAcT-3) is almost exclusively responsible for core 1 extension among seven different [beta]3GlcNAcTs and thus acts on core 1 O-glycans attached to PSGL-1. We found that transcripts encoding [beta]3GlcNAcT-3 were expressed in human neutrophils and lymphocytes but that their levels were lower than those of transcripts encoding core 2 [beta]1,6-N-acetylglucosaminyltransferase I (Core2GlcNAcT-I). Neutrophils also expressed transcripts encoding fucosyltransferase VII (FucT-VII) and Core2GlcNAcT-I, whereas lymphocytes expressed only small amounts of transcripts encoding FucT-VII. To determine the roles of sialyl Lewis x in extended core 1 O-glycans, Chinese hamster ovary (CHO) cells were stably transfected to express PSGL-1, FucT-VII, and either [beta]3GlcNAcT-3 or Core2GlcNAcT-I. Glycan structural analyses disclosed that PSGL-1 expressed in these transfected cells carried comparable amounts of sialyl Lewis x in extended core 1 and core 2 branched O-glycans. In a rolling assay, CHO cells expressing sialyl Lewis x in extended core 1 O-glycans supported a

significant degree of shear-dependent tethering and rolling of neutrophils and lymphocytes, although less than CHO cells expressing sialyl Lewis x in core 2 branched O-glycans. These results indicate that sialyl Lewis x in extended core 1 O-glycans can function as an L-selectin ligand and is potentially involved in neutrophil adhesion on neutrophils bound to activated endothelial cells.

Mitsutake, S., T.-J. Kim, et al. (2004). "Ceramide Kinase Is a Mediator of Calcium-dependent Degranulation in Mast Cells." J. Biol. Chem. **279**(17): 17570-17577.

http://www.jbc.org/cgi/content/abstract/279/17/17570

Ceramide kinase (CERK) catalyzes the conversion of ceramide to ceramide 1-phosphate (C1P) and is known to be activated by calcium. Although several groups have examined the functions of CERK and its product C1P, the functions of C1P and CERK are not understood. We studied the RBL-2H3 cell line, a widely used model for mast cells, and found that CERK and C1P are required for activation of the degranulation process in mast cells. We found that C1P formation was enhanced during activation induced by IgE/antigen or by Ca2+ ionophore A23187. The formation of C1P required the intracellular elevation of Ca2+. We generated RBL-2H3 cells that stably express CERK, and when these cells were treated with A23187, a concomitant C1P formation was observed and degranulation increased 4-fold, compared with mock transfectants. The cell-permeable N-acetylsphingosine (C2-ceramide), a poor substrate of CERK, inhibited both the formation of C1P and degranulation, indicating that C1P formation was necessary for degranulation. Exogenous introduction of CERK into permeabilized RBL-2H3 cells caused degranulation. We identified a cytosolic localization of CERK that provides exposure to cytosolic Ca2+. Taken together, these results indicate that C1P formation is a necessary step in the degranulation pathway in RBL-2H3 cells.

Miyakawa-Naito, A., P. Uhlen, et al. (2003). "Cell Signaling Microdomain with Na,K-ATPase and Inositol 1,4,5-Trisphosphate Receptor Generates Calcium Oscillations." J. Biol. Chem. **278**(50): 50355-50361.

http://www.jbc.org/cgi/content/abstract/278/50/50355

Recent studies indicate novel roles for the ubiquitous ion pump, Na,K-ATPase, in addition to its function as a key regulator of intracellular sodium and potassium concentration. We have previously demonstrated that ouabain, the endogenous ligand of Na,K-ATPase, can trigger intracellular Ca2+ oscillations, a versatile intracellular signal controlling a diverse range of cellular processes. Here we report that Na,K-ATPase and inositol 1,4,5-trisphosphate (InsP3) receptor (InsP3R) form a cell signaling microdomain that, in the presence of ouabain, generates slow Ca2+ oscillations in renal cells. Using fluorescent resonance energy transfer (FRET) measurements, we detected a close spatial proximity between Na,K-ATPase and InsP3R. Ouabain significantly enhanced FRET between Na,K-ATPase and InsP3R. The FRET effect and ouabain-induced Ca2+ oscillations were not observed following disruption of the actin cytoskeleton. Partial truncation of the NH2 terminus of Na,K-ATPase catalytic {alpha}1-subunit abolished Ca2+ oscillations and downstream activation of NF-{kappa}B. Ouabain-induced Ca2+ oscillations occurred in cells expressing an InsP3 sponge and were hence independent of InsP3 generation. Thus, we present a novel principle for a cell signaling microdomain where an ion pump serves as a receptor.

Modregger, J., A. A. Schmidt, et al. (2003). "Characterization of Endophilin B1b, a Brain-specific Membrane-associated Lysophosphatidic Acid Acyl Transferase with Properties Distinct from Endophilin A1." J. Biol. Chem. **278**(6): 4160-4167.

http://www.jbc.org/cgi/content/abstract/278/6/4160

We have characterized mammalian endophilin B1, a novel member of the endophilins and a representative of their B subgroup. The endophilins B show the same domain organization as the endophilins A, which contain an N-terminal domain responsible for lipid binding and lysophosphatidic acid acyl transferase activity, a central coiled-coil domain for oligomerization, a less conserved linker region, and a C-terminal Src homology 3 (SH3) domain. The endophilin B1 gene gives rise to at least three splice variants, endophilin B1a, which shows a widespread tissue distribution, and endophilins B1b and B1c, which appear to be brain-specific. Endophilin B1, like endophilins A, binds to palmitoyl-CoA, exhibits lysophosphatidic acid acyl transferase activity, and interacts with dynamin, amphiphysins 1 and 2, and huntingtin. However, in contrast to endophilins A, endophilin B1 does not bind to synaptojanin 1 and synapsin 1, and overexpression of its SH3 domain does not inhibit transferrin endocytosis. Consistent with this, immunofluorescence analysis of endophilin B1b transfected into fibroblasts shows an intracellular reticular staining, which in part overlaps with that of endogenous dynamin. Upon subcellular fractionation of brain and transfected fibroblasts, endophilin B1 is largely recovered in association with membranes. Together, our results suggest that the action of the endophilins is not confined to the formation of endocytic vesicles from the plasma membrane, with endophilin B1 being associated with, and presumably exerting a functional role at, intracellular membranes.

Monslow, J., J. D. Williams, et al. (2004). "Identification and Analysis of the Promoter Region of the Human Hyaluronan Synthase 2 Gene." J. Biol. Chem. **279**(20): 20576-20581.

http://www.jbc.org/cgi/content/abstract/279/20/20576

Hyaluronan (HA) is a linear glycosaminoglycan of the vertebrate extracellular matrix that is synthesized at the plasma membrane by the HA synthase (HAS) enzymes HAS1, -2 and -3. The regulation of HA synthesis has been implicated in a variety of extracellular matrix-mediated and pathological processes, including renal fibrosis. We have recently described the genomic structures of each of the human HAS genes. In the present study, we analyzed the HAS2 promoter region. In 5'-rapid amplification of cDNA ends analysis of purified mRNA from human renal epithelial proximal tubular cells, we detected an extended sequence for HAS2 exon 1, relocating the transcription initiation site 130 nucleotides upstream of the reference HAS2 mRNA sequence, GenBankTM accession number NM_005328. A luciferase reporter gene assay of nested fragments spanning the 5' terminus of NM_005328 demonstrated the constitutive promoter activity of sequences directly upstream of the repositioned transcription initiation site but not of the newly designated exonic nucleotides. Using reverse transcription-PCR, expression of this extended HAS2 mRNA was demonstrated in a variety of human cell types, and orthologous sequences were detected in mouse and rat kidney. Alignment of human, murine, and equine genomic DNA sequences upstream of the repositioned HAS2 exon 1 provided evidence for the evolutionary conservation of specific transcription factor binding sites. The location of the HAS2 promoter will facilitate analysis of the transcriptional regulation of this gene in a variety of pathological contexts as well as in developmental models in which HAS2 null animals have an embryonic lethal phenotype.

Morinobu, A., Y. Kanno, et al. (2004). "Discrete Roles for Histone Acetylation in Human T Helper 1 Cell-specific Gene Expression." J. Biol. Chem. **279**(39): 40640-40646.

http://www.jbc.org/cgi/content/abstract/279/39/40640

To better understand the control of T helper (TH) 1-expressed genes, we compared and contrasted acetylation and expression for three key genes, IFNG, TBET, and IL18RAP and found them to be distinctly regulated. The TBET and the IFNG genes, but not the IL18RAP gene, showed preferential acetylation of histones H3 and H4 during TH1 differentiation. Analysis of acetylation of specific histone residues revealed that H3(Lys-9), H4(Lys-8), and H4(Lys-12) were preferentially modified in TH1 cells, suggesting a possible contribution of acetylation of these residues for induction of these genes. On the other hand, the acetylation of IL18RAP gene occurred both in TH1 and TH2 cells the similar kinetics and on the same with residues, demonstrating that selective histone acetylation was not universally the case for all TH1-expressed genes. Histone H3 acetylation of IFNG and TBET genes occurred with different kinetics, however, and was distinctively regulated by cytokines. Interleukin (IL)-12 and IL-18 enhanced the histone acetylation of the IFNG gene. By contrast, histone acetylation of the TBET gene was markedly suppressed by IL-4, whereas IL-12 and IL-18 had only modest effects suggesting that histone acetylation during TH1 differentiation is a process that is regulated by various factors at multiple levels. By treating Th2 cells with a histone deacetylase inhibitor, we restored histone acetylation of the IFNG and TBET genes, but it did not fully restore their expression in TH2 cells, again suggesting that histone acetylation explains one but not all the aspects of TH1-specific gene expression.

Morohashi, Y., N. Hatano, et al. (2002). "Molecular Cloning and Characterization of CALP/KChIP4, a Novel EF-hand Protein Interacting with Presenilin 2 and Voltage-gated Potassium Channel Subunit Kv4." J. Biol. Chem. **277**(17): 14965-14975.

http://www.jbc.org/cgi/content/abstract/277/17/14965

Presenilin (PS) genes linked to early-onset familial Alzheimer's disease encode polytopic membrane proteins that are presumed to constitute the catalytic subunit of [gamma]-secretase, forming a high molecular weight complex with other proteins. During our attempts to identify binding partners of PS2, we cloned CALP (calsenilin-like protein)/KChIP4, a novel member of calsenilin/KChIP protein family that interacts with the C-terminal region of PS. Upon co-expression in cultured cells, CALP was directly bound to and co-localized with PS2 in endoplasmic reticulum. Overexpression of CALP did not affect the metabolism or stability of PS complex, and [gamma]-cleavage of [beta]APP or Notch site 3 cleavage was not altered. However, co-expression of CALP and a voltage-gated potassium channel subunit Kv4.2 reconstituted the features of A-type K+ currents and CALP directly bound Kv4.2, indicating that CALP functions as KChIPs that are known as components of native Kv4 channel complex. Taken together, CALP/KChIP4 is a novel EF-hand protein interacting with PS as well as with Kv4 that may modulate functions of a subset of membrane proteins in brain.

Muradov, K. G., K. K. Boyd, et al. (2003). "The GAFa Domains of Rod cGMP-phosphodiesterase 6 Determine the Selectivity of the Enzyme Dimerization." J. Biol. Chem. **278**(12): 10594-10601.

http://www.jbc.org/cgi/content/abstract/278/12/10594

Retinal rod cGMP phosphodiesterase (PDE6 family) is the effector enzyme in the vertebrate visual transduction cascade. Unlike other known PDEs that form catalytic homodimers, the rod PDE6 catalytic core is a heterodimer composed of [alpha] and [beta] subunits. A system for efficient expression of rod PDE6 is not available. Therefore, to elucidate the structural basis for specific dimerization of rod PDE6, we constructed a series of chimeric proteins between

PDE6[alpha][beta] and PDE5, which contain the N-terminal GAFa/GAFb domains, or portions thereof, of the rod enzyme. These chimeras were co-expressed in Sf9 cells in various combinations as His-, myc-, or FLAG-tagged proteins. Dimerization of chimeric PDEs was assessed using gel filtration and sucrose gradient centrifugation. The composition of formed dimeric enzymes was analyzed with Western blotting and immunoprecipitation. Consistent with the selectivity of PDE6 dimerization in vivo, efficient heterodimerization was observed between the GAF regions of PDE6[alpha] and PDE6[beta] with no significant homodimerization. In addition, PDE6[alpha] was able to form dimers with the cone PDE6[alpha]' subunit. Furthermore, our analysis indicated that the PDE6 GAFa domains contain major structural determinants for the affinity and selectivity of dimerization of PDE6 catalytic subunits. The key dimerization selectivity module of PDE6 has been localized to a small segment within the GAFa domains, PDE6[alpha]-59-74/PDE6[beta]-57-72. This study provides tools for the generation of the homodimeric [alpha][alpha] and [beta][beta] enzymes that will allow us to address the question of functional significance of the unique heterodimerization of rod PDE6.

Nagaoka, K., H. Nojima, et al. (2003). "Regulation of Blastocyst Migration, Apposition, and Initial Adhesion by a Chemokine, Interferon {gamma}-inducible Protein 10 kDa (IP-10), during Early Gestation." J. Biol. Chem. **278**(31): 29048-29056.

http://www.jbc.org/cgi/content/abstract/278/31/29048

For a pregnancy to be established, initial apposition and adhesion of the blastocyst to maternal endometrium must occur in a coordinated manner; however, a key factor(s) that mediates the trophoblast cell migration and attachment to the apical surface of the endometrium has not been identified. In this study, we examined the effect of an endometrial chemokine, interferon-{gamma}-inducible protein 10 kDa (IP-10), on conceptus migration to the endometrial epithelium. We first studied endometrial IP-10 mRNA expression, which was localized in the subepithelial stromal region, and detected the protein in the uterine flushing media during early pregnancy. Expression of IP-10 mRNA by the endometrium of cyclic animals was stimulated by the addition of a conceptus factor interferon-tau (IFN-{tau}). Immunofluorescent analysis revealed that IP-10 receptor, CXCR3, was localized in the trophoblast cells, to which biotinylated-recombinant caprine IP-10 (rcIP-10) bound. Chemotaxis assay indicated that rcIP-10 stimulated the migration of trophoblast cells, and the effects of rcIP-10 were neutralized by the pretreatment with an anti-IP-10 antibody. Adhesive activity of trophoblast cells to fibronectin was promoted by rcIP-10, and the effect was inhibited by the use of anti-IP-10 antibody. Further adhesion experiments demonstrated that binding of trophoblast cells to fibronectin was completely inhibited by a peptide of the Arg-Gly-Asp (RGD) sequence, which binds to integrins {alpha}5{beta}1, {alpha}V{beta}1, {alpha}V{beta}3, and {alpha}V{beta}5, whereas non-binding peptide containing Arg-Gly-Glu (RGE) had minimal effects. More importantly, rcIP-10 promoted the adhesion of trophoblast cells to primary cells isolated from endometrial epithelium. Furthermore, rcIP-10 stimulated the expression of integrin {alpha}5, {alpha}V, and {beta}3 subunit mRNA in trophoblast cells. These findings suggest that endometrial IP-10 regulates the establishment of apical interactions between trophoblast and epithelial cells during early gestation.

Nair, R. and C. Shaha (2003). "Diethylstilbestrol Induces Rat Spermatogenic Cell Apoptosis in Vivo through Increased Expression of Spermatogenic Cell Fas/FasL System." J. Biol. Chem. **278**(8): 6470-6481.

http://www.jbc.org/cgi/content/abstract/278/8/6470

The significant role that estrogens play in spermatogenesis has opened up an exciting area of research in male reproductive biology. The realization that estrogens are essential for proper

maintenance of spermatogenesis, as well as growing evidence pointing to the deleterious effects of estrogen-like chemicals on male reproductive health, has made it imperative to dissect the role estrogens play in the male. Using a model estrogen, diethylstilbestrol (DES), to induce spermatogenic cell apoptosis in vivo in the male rat, we provide a new insight into an estrogen-dependent regulation of the Fas-FasL system specifically in spermatogenic cells. We show a distinct increase in Fas-FasL expression in spermatogenic cells upon exposure to diethylstilbestrol. This increase is confined to the spermatid population, which correlates with increased apoptosis seen in the haploid cells. Testosterone supplementation is able to prevent DES-induced Fas-FasL up-regulation and apoptosis in the spermatogenic cells. DES-induced germ cell apoptosis does not occur in Fas-deficient lpr mice. One other important finding is that spermatogenic cells are type II cells, as the increase in Fas-FasL expression in the spermatogenic cells is followed by the cleavage of caspase-8 to its active form, following which Bax translocates to the mitochondria and precipitates the release of cytochrome c that is accompanied by a drop in mitochondrial potential. Subsequent to this, activation of caspase-9 occurs that in turn activates caspase-3 leading to the cleavage of poly(ADP-ribose) polymerase. Taken together, the data indicate that estrogen-like chemicals can precipitate apoptotic death in spermatogenic cells by increasing the expression of spermatogenic cell Fas-FasL, thus initiating apoptosis in the same lineage of cells through the activation of the apoptotic pathway chosen by type II cells.

Ngo, T. T., M. K. Bennett, et al. (2002). "A Role for Cyclic AMP Response Element-binding Protein (CREB) but Not the Highly Similar ATF-2 Protein in Sterol Regulation of the Promoter for 3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase." J. Biol. Chem. **277**(37): 33901-33905.

http://www.jbc.org/cgi/content/abstract/277/37/33901

Sterol regulatory element-binding proteins (SREBPs) activate promoters for key genes of metabolism to keep pace with the cellular demand for lipids. In each SREBP-regulated promoter, at least one ubiquitous co-regulatory factor that binds to a neighboring recognition site is also required for efficient gene induction. Some of these putative co-regulatory proteins are members of transcription factor families that all bind to the same DNA sequence elements in vitro and are often expressed in the same cells. These two observations have made it difficult to assign specific and redundant functions to the unique members of a specific gene family. We have used the chromatin immunoprecipitation (ChIP) technique coupled with a transient complementation assay in Drosophila SL2 cells to directly compare the ability of two members of the CREB/ATF family to function as co-regulatory proteins for SREBP-dependent activation of the HMG-CoA reductase promoter. Results from both of these experimental systems demonstrate that CREB is an efficient SREBP co-regulator but ATF-2 is not.

Nishino, K., N. Hattori, et al. (2004). "DNA Methylation-mediated Control of Sry Gene Expression in Mouse Gonadal Development." J. Biol. Chem. **279**(21): 22306-22313.

http://www.jbc.org/cgi/content/abstract/279/21/22306

DNA methylation at CpG sequences is involved in tissue-specific and developmentally regulated gene expression. The Sry (sex-determining region on the Y chromosome) gene encodes a master protein for initiating testis differentiation in mammals, and its expression is restricted to gonadal somatic cells at 10.5-12.5 days post-coitum (dpc) in the mouse. We found that in vitro methylation of the 5'-flanking region of the Sry gene caused suppression of reporter activity, implying that Sry gene expression could be regulated by DNA methylation-mediated gene silencing. Bisulfite restriction mapping and sodium bisulfite sequencing revealed that the 5'-flanking region of the Sry gene was hypermethylated in the 8.5-dpc embryos in which the Sry

gene was not expressed. Importantly, this region was specifically hypomethylated in the gonad at 11.5 dpc, while the hypermethylated status was maintained in tissues that do not express the Sry gene. We concluded that expression of the Sry gene is under the control of an epigenetic mechanism mediated by DNA methylation.

Nishiya, T. and A. L. DeFranco (2004). "Ligand-regulated Chimeric Receptor Approach Reveals Distinctive Subcellular Localization and Signaling Properties of the Toll-like Receptors." J. Biol. Chem. **279**(18): 19008-19017.

http://www.jbc.org/cgi/content/abstract/279/18/19008

Toll-like receptors (TLRs) are sensors for the detection of invading infectious agents and can initiate innate immune responses. Because the innate immune system induces an appropriate defense against different pathogens, different TLR signaling domains may have unique properties that are responsible for eliciting distinctive responses to different types of pathogens. To test this hypothesis, we created ligand-regulated TLR chimeric receptors composed of the extracellular region of TLR4 and the transmembrane and cytoplasmic regions of other TLRs and expressed these chimeras in macrophages lacking endogenous TLR4. Interestingly, the chimeras between TLR4 and either TLR3, TLR7, or TLR9 were localized completely intracellularly whereas other chimeras were expressed on the cell surface. Lipopolysaccharide (LPS), a ligand for these chimeras, induced the activation of nuclear factor {kappa}B and mitogen-activated protein kinases and the subsequent production of pro-inflammatory cytokines in macrophages expressing TLR4, TLR4/TLR5, or TLR4/TLR8 chimeras but not in macrophages expressing TLR4/TLR1, TLR4/TLR2, or TLR4/TLR6 chimeras. Co-expression of unresponsive chimeras in some combinations (chimeras with TLR1+TLR2 or TLR2+TLR6 but not TLR1+TLR6) resulted in LPS responsiveness, indicating functional complementarity. Furthermore, the pair of TLR2+TLR6 chimera required approximately 10-fold less LPS to induce the same responses compared with the TLR1+TLR2 pair. Finally, LPS induced effective interferon-{beta} production and subsequent Stat1 phosphorylation in macrophages expressing full-length TLR4 but not other cell surface TLR chimeras. These results suggest that the functions of TLRs are diversified not only in their extracellular regions for ligand recognition but also in their transmembrane and cytoplasmic regions for subcellular localization and signaling properties.

Ohshima, T. and K. Shimotohno (2003). "Transforming Growth Factor-{beta}-mediated Signaling via the p38 MAP Kinase Pathway Activates Smad-dependent Transcription through SUMO-1 Modification of Smad4." J. Biol. Chem. **278**(51): 50833-50842.

http://www.jbc.org/cgi/content/abstract/278/51/50833

Post-translational modifications such as ubiquitination, phosphorylation, and acetylation play important roles in the regulation of Smad-mediated functions. Here, we demonstrate that Smad4 is covalently modified by SUMO-1, which was characterized recently as a key modulator of many transcription factors. Sumoylation of Smad4 mainly occurs at lysine 159, located in the linker region, and facilitates Smad-dependent transcriptional activation. Furthermore, we show that the PIAS family proteins, PIAS1 and PIASx{beta}, function as E3 ligase factors for Smad4. Intriguingly, sumoylation of Smad4 was strongly enhanced by TGF-{beta}-induced activation of the p38 MAP kinase pathway but not the Smad pathway. Activation of p38 not only stabilized PIASx{beta} protein but also enhanced PIASx{beta} gene expression, suggesting that PIAS-mediated sumoylation of Smad4 is regulated by the p38 MAP kinase pathway. These findings illustrate a novel regulatory mechanism by which Smad-dependent transcriptional activation cooperatively modulates Smad proteins through receptor-mediated phosphorylation and sumoylation.

Ohya, T., Y. Kawasaki, et al. (2002). "The DNA Polymerase Domain of polepsilon Is Required for Rapid, Efficient, and Highly Accurate Chromosomal DNA Replication, Telomere Length Maintenance, and Normal Cell Senescence in Saccharomyces cerevisiae." J. Biol. Chem. **277**(31): 28099-28108.

http://www.jbc.org/cgi/content/abstract/277/31/28099

Saccharomyces cerevisiae POL2 encodes the catalytic subunit of DNA polymerase [epsilon]. This study investigates the cellular functions performed by the polymerase domain of Pol2p and its role in DNA metabolism. The pol2-16 mutation has a deletion in the catalytic domain of DNA polymerase [epsilon] that eliminates its polymerase and exonuclease activities. It is a viable mutant, which displays temperature sensitivity for growth and a defect in elongation step of chromosomal DNA replication even at permissive temperatures. This mutation is synthetic lethal in combination with temperature-sensitive mutants or the 3'- to 5'-exonuclease-deficient mutant of DNA polymerase [delta] in a haploid cell. These results suggest that the catalytic activity of DNA polymerase [epsilon] participates in the same pathway as DNA polymerase [delta], and this is consistent with the observation that DNA polymerases [delta] and [epsilon] colocalize in some punctate foci on yeast chromatids during S phase. The pol2-16 mutant senesces more rapidly than wild type strain and also has shorter telomeres. These results indicate that the DNA polymerase domain of Pol2p is required for rapid, efficient, and highly accurate chromosomal DNA replication in yeast.

Okuda, T., M. Okamura, et al. (2002). "Single Nucleotide Polymorphism of the Human High Affinity Choline Transporter Alters Transport Rate." J. Biol. Chem. **277**(47): 45315-45322.

http://www.jbc.org/cgi/content/abstract/277/47/45315

High affinity choline uptake plays a critical role in the regulation of acetylcholine synthesis in cholinergic neurons. Recently, we succeeded in molecular cloning of the high affinity choline transporter (CHT1), which is specifically expressed in cholinergic neurons. Here we demonstrate the presence of functionally relevant, nonsynonymous single nucleotide polymorphism in the human CHT1 gene by comprehensive sequence analysis of the exons and the intron/exon boundaries including the transcription start site. The deduced amino acid change for the polymorphism is isoleucine to valine at amino acid 89 (I89V) located within the third transmembrane domain of the protein. The allele frequency of I89V was 6% for Ashkenazi Jews. Functional assessment of the I89V transporter in mammalian cell lines revealed a 40-50% decrease in Vmax for choline uptake rate compared with the wild type, whereas there was no alteration in the apparent affinities for choline, sodium, chloride, and the specific inhibitor hemicholinum-3. There also was no change in the specific hemicholinum-3 binding activity. The decreased choline uptake was not associated with the surface expression level of the protein as assessed by biotinylation assay. These results suggest an impaired substrate translocation in the I89V transporter. The Caenorhabditis elegans ortholog of CHT1 has a valine residue at the corresponding position and a single replacement from valine to isoleucine caused a decrease in the choline uptake rate by 40%, suggesting that this hydrophobic residue is generally critical in the choline transport rate in CHT1. This polymorphism in the allelic CHT1 gene may represent a predisposing factor for cholinergic dysfunction.

Olosz, F. and T. R. Malek (2002). "Structural Basis for Binding Multiple Ligands by the Common Cytokine Receptor gamma -Chain." J. Biol. Chem. **277**(14): 12047-12052.

http://www.jbc.org/cgi/content/abstract/277/14/12047

The common [gamma]-chain ([gamma]c) that functions both in ligand binding and signal transduction is a shared subunit of the multichain receptors for interleukin (IL)-2, IL-4, IL-7, IL-9, IL-15, and IL-21. The structural basis by which the ectodomain of [gamma]c contributes to binding six distinct cytokines is only partially defined. In the present study, epitope mapping of antagonistic anti-[gamma]c monoclonal antibodies led to the identification of Asn-128 of mouse [gamma]c that represents another potential contact residue that is required for binding IL-2, IL-7, and IL-15 but not IL-4. In addition, Tyr-103, Cys-161, Cys-210, and Cys-211, previously identified to contribute to binding IL-2 and IL-7, were also found to be involved in binding IL-4 and IL-15. Collectively, these data favor a model in which [gamma]c utilizes a common mechanism for its interactions with multiple cytokines, and the binding sites are largely overlapping but not identical. Asn-128 and Tyr-103 likely act as contact residues whereas Cys-161, Cys-210, and Gly-211 may stabilize the structure of the proposed ligand-interacting surface formed by the two extracytoplasmic domains.

Ortega, A., P. Ferrer, et al. (2003). "Down-regulation of Glutathione and Bcl-2 Synthesis in Mouse B16 Melanoma Cells Avoids Their Survival during Interaction with the Vascular Endothelium." J. Biol. Chem. **278**(41): 39591-39599.

http://www.jbc.org/cgi/content/abstract/278/41/39591

B16 melanoma (B16M) cells with high GSH content show high metastatic activity. However, the molecular mechanisms linking GSH to metastatic cell survival are unclear. The possible relationship between GSH and the ability of Bcl-2 to prevent cell death was studied in B16M cells with high (F10) and low (F1) metastatic potential. Analysis of a Bcl-2 family of genes revealed that B16M-F10 cells, as compared with B16M-F1 cells, overexpressed preferentially Bcl-2 ([~]5.7-fold). Hepatic sinusoidal endothelium-induced B16M-F10 cytotoxicity in vitro increased from [~]19% (controls) to [~]97% in GSH-depleted B16M-F10 cells treated with an antisense Bcl-2 oligodeoxynucleotide (Bcl-2-AS). L-Buthionine (S,R)-sulfoximine-induced GSH depletion or Bcl-2-AS decreased the metastatic growth of B16M-F10 cells in the liver. However, the combination of L-buthionine (S,R)-sulfoximine and Bcl-2-AS abolished metastatic invasion. Bcl-2-overexpressing B16M-F1/Tet-Bcl-2 and B16M-F10/Tet-Bcl-2 cells, as compared with controls, showed an increase in GSH content, no change in the rate of GSH synthesis, and a decrease in GSH efflux. Thus, Bcl-2 overexpression may increase metastatic cell resistance against oxidative/nitrosative stress by inhibiting release of GSH. In addition, Bcl-2 availability regulates the mitochondrial GSH (mtGSH)-dependent opening of the permeability transition pore complex. Death in B16M-F10 cells was sharply activated at mtGSH levels below 30% of controls values. However, this critical threshold increased to [~]60% of control values in Bcl-2-AS-treated B16M-F10 cells. GSH ester-induced replenishment of mtGSH levels (even under conditions of cytosolic GSH depletion) prevented cell death. Our results indicate that survival of B16M cells with high metastatic potential can be challenged by inhibiting their GSH and Bcl-2 synthesis.

Ortega, A. L., J. Carretero, et al. (2003). "Tumor Cytotoxicity by Endothelial Cells. IMPAIRMENT OF THE MITOCHONDRIAL SYSTEM FOR GLUTATHIONE UPTAKE IN MOUSE B16 MELANOMA CELLS THAT SURVIVE AFTER IN VITRO INTERACTION WITH THE HEPATIC SINUSOIDAL ENDOTHELIUM." J. Biol. Chem. **278**(16): 13888-13897.

http://www.jbc.org/cgi/content/abstract/278/16/13888

High GSH content associates with high metastatic activity in B16-F10 melanoma cells cultured to

low density (LD B16M). GSH homeostasis was investigated in LD B16M cells that survive after adhesion to the hepatic sinusoidal endothelium (HSE). Invasive B16M (iB16M) cells were isolated using anti-Met-72 monoclonal antibodies and flow cytometry-coupled cell sorting. HSE-derived NO and H2O2 caused GSH depletion and a decrease in [gamma]-glutamylcysteine synthetase activity in iB16M cells. Overexpression of [gamma]-glutamylcysteine synthetase heavy and light subunits led to a rapid recovery of cytosolic GSH, whereas mitochondrial GSH (mtGSH) further decreased during the first 18 h of culture. NO and H2O2 damaged the mitochondrial system for GSH uptake (rates in iB16M were approximately 75% lower than in LD B16M cells). iB16M cells also showed a decreased activity of mitochondrial complexes II, III, and IV, less O2 consumption, lower ATP levels, higher O[IMG]img001.gif" ALT="<UP>₂</UP>"> and H2O2 production, and lower mitochondrial membrane potential. In vitro growing iB16M cells maintained high viability (>98%) and repaired HSE-induced mitochondrial damages within 48 h. However, iB16M cells with low mtGSH levels were highly susceptible to TNF-[alpha]-induced oxidative stress and death. Therefore depletion of mtGSH levels may represent a critical target to challenge survival of invasive cancer cells.

Osada, M., S. Imaoka, et al. (2002). "NADPH-Cytochrome P-450 Reductase in the Plasma Membrane Modulates the Activation of Hypoxia-inducible Factor 1." J. Biol. Chem. **277**(26): 23367-23373.

http://www.jbc.org/cgi/content/abstract/277/26/23367

Hypoxia induces a group of physiologically important genes that include erythropoietin (EPO) and vascular endothelial growth factor (VEGF). Hypoxia-inducible factor 1 (HIF-1) was identified as a hypoxia-activated transcription factor; however, the molecular mechanisms that underlie hypoxia signal transduction in mammalian cells remain undefined. In this study, we found that a flavoprotein, NADPH-P450 reductase (NPR), could regulate the induction of EPO mRNA under hypoxic conditions. Hypoxic EPO mRNA induction in Hep3B cells was inhibited by diphenyleneiodonium chloride, which is an inhibitor of NADPH-dependent enzymes. NPR antisense cDNA was transfected into Hep3B cells, and NPR-deficient hepatocyte cells (NPR[-] cells) were established. NPR[-] cells lacked EPO induction under hypoxia, and HIF-1[alpha] in NPR[-] cells did not respond to either transcriptional activation or translocation to the nucleus based on electrophoretic mobility shift assays and reporter gene assay including hypoxia response element. In contrast, NPR overexpression in Hep3B cells enhanced the DNA binding activity of HIF-1[alpha] by luciferase reporter gene assay. A study with HeLa S3 cells produced the same results. Furthermore, anti-NPR IgG inhibited EPO induction. EPO induction inhibited by diphenyleneiodonium chloride was recovered by bovine serum albumin-NADPH (a covalent binding complex of bovine serum albumin and NADPH) as well as NADPH. These results suggested that NPR located at the plasma membrane regulates EPO expression in hypoxia, including HIF-1 activation and translocation. We further studied the expression of NPR and VEGF mRNAs in human tumor tissues and found that the NPR mRNA levels were correlated with the VEGF mRNA levels, suggesting that NPR might be an important factor in the hypoxic induction of genes such as VEGF in vivo.

Pankonin, M. S., J. T. Gallagher, et al. (2005). "Specific Structural Features of Heparan Sulfate Proteoglycans Potentiate Neuregulin-1 Signaling." J. Biol. Chem. **280**(1): 383-388.

http://www.jbc.org/cgi/content/abstract/280/1/383

Neuregulins are a family of growth and differentiation factors that act through activation of cell-surface erbB receptor tyrosine kinases and have essential functions both during development and on the growth of cancer cells. One alternatively spliced neuregulin-1 form has a distinct heparin-binding immunoglobulin-like domain that enables it to adhere to heparan sulfate proteoglycans at

key locations during development and substantially potentiates its activity. We examined the structural specificity needed for neuregulin-1-heparin interactions using a gel mobility shift assay together with an assay that measures the ability of specific oligosaccharides to block erbB receptor phosphorylation in L6 muscle cells. Whereas the N-sulfate group of heparin was most important, the 2-O-sulfate and 6-O-sulfate groups also contributed to neuregulin-1 binding in these two assays. Optimal binding to neuregulin-1 required eight or more heparin disaccharides; however, as few as two disaccharides were still able to bind neuregulin-1 to a lesser extent. The physiological importance of this specificity was shown both by chemical and siRNA treatment of cultured muscle cells. Pretreatment of muscle cells with chlorate that blocks all sulfation or with an siRNA that selectively blocks N-sulfation significantly reduced erbB receptor activation by neuregulin-1 but had no effect on the activity of neuregulin-1 that lacks the heparin-binding domain. These results suggest that the regulation of glycosaminoglycan sulfation is an important biological mechanism that can modulate both the localization and potentiation of neuregulin-1 signaling.

Papoucheva, E., A. Dumuis, et al. (2004). "The 5-Hydroxytryptamine(1A) Receptor Is Stably Palmitoylated, and Acylation Is Critical for Communication of Receptor with Gi Protein." J. Biol. Chem. **279**(5): 3280-3291.

http://www.jbc.org/cgi/content/abstract/279/5/3280

In the present study, we verified that the mouse 5-hydroxytryptamine(1A) (5-HT1A) receptor is modified by palmitic acid, which is covalently attached to the protein through a thioester-type bond. Palmitoylation efficiency was not modulated by receptor stimulation with agonists. Block of protein synthesis by cycloheximide resulted in a significant reduction of receptor acylation, suggesting that palmitoylation occurs early after synthesis of the 5-HT1A receptor. Furthermore, pulse-chase experiments demonstrated that fatty acids are stably attached to the receptor. Two conserved cysteine residues 417 and 420 located in the proximal C-terminal domain were identified as acylation sites by site-directed mutagenesis. To address the functional role of 5-HT1A receptor acylation, we have analyzed the ability of acylation-deficient mutants to interact with heterotrimeric Gi protein and to modulate downstream effectors. Replacement of individual cysteine residues (417 or 420) resulted in a significantly reduced coupling of receptor with Gi protein and impaired inhibition of adenylyl cyclase activity. When both palmitoylated cysteines were replaced, the communication of receptors with G{alpha}i subunits was completely abolished. Moreover, non-palmitoylated mutants were no longer able to inhibit forskolin-stimulated cAMP formation, indicating that palmitoylation of the 5-HT1A receptor is critical for the enabling of Gi protein coupling/effector signaling. The receptor-dependent activation of extracellular signal-regulated kinase was also affected by acylation-deficient mutants, suggesting the importance of receptor palmitoylation for the signaling through the G{beta}{gamma}-mediated pathway, in addition to the G{alpha}i-mediated signaling.

Patnaik, S. K., B. Potvin, et al. (2004). "LEC12 and LEC29 Gain-of-Function Chinese Hamster Ovary Mutants Reveal Mechanisms for Regulating VIM-2 Antigen Synthesis and E-selectin Binding." J. Biol. Chem. **279**(48): 49716-49726.

http://www.jbc.org/cgi/content/abstract/279/48/49716

LEC12 and LEC29 are two gain-of-function Chinese hamster ovary glycosylation mutants that express the Fut9 gene encoding {alpha}(1,3)fucosyltransferase IX ({alpha}(1,3) Fuc-TIX). Both mutants express the Lewis X (LeX) determinant Gal{beta}(1,4)[Fuc{alpha}(1,3)]GlcNAc, and LEC12, but not LEC29 cells, also express the VIM-2 antigen SA{alpha}(2,3)-Gal{beta}(1,4)GlcNAc{beta}(1,3)Gal{beta}(1,4)[Fuc{alpha}(1,3)]GlcNAc. Here we show that

LEC29 cells transfected with a Fut9 cDNA express VIM-2, and thus LEC29 cells synthesize appropriate acceptors to generate the VIM-2 epitope. Semiquantitative reverse transcription-PCR showed that LEC12 has 10- to 20-fold less Fut9 gene transcripts than LEC29. However, Western analysis revealed that LEC12 has [~]20 times more Fut9 protein than LEC29. The latter finding was consistent with our previous observation that LEC12 has [~]40 times more in vitro {alpha}(1,3)Fuc-T activity than LEC29. The basis for the difference in Fut9 protein levels was found to lie in sequence differences in the 5'-untranslated regions (5'-UTR) of LEC12 and LEC29 Fut9 gene transcripts. Whereas reporter assays with the respective 5'-UTR regions linked to luciferase did not indicate a reduced translation efficiency caused by the LEC29 5'-UTR, transfected full-length LEC29 Fut9 cDNA or in vitro-synthesized full-length LEC29 Fut9 RNA gave less Fut9 protein than similar constructs with a LEC12 5'-UTR. This difference appears to be largely responsible for the reduced {alpha}(1,3)Fuc-TIX activity and lack of VIM-2 expression of LEC29 cells. This could be of physiological relevance, because LEC29 and parent Chinese hamster ovary cells transiently expressing a Fut9 cDNA were able to bind mouse E-selectin, although they did not express sialyl-LeX.

Paulussen, A., A. Raes, et al. (2002). "A Novel Mutation (T65P) in the PAS Domain of the Human Potassium Channel HERG Results in the Long QT Syndrome by Trafficking Deficiency." J. Biol. Chem. **277**(50): 48610-48616.

http://www.jbc.org/cgi/content/abstract/277/50/48610

The congenital long QT syndrome is a cardiac disease characterized by an increased susceptibility to ventricular arrhythmias. The clinical hallmark is a prolongation of the QT interval, which reflects a delay in repolarization caused by mutations in cardiac ion channel genes. Mutations in the HERG (human ether-a-go-go-related gene KCNH2 can cause a reduction in IKr, one of the currents responsible for cardiac repolarization. We describe the identification and characterization of a novel missense mutation T65P in the PAS (Per-Arnt-Sim) domain of HERG, resulting in defective trafficking of the protein to the cell membrane. Defective folding of the mutant protein could be restored by decreased cell incubation temperature and pharmacologically by cisapride and E-4031. When trafficking was restored by growing cells at 27 {degrees}C, the kinetics of the mutated channel resembled that of wild-type channels although the rate of activation, deactivation, and recovery from inactivation were accelerated. No positive evidence for the formation of heterotetramers was obtained by co-expression of wild-type with mutant subunits at 37 {degrees}C. As a consequence the clinical symptoms may be explained rather by haploinsufficiency than by dominant negative effects. This study is the first to relate a PAS domain mutation in HERG to a trafficking deficiency at body temperature, apart from effects on channel deactivation.

Pelicano, H., L. Feng, et al. (2003). "Inhibition of Mitochondrial Respiration: A NOVEL STRATEGY TO ENHANCE DRUG-INDUCED APOPTOSIS IN HUMAN LEUKEMIA CELLS BY A REACTIVE OXYGEN SPECIES-MEDIATED MECHANISM." J. Biol. Chem. **278**(39): 37832-37839.

http://www.jbc.org/cgi/content/abstract/278/39/37832

Cancer cells are under intrinsic increased oxidative stress and vulnerable to free radical-induced apoptosis. Here, we report a strategy to hinder mitochondrial electron transport and increase superoxide [IMG]f1.gif" BORDER="0"> radical generation in human leukemia cells as a novel mechanism to enhance apoptosis induced by anticancer agents. This strategy was first tested in a proof-of-principle study using rotenone, a specific inhibitor of mitochondrial electron transport complex I. Partial inhibition of mitochondrial respiration enhances electron leakage from the transport chain, leading to an increase in [IMG]f1.gif" BORDER="0"> generation and sensitization

of the leukemia cells to anticancer agents whose action involve free radical generation. Using leukemia cells with genetic alterations in mitochondrial DNA and biochemical approaches, we further demonstrated that As2O3, a clinically active anti-leukemia agent, inhibits mitochondrial respiratory function, increases free radical generation, and enhances the activity of another [IMG]f2.gif" BORDER="0"> agent against cultured leukemia cells and primary leukemia cells isolated from patients. Our study shows that interfering mitochondrial respiration is a novel mechanism by which As2O3 increases generation of free radicals. This novel mechanism of action provides a biochemical basis for developing new drug combination strategies using As2O3 to enhance the activity of anticancer agents by promoting generation of free radicals.

Perrier, E., R. Perrier, et al. (2004). "Ca2+ Controls Functional Expression of the Cardiac K+ Transient Outward Current via the Calcineurin Pathway." J. Biol. Chem. **279**(39): 40634-40639.

http://www.jbc.org/cgi/content/abstract/279/39/40634

The transient outward K+ current (Ito) modulates transmembrane Ca2+ influx into cardiomyocytes, which, in turn, might act on Ito. Here, we investigated whether Ca2+ modifies functional expression of Ito. Whole-cell Ito were recorded using the patch clamp technique in single right ventricular myocytes isolated from adult rats and incubated for 24 h at 37 {degrees}C in a serum-free medium containing various Ca2+ concentrations ([Ca2+]o). Increasing the [Ca2+]o from 0.5 to 1.0 and 2.5 mM produced a gradual decrease in Ito density without change in current kinetics. Quantitativereverse transcriptase-PCR showed that a decrease of the Kv4.2 mRNA could account for this decrease. In the acetoxymethyl ester form of 1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (BAPTA-AM)-loaded myocytes (a permeant Ca2+ chelator), Ito density increased significantly when cells were exposed for 24 h to either 1 or 2.5 mM [Ca2+]o. Moreover, 24-h exposure to the Ca2+ channel agonist, Bay K8644, in 1 mM [Ca2+]o induced a decrease in Ito density, whereas the Ca2+ channel antagonist, nifedipine, blunted Ito decrease in 2.5 mM [Ca2+]o. The decrease of Ito in 2.5 mM [Ca2+]o was also prevented by co-incubation with either the calmodulin inhibitor W7 or the calcineurin inhibitors FK506 or cyclosporin A. Furthermore, in myocytes incubated for 24 h with 2.5 mM [Ca2+]o, calcineurin activity was significantly increased compared with 1 mM [Ca2+]o. Our data suggest that modulation of [Ca2+]i via L-type Ca2+ channels, which appears to involve the Ca2+/calmodulin-regulated protein phosphatase calcineurin, down-regulates the functional expression of Ito. This effect might be involved in many physiological and pathological modulations of Ito channel expression in cardiac cells, as well other cell types.

Piechotta, K., J. Lu, et al. (2002). "Cation Chloride Cotransporters Interact with the Stress-related Kinases Ste20-related Proline-Alanine-rich Kinase (SPAK) and Oxidative Stress Response 1 (OSR1)." J. Biol. Chem. **277**(52): 50812-50819.

http://www.jbc.org/cgi/content/abstract/277/52/50812

Cells respond to stress stimuli by mounting specific responses. During osmotic and oxidative stress, cation chloride cotransporters, e.g. Na-K-2Cl and K-Cl cotransporters, are activated to maintain fluid/ion homeostasis. Here we report the interaction of the stress-related serine-threonine kinases Ste20-related proline-alanine-rich kinase (SPAK) and oxidative stress response 1 (OSR1) with the cotransporters KCC3, NKCC1, and NKCC2 but not KCC1 and KCC4. The interaction was identified using yeast two-hybrid assays and confirmed via glutathione S-transferase pull-down experiments. Evidence for in vivo interaction was established by co-immunoprecipitation of SPAK from mouse brain with anti-NKCC1 antibody. The interacting region of both kinases comprises the last 100 amino acids of the protein. The SPAK/OSR1 binding motif on the cotransporters consists of nine residues, starting with an (R/K)FX(V/I) sequence followed

by five additional residues that are essential for binding but for which no consensus was found. Immunohistochemical analysis of choroid plexus epithelium revealed co-expression of NKCC1 and SPAK on the apical membrane. In contrast, in choroid plexus epithelium from NKCC1 null mice, SPAK immunostaining was found in the cytoplasm. We conclude that several cation chloride co-transporters interact with SPAK and/or OSR1, and we hypothesize that this interaction might play a role during the initiation of the cellular stress response.

Pinkoski, M. J., N. M. Droin, et al. (2002). "Tumor Necrosis Factor alpha Up-regulates Non-lymphoid Fas-ligand following Superantigen-induced Peripheral Lymphocyte Activation." J. Biol. Chem. **277**(44): 42380-42385.

http://www.jbc.org/cgi/content/abstract/277/44/42380

Members of the tumor necrosis factor (TNF) and TNF receptor families play important roles in inducing apoptosis and mediating the inflammatory response. Activated T lymphocytes can trigger the expression of Fas-ligand on non-lymphoid tissue, such as intestinal epithelial cells (IEC), and this, in turn, can induce apoptosis in the T cells. Here, we examine the role of TNF[alpha] in this feedback regulation. Injection of TNF[alpha] into mice caused a rapid up-regulation of Fas-ligand mRNA in IEC. TNF[alpha]-induced activation of the Fas-ligand promoter in IEC requires NF-[kappa]B as this was blocked by an I-[kappa]B[alpha]M super-repressor and by mutation of an NF-[kappa]B site in the Fas-ligand promoter. Activation of T cells by antigen induced Fas-ligand expression in IEC in vivo in wild type, but not in TNF[alpha][-]/[-] or TNFR1[-]/[-] mice. These results define a novel pathway wherein TNF[alpha], produced by activated T cells in the intestine, induce Fas-ligand expression in IEC. This is the first observation that one member of the TNF superfamily mediates the regulation of another family member and represents a potential feedback mechanism controlling lymphocyte infiltration and inflammation in the small intestine.

Ponimaskin, E. G., M. Heine, et al. (2002). "The 5-Hydroxytryptamine(4a) Receptor Is Palmitoylated at Two Different Sites, and Acylation Is Critically Involved in Regulation of Receptor Constitutive Activity." J. Biol. Chem. **277**(4): 2534-2546.

http://www.jbc.org/cgi/content/abstract/277/4/2534

We have reported recently that the mouse 5-hydroxytryptamine(4a) (5-HT4(a)) receptor undergoes dynamic palmitoylation (Ponimaskin, E. G., Schmidt, M. F., Heine, M., Bickmeyer, U., and Richter, D. W. (2001) Biochem. J. 353, 627-663). In the present study, conserved cysteine residues 328/329 in the carboxyl terminus of the 5-HT4(a) receptor were identified as potential acylation sites. In contrast to other palmitoylated G-protein-coupled receptors, the additional cysteine residue 386 positioned close to the COOH-terminal end of the receptor was also found to be palmitoylated. Using pulse and pulse-chase labeling techniques, we demonstrated that palmitoylation of individual cysteines is a reversible process and that agonist stimulation of the 5-HT4(a) receptor independently increases the rate of palmitate turnover for both acylation sites. Analysis of acylation-deficient mutants revealed that non-palmitoylated 5-HT4(a) receptors were indistinguishable from the wild type in their ability to interact with Gs, to stimulate the adenylyl cyclase activity and to activate cyclic nucleotide-sensitive cation channels after agonist stimulation. The most distinctive finding of the present study was the ability of palmitoylation to modulate the agonist-independent constitutive 5-HT4(a) receptor activity. We demonstrated that mutation of the proximal palmitoylation site (Cys328 [right-arrow] Ser/Cys329 [right-arrow] Ser) significantly increases the capacity of receptors to convert from the inactive (R) to the active (R*) form in the absence of agonist. In contrast, the rate of isomerization from R to R* for the Cys386 [right-arrow] Ser as well as for the triple, non-palmitoylated mutant (Cys328 [right-arrow]

Ser/Cys329 [right-arrow] Ser/Cys386 [right-arrow]Ser) was similar to that obtained for the wild type.

Priest, J. W. and S. L. Hajduk (2003). "Trypanosoma brucei Cytochrome c1 Is Imported into Mitochondria Along an Unusual Pathway." J. Biol. Chem. **278**(17): 15084-15094.

http://www.jbc.org/cgi/content/abstract/278/17/15084

In most eukaryotic organisms, cytochrome c1 is encoded in the nucleus, translated on cytosolic ribosomes, and directed to its final destination in the mitochondrial inner membrane by a bipartite, cleaved, amino-terminal presequence. However, in the kinetoplastids and euglenoids, the cytochrome c1 protein has been shown to lack a cleaved presequence; a single methionine is removed from the amino terminus upon maturation, and the sequence upstream of the heme-binding site is generally shorter than that of the other eukaryotic homologs. We have used a newly developed mitochondrial protein import assay system from Trypanosoma brucei to demonstrate that the T. brucei cytochrome c1 protein is imported along a non-conservative pathway similar to that described for the inner membrane carrier proteins of other organisms. This pathway requires external ATP and an external protein receptor but is not absolutely dependent on a membrane potential or on ATP hydrolysis in the mitochondrial matrix. We propose the cytochrome c1 import in T. brucei is a two-step process first involving a membrane potential independent translocation across the outer mitochondrial membrane followed by heme attachment and a membrane potential-dependent insertion into the inner membrane.

Pruitt, K., A. S. Ulku, et al. (2005). "RAS mediated loss of the pro-apoptotic response protein par-4 is mediated by DNA hypermethylation through RAF-independent and RAF-dependent signaling cascades in epithelial cells." J. Biol. Chem.: M503083200.

http://www.jbc.org/cgi/content/abstract/M503083200v1

The apoptosis promoting protein, Par-4, has been shown to be downregulated in Ras-transformed NIH 3T3 fibroblasts through the Raf/MEK/ERK mitogen-activated protein kinase (MAPK) pathway. Since mutations of the ras gene are most often found in tumors of epithelial origin, we explored the signaling pathways utilized by oncogenic Ras to downregulate Par-4 in RIE-1 and ROSE epithelial cells. We determined that constitutive activation of the Raf, phosphatidylinositol 3-kinase (PI3K), or Ral guanine nucleotide exchange factor effector pathway alone was not sufficient to downregulate Par-4 in RIE-1 or ROSE cells. However, treatment of Ras-transformed RIE-1 or ROSE cells with the MEK inhibitors U0126 or PD98059 increased Par-4 protein expression. Thus, while oncogenic Ras utilizes the Raf/MEK/ERK pathway to down modulate Par-4 in both fibroblasts and epithelial cells, Ras activation of an additional signaling pathway(s) is required to achieve the same outcome in epithelial cells. Methylation-specific PCR showed the Par-4 promoter is methylated in Ras transformed cells through a MEK-dependent pathway and treatment with the DNA methyltransferase inhibitor, azadeoxycytidine, restored Par-4 mRNA transcript and protein levels suggesting that the mechanism for Ras-mediated downregulation of Par-4 is by promoter methylation. Support for this possibility is provided by our observation that Ras transformation was associated with upregulated expression of the Dnmt1 and Dnmt3 DNA methyltransferases. Finally, ectopic Par-4 expression significantly reduced Ras-mediated growth in soft agar but not morphological transformation highlighting the importance of Par-4 downregulation in specific aspects of Ras-mediated transformation of epithelial cells.

Pulliainen, A. T., S. Haataja, et al. (2003). "Molecular Basis of H2O2 Resistance Mediated by Streptococcal Dpr. DEMONSTRATION OF THE FUNCTIONAL INVOLVEMENT OF THE PUTATIVE FERROXIDASE CENTER BY SITE-DIRECTED MUTAGENESIS IN STREPTOCOCCUS SUIS." J. Biol. Chem. **278**(10): 7996-8005.

http://www.jbc.org/cgi/content/abstract/278/10/7996

H2O2 is an unavoidable cytotoxic by-product of aerobic life. Dpr, a recently discovered member of the Dps protein family, provides a means for catalase-negative bacteria to tolerate H2O2. Potentially, Dpr could bind free intracellular iron and thus inhibit the Fenton chemistry-catalyzed formation of toxic hydroxyl radicals (H2O2 + Fe2+ [right-arrow] {middle dot}OH + -OH + Fe3+). We explored the in vivo function of Dpr in the catalase- and NADH peroxidase-negative pig and human pathogen Streptococcus suis. We show that: (i) a Dpr allelic exchange knockout mutant was hypersensitive (~106-fold) to H2O2, (ii) Dpr incorporated iron in vivo, (iii) a putative ferroxidase center was present in Dpr, (iv) single amino acid substitutions D74A or E78A to the putative ferroxidase center abolished the in vivo iron incorporation, and (v) the H2O2 hypersensitive phenotype was complemented by wild-type Dpr or by a membrane-permeating iron chelator, but not by the site-mutated forms of Dpr. These results demonstrate that the putative ferroxidase center of Dpr is functionally active in iron incorporation and that the H2O2 resistance is mediated by Dpr in vivo by its iron binding activity.

Rafiee, P., Y. Shi, et al. (2003). "Cellular Redistribution of Inducible Hsp70 Protein in the Human and Rabbit Heart in Response to the Stress of Chronic Hypoxia: ROLE OF PROTEIN KINASES." J. Biol. Chem. **278**(44): 43636-43644.

http://www.jbc.org/cgi/content/abstract/278/44/43636

Many infants who undergo cardiac surgery have a congenital cyanotic defect where the heart is chronically perfused with hypoxemic blood. Infant hearts adapt to chronic hypoxemia by activation of intracellular protein kinase signal transduction pathways. However, the involvement of heat shock protein 70 in adaptation to chronic hypoxemia and its role in protein kinase signaling pathways is unknown. We determined expression of message and subcellular protein distribution for inducible (Hsp70i) and constitutive heat shock protein 70 (Hsc70) in chronically hypoxic and normoxic infant human and rabbit hearts and their relationship to protein kinases. In chronically hypoxic human and rabbit hearts message levels for Hsp70i were elevated 4- to 5-fold compared with normoxic hearts, Hsp70i protein was redistributed from the particulate to the cytosolic fraction. In normoxic infants Hsp70i protein was distributed almost equally between the cytosolic and particulate fractions. Hsc70 message and subcellular distribution of Hsc70 protein were unaffected by chronic hypoxia. We then determined if protein kinases influence Hsp70i protein subcellular distribution. In rabbit hearts SB203580 and chelerythrine reduced Hsp70i message levels, whereas SB203580, chelerythrine, and curcumin reversed the subcellular redistribution of Hsp70i protein caused by chronic hypoxia, with no effect in normoxic hearts, indicating regulation of Hsp70i message and subcellular distribution of Hsp70i protein in chronically hypoxic rabbit hearts is influenced by protein kinase C and mitogen-activated protein kinases, specifically p38 MAPK and JNK. We conclude the Hsp70 signal transduction pathway plays an important role in adaptation of infant human and rabbit hearts to chronic hypoxemia.

Rahman, A. S., J. Hothersall, et al. (2005). "Tandemly Duplicated Acyl Carrier Proteins, Which Increase Polyketide Antibiotic Production, Can Apparently Function Either in Parallel or in Series." J. Biol. Chem. **280**(8): 6399-6408.

http://www.jbc.org/cgi/content/abstract/280/8/6399

Polyketide biosynthesis involves the addition of subunits commonly derived from malonate or methylmalonate to a starter unit such as acetate. Type I polyketide synthases are multifunctional polypeptides that contain one or more modules, each of which normally contains all the enzymatic domains for a single round of extension and modification of the polyketide backbone. Acyl carrier proteins (ACP(s)) hold the extender unit to which the starter or growing chain is added. Normally there is one ACP for each ketosynthase module. However, there are an increasing number of known examples of tandemly repeated ACP domains, whose function is as yet unknown. For the doublet and triplet ACP domains in the biosynthetic pathway for the antibiotic mupirocin from Pseudomonas fluorescens NCIMB10586 we have inactivated ACP domains by inframe deletion and amino acid substitution of the active site serine. By deletion analysis each individual ACP from a cluster can provide a basic but reduced activity for the pathway. In the doublet cluster, substitution analysis indicates that the pathway may follow two parallel routes, one via each of the ACPs, thus increasing overall pathway flow. In the triplet cluster, substitution in ACP5 blocked the pathway. Thus ACP5 appears to be arranged "in series" to ACP6 and ACP7. Thus although both the doublet and triplet clusters increase antibiotic production, the mechanisms by which they do this appear to be different and depend specifically on the biosynthetic stage involved. The function of some ACPs may be determined by their location in the protein rather than absolute enzymic activity.

Ramakrishnan, S. N., P. Lau, et al. (2005). "Rev-erb{beta} Regulates the Expression of Genes Involved in Lipid Absorption in Skeletal Muscle Cells: EVIDENCE FOR CROSS-TALK BETWEEN ORPHAN NUCLEAR RECEPTORS AND MYOKINES." J. Biol. Chem. **280**(10): 8651-8659.

http://www.jbc.org/cgi/content/abstract/280/10/8651

Rev-erb{beta} is an orphan nuclear receptor that selectively blocks trans-activation mediated by the retinoic acid-related orphan receptor-{alpha} (ROR{alpha}). ROR{alpha} has been implicated in the regulation of high density lipoprotein cholesterol, lipid homeostasis, and inflammation. Reverb{beta} and ROR{alpha} are expressed in similar tissues, including skeletal muscle; however, the pathophysiological function of Rev-erb{beta} has remained obscure. We hypothesize from the similar expression patterns, target genes, and overlapping cognate sequences of these nuclear receptors that Rev-erb{beta} regulates lipid metabolism in skeletal muscle. This lean tissue accounts for >30% of total body weight and 50% of energy expenditure. Moreover, this metabolically demanding tissue is a primary site of glucose disposal, fatty acid oxidation, and cholesterol efflux. Consequently, muscle has a significant role in insulin sensitivity, obesity, and the blood-lipid profile. We utilize ectopic expression in skeletal muscle cells to understand the regulatory role of Rev-erb{beta} in this major mass peripheral tissue. Exogenous expression of a dominant negative version of mouse Rev-erb{beta} decreases the expression of many genes involved in fatty acid/lipid absorption (including Cd36, and Fabp-3 and -4). Interestingly, we observed a robust induction (>15-fold) in mRNA expression of interleukin-6, an "exercise-induced myokine" that regulates energy expenditure and inflammation. Furthermore, we observed the dramatic repression (>20-fold) of myostatin mRNA, another myokine that is a negative regulator of muscle hypertrophy and hyperplasia that impacts on body fat accumulation. This study implicates Rev-erb{beta} in the control of lipid and energy homoeostasis in skeletal muscle. In conclusion, we speculate that selective modulators of Rev-erb{beta} may have therapeutic utility in the treatment of dyslipidemia and regulation of muscle growth.

Rees, M. I., K. Harvey, et al. (2003). "Isoform Heterogeneity of the Human Gephyrin Gene (GPHN), Binding Domains to the Glycine Receptor, and Mutation Analysis in Hyperekplexia." J. Biol. Chem. **278**(27): 24688-24696.

http://www.jbc.org/cgi/content/abstract/278/27/24688

Gephyrin (GPHN) is an organizational protein that clusters and localizes the inhibitory glycine (GlyR) and GABAA receptors to the microtubular matrix of the neuronal postsynaptic membrane. Mice deficient in gephyrin develop a hereditary molybdenum cofactor deficiency and a neurological phenotype that mimics startle disease (hyperekplexia). This neuromotor disorder is associated with mutations in the GlyR {alpha}1 and {beta} subunit genes (GLRA1 and GLRB). Further genetic heterogeneity is suspected, and we hypothesized that patients lacking mutations in GLRA1 and GLRB might have mutations in the gephyrin gene (GPHN). In addition, we adopted a yeast two-hybrid screen, using the GlyR {beta} subunit intracellular loop as bait, in an attempt to identify further GlyR-interacting proteins implicated in hyperekplexia. Gephyrin cDNAs were isolated, and subsequent RT-PCR analysis from human tissues demonstrated the presence of five alternatively spliced GPHN exons concentrated in the central linker region of the gene. This region generated 11 distinct GPHN transcript isoforms, with 10 being specific to neuronal tissue. Mutation analysis of GPHN exons in hyperekplexia patients revealed a missense mutation (A28T) in one patient causing an amino acid substitution (N10Y). Functional testing demonstrated that GPHNN10Y does not disrupt GlyR-gephyrin interactions or collybistininduced cell-surface clustering. We provide evidence that GlyR-gephyrin binding is dependent on the presence of an intact C-terminal MoeA homology domain. Therefore, the N10Y mutation and alternative splicing of GPHN transcripts do not affect interactions with GlyRs but may affect other interactions with the cytoskeleton or gephyrin accessory proteins.

Reglier-Poupet, H., C. Frehel, et al. (2003). "Maturation of Lipoproteins by Type II Signal Peptidase Is Required for Phagosomal Escape of Listeria monocytogenes." J. Biol. Chem. **278**(49): 49469-49477.

http://www.jbc.org/cgi/content/abstract/278/49/49469

Lipoproteins of Gram-positive bacteria are involed in a broad range of functions such as substrate binding and transport, antibiotic resistance, cell signaling, or protein export and folding. Lipoproteins are also known to initiate both innate and adaptative immune responses. However, their role in the pathogenicity of intracellular microorganisms is yet poorly understood. In Listeria monocytogenes, a Gram-positive facultative intracellular human pathogen, surface proteins have important roles in the interactions of the microorganism with the host cells. Among the putative surface proteins of L. monocytogenes, lipoproteins constitute the largest family. Here, we addressed the role of the signal peptidase (SPase II), responsible for the maturation of lipoproteins in listerial pathogenesis. We identified a gene, lsp, encoding a SPase II in the genome of L. monocytogenes and constructed a {Delta}lsp chromosomal deletion mutant. The mutant strain fails to process several lipoproteins demonstrating that lsp encodes a genuine SPase II. This defect is accompanied by a reduced efficiency of phagosomal escape during infection of eucaryotic cells, and leads to an attenuated virulence. We show that lsp gene expression is strongly induced when bacteria are still entrapped inside phagosomes of infected macrophages. The data presented establish, thus, that maturation of lipoproteins is critical for efficient phagosomal escape of L. monocytogenes, a process temporally controlled by the regulation of Lsp production in infected cells.

Richer, J. K., B. M. Jacobsen, et al. (2002). "Differential Gene Regulation by the Two Progesterone Receptor Isoforms in Human Breast Cancer Cells." J. Biol. Chem. **277**(7): 5209-5218.

http://www.jbc.org/cgi/content/abstract/277/7/5209

The PR-A and PR-B isoforms of progesterone receptors (PR) have different physiological functions, and their ratio varies widely in breast cancers. To determine whether the two PR regulate different genes, we used human breast cancer cell lines engineered to express one or the other isoform. Cells were treated with progesterone in triplicate, time-separated experiments, allowing statistical analyses of microarray gene expression data. Of 94 progesterone-regulated genes, 65 are uniquely regulated by PR-B, 4 uniquely by PR-A, and only 25 by both. Almost half the genes encode proteins that are membrane-bound or involved in membrane-initiated signaling. We also find an important set of progesterone-regulated genes involved in mammary gland development and/or implicated in breast cancer. This first, large scale study of PR gene regulation has important implications for the measurement of PR in breast cancers and for the many clinical uses of synthetic progestins. It suggests that it is important to distinguish between the two isoforms in breast cancers and that isoform-specific genes can be used to screen for ligands that selectively modulate the activity of PR-A or PR-B. Additionally, use of natural target genes, rather than "consensus" response elements, for transcription studies should improve our understanding of steroid hormone action.

Robichaud, G. A., M. Nardini, et al. (2004). "Human Pax-5 C-terminal Isoforms Possess Distinct Transactivation Properties and Are Differentially Modulated in Normal and Malignant B Cells." J. Biol. Chem. **279**(48): 49956-49963.

http://www.jbc.org/cgi/content/abstract/279/48/49956

The transcription factor Pax-5 occupies a central role in B cell differentiation and has been implicated in the development of B cell lymphoma. The transcriptional activation function of Pax-5 requires an intact N-terminal DNA-binding domain and is strongly influenced by the C-terminal transactivation domain. We report the identification and characterization of five human Pax-5 isoforms, which occur through the alternative splicing of exons that encode for the C-terminal transactivation domain. These isoforms arise from the inclusion or exclusion of exon 7, exon 8, and/or exon 9. Three of the Pax-5 isoforms generate novel protein sequences rich in proline, serine, and threonine amino acids that are the hallmarks of transactivation domains. The Pax-5 isoforms are expressed in peripheral blood mononuclear cells, cancerous and non-cancerous B cell lines, as well as in primary B cell lymphoma tissue. Electrophoretic mobility shift assays demonstrate that the isoforms possess specific DNA binding activity and recognize the PAX-5 consensus binding sites. In reporter assays using the CD19 promoter, the transactivation properties of the various isoforms were significantly influenced by the changes in the C-terminal protein sequence. Finally, we demonstrate, for the first time, that human Pax-5 isoform expression is modulated by specific signaling pathways in B lymphocytes.

Rumberger, J. M., T. Wu, et al. (2003). "Role of Hexosamine Biosynthesis in Glucose-mediated Up-regulation of Lipogenic Enzyme mRNA Levels: EFFECTS OF GLUCOSE, GLUTAMINE, AND GLUCOSAMINE ON GLYCEROPHOSPHATE DEHYDROGENASE, FATTY ACID SYNTHASE, AND ACETYL-CoA CARBOXYLASE mRNA LEVELS." J. Biol. Chem. **278**(31): 28547-28552.

http://www.jbc.org/cgi/content/abstract/278/31/28547

Glucose uptake into adipose and liver cells is known to up-regulate mRNA levels for various lipogenic enzymes such as fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC). To determine whether the hexosamine biosynthesis pathway (HBP) mediates glucose regulation of mRNA expression, we treated primary cultured adipocytes for 18 h with insulin (25 ng/ml) and either glucose (20 mM) or glucosamine (2 mM). A ribonuclease protection assay was used to quantitate mRNA levels for FAS, ACC, and glycerol-3-P dehydrogenase (GPDH). Treatment with insulin and various concentrations of D-glucose increased mRNA levels for FAS (280%), ACC

(93%), and GPDH (633%) in a dose-dependent manner (ED50 8-16 mM). Mannose similarly elevated mRNA levels, but galactose and fructose were only partially effective. L-glucose had no effect. Omission of glutamine from the culture medium markedly diminished the stimulatory effect of glucose on mRNA expression. Since glutamine is a crucial amide donor in hexosamine biosynthesis, we interpret these data to mean that glucose flux through the HBP is linked to regulation of lipogenesis through control of gene expression. Further evidence for hexosamine regulation was obtained using glucosamine, which is readily transported into adipocytes where it directly enters the HBP. Glucosamine was 15-30 times more potent than glucose in elevating FAS, ACC, and GPDH mRNA levels (ED50 [~]0.5 mM). In summary: 1) GPDH, FAS, and ACC mRNA levels are upregulated by glucose; 2) glucose-induced up-regulation requires glutamine; and 3) mRNA levels for lipogenic enzymes are up-regulated by glucosamine. Hyperglycemia is the hallmark of diabetes mellitus and leads to insulin resistance, impaired glucose metabolism, and dyslipidemia. We postulate that disease pathophysiology may have a common underlying factor, excessive glucose flux through the HBP.

Sancho, R., N. Marquez, et al. (2004). "Imperatorin Inhibits HIV-1 Replication through an Sp1-dependent Pathway." J. Biol. Chem. **279**(36): 37349-37359.

http://www.jbc.org/cgi/content/abstract/279/36/37349

Coumarins and structurally related compounds have been recently shown to present anti-human immunodeficiency virus, type 1 (HIV-1) activity. Among them, the dietary furanocoumarin imperatorin is present in citrus fruits, in culinary herbs, and in some medicinal plants. In this study we report that imperatorin inhibits either vesicular stomatitis virus-pseudotyped or gp160-enveloped recombinant HIV-1 infection in several T cell lines and in HeLa cells. These recombinant viruses express luciferase as a marker of viral replication. Imperatorin did not inhibit the reverse transcription nor the integration steps in the viral cell cycle. Using several 5' long terminal repeat-HIV-1 constructs where critical response elements were either deleted or mutated, we found that the transcription factor Sp1 is critical for the inhibitory activity of imperatorin induced by both phorbol 12-myristate 13-acetate and HIV-1 Tat. Moreover in transient transfections imperatorin specifically inhibited phorbol 12-myristate 13-acetate-induced transcriptional activity of the Gal4-Sp1 fusion protein. Since Sp1 is also implicated in cell cycle progression we further studied the effect of imperatorin on cyclin D1 gene transcription and protein expression and in HeLa cell cycle progression. We found that imperatorin strongly inhibited cyclin D1 expression and arrested the cells at the G1 phase of the cell cycle. These results highlight the potential of Sp1 transcription factor as a target for natural anti-HIV-1 compounds such as furanocoumarins that might have a potential therapeutic role in the management of AIDS.

Satake, H., H. Y. Chen, et al. (2003). "Genes Modulated by Expression of GD3 Synthase in Chinese Hamster Ovary Cells. EVIDENCE THAT THE Tis21 GENE IS INVOLVED IN THE INDUCTION OF GD3 9-O-ACETYLATION." J. Biol. Chem. **278**(10): 7942-7948.

http://www.jbc.org/cgi/content/abstract/278/10/7942

9-O-Acetylation is a common sialic acid modification, expressed in a developmentally regulated and tissue/cell type-specific manner. The relevant 9-O-acetyltransferase(s) have not been isolated or cloned; nor have mechanisms for their regulation been elucidated. We previously showed that transfection of the GD3 synthase (ST8Sia-I) gene into Chinese hamster ovary (CHO)-K1 cells gave expression of not only the disialoganglioside GD3 but also 9-O-acetyl-GD3. We now use differential display PCR between wild type CHO-K1 cells and clones stably expressing GD3 synthase (CHO-GD3 cells) to detect any increased expression of other genes

and explore the possible induction of a 9-O-acetyltransferase. The four CHO mRNAs showing major up-regulation were homologous to VCAM-1, Tis21, the KC-protein-like protein, and a functionally unknown type II transmembrane protein. A moderate increase in expression of the FxC1 and SPR-1 genes was also seen. Interestingly, these are different from genes observed by others to be up-regulated after transfection of GD3 synthase into a neuroblastoma cell line. We also isolated a CHO-GD3 mutant lacking 9-O-acetyl-GD3 following chemical mutagenesis (CHO-GD3-OAc[-]). Analysis of the above differential display PCR-derived genes in these cells showed that expression of Tis21 was selectively reduced. Transfection of a mouse Tis21 cDNA into the CHO-GD3-OAc[-] mutant cells restored 9-O-acetyl-GD3 expression. Since the only major gangliosides expressed by CHO-GD3 cells are GD3 and 9-O-acetyl-GD3 (in addition to GM3, the predominant ganglioside type in wild-type CHO-K1 cells), we conclude that GD3 enhances its own 9-O-acetylation via induction of Tis21. This is the first known nuclear inducible factor for 9-O-acetylation and also the first proof that 9-O-acetylation can be directly regulated by GD3 synthase. Finally, transfection of CHO-GD3-OAc[-] mutant cells with ST6Gal-I induced 9-O-acetylation specifically on sialylated N-glycans, in a manner similar to wild-type cells. This indicates separate machineries for 9-O-acetylation on [alpha]2-8-linked sialic acids of gangliosides and on [alpha]2-6-linked sialic acids on N-glycans.

Schmidt, C., B. Li, et al. (2003). "Random Mutagenesis of the M3 Muscarinic Acetylcholine Receptor Expressed in Yeast: IDENTIFICATION OF POINT MUTATIONS THAT "SILENCE" A CONSTITUTIVELY ACTIVE MUTANT M3 RECEPTOR AND GREATLY IMPAIR RECEPTOR/G PROTEIN COUPLING." J. Biol. Chem. **278**(32): 30248-30260.

http://www.jbc.org/cgi/content/abstract/278/32/30248

The M3 muscarinic receptor is a prototypical member of the class I family of G protein-coupled receptors (GPCRs). To facilitate studies on the structural mechanisms governing M3 receptor activation, we generated an M3 receptor-expressing yeast strain (Saccharomyces cerevisiae) that requires agonist-dependent M3 receptor activation for cell growth. By using receptor random mutagenesis followed by a genetic screen in yeast, we initially identified a point mutation at the cytoplasmic end of transmembrane domain (TM) VI (Q490L) that led to robust agonist-independent M3 receptor signaling in both yeast and mammalian cells. To explore further the molecular mechanisms by which point mutations can render GPCRs constitutively active, we subjected a region of the Q490L mutant M3 receptor that included TM V-VII to random mutagenesis. We then applied a yeast genetic screen to identify second-site mutations that could suppress the activating effects of the Q490L mutation and restore wild-type receptor-like function to the Q490L mutant receptor. This analysis led to the identification of 12 point mutations that allowed the Q490L mutant receptor to function in a fashion similar to the wild-type receptor. These amino acid substitutions mapped to two distinct regions of the M3 receptor, the exofacial segments of TM V and VI and the cytoplasmic ends of TM V-VII. Strikingly, in the absence of the activating Q490L mutation, all recovered point mutations severely reduced the efficiency of receptor/G protein coupling, indicating that the targeted residues play important roles in receptor activation and/or receptor/G protein coupling. This strategy should be generally applicable to identify sites in GPCRs that are critically involved in receptor function.

Schwarzer, C., T. E. Machen, et al. (2004). "NADPH Oxidase-dependent Acid Production in Airway Epithelial Cells." J. Biol. Chem. **279**(35): 36454-36461.

http://www.jbc.org/cgi/content/abstract/279/35/36454

The purpose of this study was to determine the role of NADPH oxidase in H+ secretion by airway epithelia. In whole cell patch clamp recordings primary human tracheal epithelial cells (hTE) and

the human serous gland cell line Calu-3 expressed a functionally similar zincblockable plasma membrane H+ conductance. However, the rate of H+ secretion of confluent epithelial monolayers measured in Ussing chambers was 9-fold larger in hTE compared with Calu-3. In hTE H+ secretion was blocked by mucosal ZnCl2 and the NADPH oxidase blockers acetovanillone and 4-(2-aminoethyl)benzenesulfonyl fluoride (AEBSF), whereas these same blockers had no effect in Calu-3. We determined levels of transcripts for the NADPH oxidase transmembrane isoforms (Nox1 through -5, Duox1 and -2, and p22phox) and found Duox1, -2, and p22phox to be highly expressed in hTE, as well as the intracellular subunits p40phox, p47phox, and p67phox. In contrast, Calu-3 lacked transcripts for Duox1, p40phox, and p47phox. Anti-Duox antibody staining resulted in prominent apical staining in hTE but no significant staining in Calu-3. When treated with amiloride to block the Na+/H+ exchanger, intracellular pH in hTE acidified at significantly higher rates than in Calu-3, and treatment with AEBSF blocked acidification. These data suggest a role for an apically located Duox-based NADPH oxidase during intracellular H+ production and H+ secretion, but not in H+ conduction.

Smith, S. B., R. Gasa, et al. (2003). "Neurogenin3 and Hepatic Nuclear Factor 1 Cooperate in Activating Pancreatic Expression of Pax4." J. Biol. Chem. **278**(40): 38254-38259.

http://www.jbc.org/cgi/content/abstract/278/40/38254

During fetal development, paired/homeodomain transcription factor Pax4 controls the formation of the insulin-producing {beta} cells and the somatostatin-producing {delta} cells in the islets of Langerhans in the pancreas. Targeting of Pax4 expression to the islet lineage in the fetal pancreas depends on a short sequence located [~]2 kb upstream of the transcription initiation site of the PAX4 gene. This short sequence contains binding sites for homeodomain transcription factors PDX1 and hepatic nuclear factor (HNF)1, nuclear receptor HNF4{alpha}, and basic helix-loop-helix factor Neurogenin3. In the current study we demonstrate that the HNF1{alpha} and Neurogenin3 binding sites are critical for activity of the region through synergy between the two proteins. Synergy involves a physical interaction between the factors and requires the activation domains of both factors. Furthermore, exogenous expression of Neurogenin3 is sufficient to induce expression of the endogenous pax4 gene in the mouse pancreatic ductal cell line mPAC, which already expresses HNF1{alpha}, whereas expression of both Neurogenin3 and HNF1{alpha} are necessary to activate the pax4 gene in the fibroblast cell line NIH3T3. These data demonstrate how Neurogenin3 and HNF1{alpha} activate the pax4 gene during the cascade of gene expression events that control pancreatic endocrine cell development.

Sorger, D., K. Athenstaedt, et al. (2004). "A Yeast Strain Lacking Lipid Particles Bears a Defect in Ergosterol Formation." J. Biol. Chem. **279**(30): 31190-31196.

http://www.jbc.org/cgi/content/abstract/279/30/31190

Lipid particles of the yeast Saccharomyces cerevisiae are storage compartments for triacylglycerols (TAG) and steryl esters (STE). Four gene products, namely the TAG synthases Dga1p and Lro1p, and the STE synthases Are1p and Are2p contribute to storage lipid synthesis. A yeast strain lacking the four respective genes is devoid of lipid particles thus providing a valuable tool to study the physiological role of storage lipids and lipid particles. Using a dga1lro1are1are2 quadruple mutant transformed with plasmids bearing inducible DGA1, LRO1, or ARE2 we demonstrate that TAG synthesis contributes more efficiently to lipid particle proliferation than synthesis of STE. Moreover, we show that proteins typically located to lipid particles in wild type such as Erg1p, Erg6p, Erg7p, and Ayr1p are refined to microsomal fractions of the dga1lro1are1are2 quadruple mutant. This result confirms the close relationship between lipid particles and endoplasmic reticulum. Most interestingly, the amount of the squalene

epoxidase Erg1p, which is dually located in lipid particles and endoplasmic reticulum of wild type, is decreased in the quadruple mutant, whereas amounts of other lipid particle proteins tested were not reduced. This decrease is not caused by down-regulation of ERG1 transcription but by the low stability of Erg1p in the quadruple mutant. Because a similar effect was also observed in are1are2 mutants this finding can be mainly attributed to the lack of STE. The quadruple mutant, however, was more sensitive to terbinafine, an inhibitor of Erg1p, than the are1are2 strain suggesting that the presence of TAG and/or intact lipid particles has an additional protective effect. In a strain lacking the two STE synthases, Are1p and Are2p, incorporation of ergosterol into the plasma membrane was reduced, although the total cellular amount of free ergosterol was higher in the mutant than in wild type. Thus, an esterification/deacylation mechanism appears to contribute to the supply of ergosterol to the plasma membrane.

Tabuchi, A., H. Sakaya, et al. (2002). "Involvement of an Upstream Stimulatory Factor as Well as cAMP-responsive Element-binding Protein in the Activation of Brain-derived Neurotrophic Factor Gene Promoter I." J. Biol. Chem. **277**(39): 35920-35931.

http://www.jbc.org/cgi/content/abstract/277/39/35920

The use of different brain-derived neurotrophic factor (BDNF) gene promoters results in the differential production of 5'-alternative transcripts, suggesting versatile functions of BDNF in neurons. Among four BDNF promoters I, II, III, and IV (BDNF-PI, -PII, -PIII, and -PIV), BDNF-PI was markedly activated, as well as BDNF-PIII, by Ca2+ signals evoked via neuronal activity. However, little is known about the mechanisms for the transcriptional activation of BDNF-PI. Using rat cortical neurons in culture, we assigned the promoter sequences responsible for the Ca2+ signal-mediated activation of BDNF-PI and found that the Ca2+-responsive elements were located in two separate (distal and proximal) regions and that the DNA sequences in the proximal region containing cAMP-responsive element (CRE), which is overlapped by the upstream stimulatory factor (USF)-binding element, were largely responsible for the activation of BDNF-PI. CRE-binding protein (CREB) family transcription factors and USF1/USF2 bind to this overlapping site, depending upon their preferred sequences which also control the magnitude of the activation. Overexpression of dominant negative CREB or USF reduced the BDNF-PI activation. These findings support that not only CREB but also USF1/USF2 contributes to Ca2+ signal-mediated activation of BDNF-PI through the recognition of an overlapping CRE and USF-binding element.

Tagami, S., J.-i. Inokuchi, et al. (2002). "Ganglioside GM3 Participates in the Pathological Conditions of Insulin Resistance." J. Biol. Chem. **277**(5): 3085-3092.

http://www.jbc.org/cgi/content/abstract/277/5/3085

Gangliosides are known as modulators of transmembrane signaling by regulating various receptor functions. We have found that insulin resistance induced by tumor necrosis factor-[alpha] (TNF-[alpha]) in 3T3-L1 adipocytes was accompanied by increased GM3 ganglioside expression caused by elevating GM3 synthase activity and its mRNA. We also demonstrated that TNF-[alpha] simultaneously produced insulin resistance by uncoupling insulin receptor activity toward insulin receptor substrate-1 (IRS-1) and suppressing insulin-sensitive glucose transport. Pharmacological depletion of GM3 in adipocytes by an inhibitor of glucosylceramide synthase prevented the TNF-[alpha]-induced defect in insulin-dependent tyrosine phosphorylation of IRS-1 and also counteracted the TNF-[alpha]-induced serine phosphorylation of IRS-1. Moreover, when the adipocytes were incubated with exogenous GM3, suppression of tyrosine phosphorylation of insulin receptor and IRS-1 and glucose uptake in response to insulin stimulation was observed, demonstrating that GM3 itself is able to mimic the effects of TNF on insulin signaling. We used

the obese Zucker fa/fa rat and ob/ob mouse, which are known to overproduce TNF-[alpha] mRNA in adipose tissues, as typical models of insulin resistance. We found that the levels of GM3 synthase mRNA in adipose tissues of these animals were significantly higher than in their lean counterparts. Taken together, the increased synthesis of cellular GM3 by TNF may participate in the pathological conditions of insulin resistance in type 2 diabetes.

Tang, S. and Z.-M. Zheng (2002). "Kaposi's Sarcoma-associated Herpesvirus K8 Exon 3 Contains Three 5'-Splice Sites and Harbors a K8.1 Transcription Start Site." J. Biol. Chem. **277**(17): 14547-14556.

http://www.jbc.org/cgi/content/abstract/277/17/14547

Kaposi's sarcoma-associated herpesvirus (KSHV) K8 and K8.1 open reading frames are juxtaposed and span from nucleotide (nt) 74850 to 76695 of the virus genome. A K8 pre-mRNA overlaps the entire K8.1 coding region, and alternative splicing of KSHV K8 and K8.1 pre-mRNAs each produces three isoforms ([alpha], [beta], and [gamma]) of the mRNAs. We have mapped the 5' end of the K8.1 RNA in butyrate-induced KSHV-positive JSC-1 cells to nt 75901 in the KSHV genome and have shown that exon 3 of the K8 pre-mRNA in JSC-1 cells covers most part of the intron 3 defined previously and has three 5'-splice sites (ss), respectively, at nt 75838, 76155, and 76338. Selection of the nt 75838 5'-ss dictates the K8 mRNA production and overwhelms the RNA processing. Alternative selection of other two 5'-ss is feasible and leads to production of two additional bicistronic mRNAs, K8/K8.1[alpha] and -[beta]. However, the novel bicistronic K8/K8.1 mRNAs translated a little K8 and no detectable K8.1 proteins in 293 cells. Data suggest that production of the K8/K8.1 mRNAs may be an essential way to control K8 mRNAs, especially K8[alpha], to a threshold at RNA processing level.

Tanner, J. A., R. M. Watt, et al. (2003). "The Severe Acute Respiratory Syndrome (SARS) Coronavirus NTPase/Helicase Belongs to a Distinct Class of 5' to 3' Viral Helicases." J. Biol. Chem. **278**(41): 39578-39582.

http://www.jbc.org/cgi/content/abstract/278/41/39578

The putative NTPase/helicase protein from severe acute respiratory syndrome coronavirus (SARS-CoV) is postulated to play a number of crucial roles in the viral life cycle, making it an attractive target for anti-SARS therapy. We have cloned, expressed, and purified this protein as an N-terminal hexahistidine fusion in Escherichia coli and have characterized its helicase and NTPase activities. The enzyme unwinds double-stranded DNA, dependent on the presence of a 5' single-stranded overhang, indicating a 5'o 3' polarity of activity, a distinct characteristic of coronaviridae helicases. We provide the first quantitative analysis of the polynucleic acid binding and NTPase activities of a Nidovirus helicase, using a high throughput phosphate release assay that will be readily adaptable to the future testing of helicase inhibitors. All eight common NTPs and dNTPs were hydrolyzed by the SARS helicase in a magnesium-dependent reaction, stimulated by the presence of either single-stranded DNA or RNA. The enzyme exhibited a preference for ATP, dATP, and dCTP over the other NTP/dNTP substrates. Homopolynucleotides significantly stimulated the ATPase activity (15-25-fold) with the notable exception of poly(G) and poly(dG), which were non-stimulatory. We found a large variation in the apparent strength of binding of different homopolynucleotides, with dT24 binding over 10 times more strongly than dA24 as observed by the apparent Km.

Tardif, K. D., K. Mori, et al. (2004). "Hepatitis C Virus Suppresses the IRE1-XBP1 Pathway of the Unfolded Protein Response." J. Biol. Chem. **279**(17): 17158-17164.

http://www.jbc.org/cgi/content/abstract/279/17/17158

Hepatitis C virus (HCV) gene expression disrupts normal endoplasmic reticulum (ER) functions and induces ER stress. ER stress results from the accumulation of unfolded or misfolded proteins in the ER; cells can alleviate this stress by degrading or refolding these proteins. The IRE1-XBP1 pathway directs both protein refolding and degradation in response to ER stress. Like IRE1-XBP1, other branches of the ER stress response mediate protein refolding. However, IRE1-XBP1 can also specifically activate protein degradation. We show here that XBP1 expression is elevated in cells carrying HCV subgenomic replicons, but XBP1 trans-activating activity is repressed. This prevents the IRE1-XBP1 transcriptional induction of EDEM (ER degradation-enhancing {alpha}-mannosidase-like protein). The mRNA expression of EDEM is required for the degradation of misfolded proteins. Consequently, misfolded proteins are stable in cells expressing HCV replicons. HCV may suppress the IRE1-XBP1 pathway to stimulate the synthesis of its viral proteins. IRE1{alpha}-null MEFs, a cell line with a defective IRE1-XBP1 pathway, show elevated levels of HCV IRES-mediated translation. Therefore, HCV may suppress the IRE1-XBP1 pathway to not only promote HCV expression but also to contribute to the persistence of the virus in infected hepatocytes.

Tian, B., D. E. Nowak, et al. (2005). "Identification of Direct Genomic Targets Downstream of the Nuclear Factor-{kappa}B Transcription Factor Mediating Tumor Necrosis Factor Signaling." J. Biol. Chem. **280**(17): 17435-17448.

http://www.jbc.org/cgi/content/abstract/280/17/17435

Tumor necrosis factor (TNF) is a pro-inflammatory cytokine that controls expression of inflammatory genetic networks. Although the nuclear factor-{kappa}B (NF-{kappa}B) pathway is crucial for mediating cellular TNF responses, the complete spectrum of NF-{kappa}B-dependent genes is unknown. In this study, we used a tetracycline-regulated cell line expressing an NF-{kappa}B inhibitor to systematically identify NF-{kappa}B-dependent genes. A microarray data set generated from a time course of TNF stimulation in the presence or absence of NF-{kappa}B signaling was analyzed. We identified 50 unique genes that were regulated by TNF (Pr(F) <0.001) and demonstrated a change in signal intensity of {+/-} 3-fold relative to control. Of these, 28 were NF-{kappa}B-dependent, encoding proteins involved in diverse cellular activities. Quantitative real-time PCR assays of eight characterized NF-{kappa}B-dependent genes and five genes not previously known to be NF-{kappa}B-dependent (Gro-{beta} and-{gamma}, I{kappa}B{epsilon}, interleukin (IL)-7R, and Naf-1) were used to determine whether they were directly or indirectly NF-{kappa}B regulated. Expression of constitutively active enhanced green fluorescent{middle dot}NF-{kappa}B/Rel A fusion protein transactivated all but IL-6 and IL-7R in the absence of TNF stimulation. Moreover, TNF strongly induced all 12 genes in the absence of new protein synthesis. High probability NF-{kappa}B sites in novel genes were predicted by binding site analysis and confirmed by electrophoretic mobility shift assay. Chromatin immunoprecipitation assays show the endogenous I{kappa}B{alpha}/{epsilon}, Gro-{beta}/{gamma}, and Naf-1 promoters directly bound NF-{kappa}B/Rel A in TNF-stimulated cells. Together, these studies systematically identify the direct NF-{kappa}B-dependent gene network downstream of TNF signaling, extending our knowledge of biological processes regulated by this pathway.

Torres, B., G. Porras, et al. (2003). "Regulation of the mhp Cluster Responsible for 3-(3-Hydroxyphenyl)propionic Acid Degradation in Escherichia coli." J. Biol. Chem. **278**(30): 27575-

27585.

http://www.jbc.org/cgi/content/abstract/278/30/27575

The mhp gene cluster from Escherichia coli constitutes a model system to study bacterial degradation of 3-(3-hydroxyphenyl)propionic acid (3HPP). In this work the regulation of the inducible mhp catabolic genes has been studied by genetic and biochemical approaches. The Pr and Pa promoters, which control the expression of the divergently transcribed mhpR regulatory gene and mhp catabolic genes, respectively, show a peculiar arrangement leading to transcripts that are complementary at their 5'-ends. By using Pr-lacZ and Pa-lacZ translational fusions and gel retardation assays, we have shown that the mhpR gene product behaves as a 3HPP-dependent activator of the Pa promoter, being the expression from Pr constitutive and MhpR-independent. DNase I footprinting experiments and mutational analysis mapped an MhpR-protected region, centered at position -58 with respect to the Pa transcription start site, which is indispensable for MhpR binding and in vivo activation of the Pa promoter. Superimposed in the specific MhpR-mediated regulation of the Pa promoter, we have observed a strict catabolite repression control carried out by the cAMP receptor protein (CRP) that allows expression of the mhp catabolic genes when the preferred carbon source (glucose) is not available and 3HPP is present in the medium. Gel retardation assays revealed that the specific activator, MhpR, is essential for the binding of the second activator, CRP, to the Pa promoter. Such peculiar synergistic transcription activation has not yet been observed in other aromatic catabolic pathways, and the MhpR activator becomes the first member of the IclR family of transcriptional regulators that is indispensable for recruiting CRP to the target promoter.

Tremper-Wells, B. and M. L. Vallano (2005). "Nuclear Calpain Regulates Ca2+-dependent Signaling via Proteolysis of Nuclear Ca2+/Calmodulin-dependent Protein Kinase Type IV in Cultured Neurons." J. Biol. Chem. **280**(3): 2165-2175.

http://www.jbc.org/cgi/content/abstract/280/3/2165

Accumulating evidence indicates that calpains can reside in or translocate to the cell nucleus, but their functions in this compartment remain poorly understood. Dissociated cultures of cerebellar granule cells (GCs) demonstrate improved long-term survival when their growth medium is supplemented with depolarizing agents that stimulate Ca2+ influx and activate calmodulin-dependent signaling cascades, notably 20 mM KCl. We previously observed Ca2+-dependent down-regulation of Ca2+/calmodulin-dependent protein kinase (CaMK) type IV, which was attenuated by calpain inhibitors, in GCs supplemented with 20 mM KCl (Tremper-Wells, B., Mathur, A., Beaman-Hall, C. M., and Vallano, M. L. (2002) J. Neurochem. 81, 314-324). CaMKIV is highly enriched in the nucleus and thought to be critical for improved survival. Here, we demonstrate by immunolocalization/confocal microscopy and subcellular fractionation that the regulatory and catalytic subunits of m-calpain are enriched in GC nuclei, including GCs grown in medium containing 5 mM KCl. Calpain-mediated proteolysis of CaMKIV is selective, as several other nuclear and non-nuclear calpain substrates were not degraded under chronic depolarizing culture conditions. Depolarization and Ca2+-dependent down-regulation of CaMKIV were associated with significant alterations in other components of the Ca2+-CaMKIV signaling cascade: the ratio of phosphorylated to total cAMP response element-binding protein (a downstream CaMKIV substrate) was reduced by [~]10-fold, and the amount of CaMK kinase (an upstream activator of CaMKIV) protein and mRNA was significantly reduced. We hypothesize that calpain-mediated CaMKIV proteolysis is an autoregulatory feedback response to sustained activation of a Ca2+-CaMKIV signaling pathway, resulting from growth of cultures in medium containing 25 mM KCl. This study establishes nuclear m-calpain as a regulator of CaMKIV and associated signaling molecules under conditions of sustained Ca2+ influx.

Tuli, R., S. Tuli, et al. (2003). "Transforming Growth Factor-{beta}-mediated Chondrogenesis of Human Mesenchymal Progenitor Cells Involves N-cadherin and Mitogen-activated Protein Kinase and Wnt Signaling Cross-talk." J. Biol. Chem. **278**(42): 41227-41236.

http://www.jbc.org/cgi/content/abstract/278/42/41227

The multilineage differentiation potential of adult tissue-derived mesenchymal progenitor cells (MPCs), such as those from bone marrow and trabecular bone, makes them a useful model to investigate mechanisms regulating tissue development and regeneration, such as cartilage. Treatment with transforming growth factor-{beta} (TGF-{beta}) superfamily members is a key requirement for the in vitro chondrogenic differentiation of MPCs. Intracellular signaling cascades, particularly those involving the mitogen-activated protein (MAP) kinases, p38, ERK-1, and JNK, have been shown to be activated by TGF-{beta}s in promoting cartilage-specific gene expression. MPC chondrogenesis in vitro also requires high cell seeding density, reminiscent of the cellular condensation requirements for embryonic mesenchymal chondrogenesis, suggesting common chondro-regulatory mechanisms. Prompted by recent findings of the crucial role of the cell adhesion protein, N-cadherin, and Wnt signaling in condensation and chondrogenesis, we have examined here their involvement, as well as MAP kinase signaling, in TGF-{beta}1-induced chondrogenesis of trabecular bone-derived MPCs. Our results showed that TGF-{beta}1 treatment initiates and maintains chondrogenesis of MPCs through the differential chondro-stimulatory activities of p38, ERK-1, and to a lesser extent, JNK. This regulation of MPC chondrogenic differentiation by the MAP kinases involves the modulation of N-cadherin expression levels, thereby likely controlling condensation-like cell-cell interaction and progression to chondrogenic differentiation, by the sequential up-regulation and progressive down-regulation of N-cadherin. TGF-{beta}1-mediated MAP kinase activation also controls WNT-7A gene expression and Wnt-mediated signaling through the intracellular {beta}-catenin-TCF pathway, which likely regulates N-cadherin expression and subsequent N-cadherin-mediated cell-adhesion complexes during the early steps of MPC chondrogenesis.

Tynan, F. E., N. A. Borg, et al. (2005). "The high resolution structures of highly bulged viral epitopes bound to the major histocompatability class I: Implications for T-cell receptor engagement and T-cell immunodominance." J. Biol. Chem.: M503060200.

http://www.jbc.org/cgi/content/abstract/M503060200v1

Although HLA class I alleles can bind epitopes up to 14 amino acids in length, little is known about the immunogenicity or the responding T-cell repertoire against such determinants. Here we describe a HLA-B*3508-restricted CTL response to a 13-mer viral epitope (LPEPLPQGQLTAY). The rigid, centrally-bulged epitope generates a biased T-cell response. Only the N-terminal face of the peptide bulge is critical for recognition by the dominant clonotype, SB27. The SB27 public TcR associates slowly onto the bulged pMHC complex, suggesting significant remodelling upon engagement. The broad antigen binding cleft of HLA-B*3508 represents a critical feature for engagement of the public TcR, as the narrower binding cleft of HLA-B*3501LPEPLPQGQLTAY, which differs from HLA-B*3508 by a single amino acid polymorphism (Arg 156'Leu), interacts poorly with the dominant TcR. Biased TcR usage in this CTL response appears to reflect a dominant role of the prominent pMHC-I surface.

Uchimura, K., K. Kadomatsu, et al. (2002). "Functional Analysis of the Chondroitin 6-Sulfotransferase Gene in Relation to Lymphocyte Subpopulations, Brain Development, and Oversulfated Chondroitin Sulfates." J. Biol. Chem. **277**(2): 1443-1450.

http://www.jbc.org/cgi/content/abstract/277/2/1443

Chondroitin 6-sulfotransferase (C6ST) catalyzes the transfer of sulfate to position 6 of the N-acetylgalactosamine residue of chondroitin. To obtain direct evidence regarding the function of C6ST and its product, chondroitin 6-sulfate, in vivo, we isolated the mouse C6ST gene (C6st) and generated mice deficient in this gene (C6st[-]/[-]) by embryonic stem cell technology. C6st[-]/[-] mice were born at approximately the expected frequency and were viable through adulthood. In the spleen of C6st[-]/[-] mice, the level of chondroitin 6-sulfate became almost undetectable. Analyses of these knockout mice provided insights into the biosynthesis of oversulfated chondroitin sulfates in mice; chondroitin sulfate D in the brain of null mice and the cartilage and telencephalon of null embryos disappeared, whereas the chondroitin sulfate E level in the spleen and brain of the null mice was unchanged. Despite the disappearance of chondroitin sulfate D structure, brain development was normal in the C6st[-]/[-] mice. Further analysis revealed that the number of CD62L+CD44low T lymphocytes corresponding to naive T lymphocytes in the spleen of 5-6-week-old C6st[-]/[-] mice was significantly decreased, whereas those in other secondary lymphoid organs were unchanged. This finding suggested that chondroitin 6-sulfate plays a role in the maintenance of naive T lymphocytes in the spleen of young mice.

Unnikrishnan, I., S. Miller, et al. (2003). "Multiple Positive and Negative Elements Involved in the Regulation of Expression of GSY1 in Saccharomyces cerevisiae." J. Biol. Chem. **278**(29): 26450-26457.

http://www.jbc.org/cgi/content/abstract/278/29/26450

GSY1 is one of the two genes encoding glycogen synthase in Saccharomyces cerevisiae. Both the GSY1 message and the protein levels increased as cells approached stationary phase. A combination of deletion analysis and site-directed mutagenesis revealed a complex promoter containing multiple positive and negative regulatory elements. Expression of GSY1 was dependent upon the presence of a TATA box and two stress response elements (STREs). Expression was repressed by Mig1, which mediates responses to glucose, and Rox1, which mediates responses to oxygen. Characterization of the GSY1 promoter also revealed a novel negative element. This element, N1, can repress expression driven by either an STRE or a heterologous element, the UAS of CYC1. Repression by N1 is dependent on the number of these elements that are present, but is independent of their orientation. N1 repressed expression when placed either upstream or downstream of the UAS, although the latter position is more effective. Gel shift analysis detected a factor that appears to bind to the N1 element. The complexity of the GSY1 promoter, which includes two STREs and three distinct negative elements, was surprising. This complexity may allow GSY1 to respond to a wide range of environmental stresses.

Valcourt, U., J. Gouttenoire, et al. (2002). "Functions of Transforming Growth Factor-beta Family Type I Receptors and Smad Proteins in the Hypertrophic Maturation and Osteoblastic Differentiation of Chondrocytes." J. Biol. Chem. **277**(37): 33545-33558.

http://www.jbc.org/cgi/content/abstract/277/37/33545

We investigated the effects of bone morphogenetic protein (BMP)-2, a member of the transforming growth factor-[beta] superfamily, on the regulation of the chondrocyte phenotype, and we identified signaling molecules involved in this regulation. BMP-2 triggers three concomitant responses in mouse primary chondrocytes and chondrocytic MC615 cells. First, BMP-2 stimulates expression or synthesis of type II collagen. Second, BMP-2 induces expression of molecular markers characteristic of pre- and hypertrophic chondrocytes, such as Indian

hedgehog, parathyroid hormone/parathyroid hormone-related peptide receptor, type X collagen, and alkaline phosphatase. Third, BMP-2 induces osteocalcin expression, a specific trait of osteoblasts. Constitutively active forms of transforming growth factor-[beta] family type I receptors and Smad proteins were overexpressed to address their role in this process. Activin receptor-like kinase (ALK)-1, ALK-2, ALK-3, and ALK-6 were able to reproduce the hypertrophic maturation of chondrocytes induced by BMP-2. In addition, ALK-2 mimicked further the osteoblastic differentiation of chondrocytes induced by BMP-2. In the presence of BMP-2, Smad1, Smad5, and Smad8 potentiated the hypertrophic maturation of chondrocytes, but failed to induce osteocalcin expression. Smad6 and Smad7 impaired chondrocytic expression and osteoblastic differentiation induced by BMP-2. Thus, our results indicate that Smad-mediated pathways are essential for the regulation of the different steps of chondrocyte and osteoblast differentiation and suggest that additional Smad-independent pathways might be activated by ALK-2.

Valineva, T., J. Yang, et al. (2005). "The Transcriptional Co-activator Protein p100 Recruits Histone Acetyltransferase Activity to STAT6 and Mediates Interaction between the CREB-binding Protein and STAT6." J. Biol. Chem. **280**(15): 14989-14996.

http://www.jbc.org/cgi/content/abstract/280/15/14989

STAT6 is a critical regulator of transcription for interleukin-4 (IL-4)-induced genes. Activation of gene expression involves recruitment of coactivator proteins that function as bridging factors connecting sequence-specific transcription factors to the basal transcription machinery, and as chromatin-modifying enzymes. Coactivator proteins CBP/p300 have been implicated in regulation of transcription in all STATs. CBP is also required for STAT6-mediated gene activation, but the underlying molecular mechanisms are still elusive. In this study we investigated the mechanisms by which STAT6 recruits CBP and chromatin-modifying activities to the promoter. Our results indicate that while STAT1-interacted directly with CBP, the interaction between STAT6 and CBP was found to be mediated through p100 protein, a coactivator protein that has previously been shown to stimulate the transcription of IL-4-induced genes. The staphylococcal nuclease-like (SN)-domains of p100 directly interacted with amino acids 1099-1758 of CBP, while p100 did not associate with SRC-1, another coactivator of STAT6. p100 was found to recruit histone acetyltransferase (HAT) activity to STAT6 in vivo. Chromatin immunoprecipitation studies demonstrated that p100 increases the STAT6-p100-CBP ternary complex formation in the human Ig{epsilon} promoter. p100 also increased the amount of acetylated histone H4 at the Ig{epsilon} promoter, and siRNAs directed against p100 effectively inhibited Ig{epsilon} reporter gene expression. Our results suggest that p100 has an important role in the assembly of STAT6 transcriptosome, and that p100 stimulates IL-4-dependent transcription by mediating interaction between STAT6 and CBP and recruiting chromatin modifying activities to STAT6-responsive promoters.

van der Slot, A. J., A.-M. Zuurmond, et al. (2003). "Identification of PLOD2 as Telopeptide Lysyl Hydroxylase, an Important Enzyme in Fibrosis." J. Biol. Chem. **278**(42): 40967-40972.

http://www.jbc.org/cgi/content/abstract/278/42/40967

The hallmark of fibrotic processes is an excessive accumulation of collagen. The deposited collagen shows an increase in pyridinoline cross-links, which are derived from hydroxylated lysine residues within the telopeptides. This change in cross-linking is related to irreversible accumulation of collagen in fibrotic tissues. The increase in pyridinoline cross-links is likely to be the result of increased activity of the enzyme responsible for the hydroxylation of the telopeptides (telopeptide lysyl hydroxylase, or TLH). Although the existence of TLH has been postulated, the gene encoding TLH has not been identified. By analyzing the genetic defect of Bruck syndrome,

which is characterized by a pyridinoline deficiency in bone collagen, we found two missense mutations in exon 17 of PLOD2, thereby identifying PLOD2 as a putative TLH gene. Subsequently, we investigated fibroblasts derived from fibrotic skin of systemic sclerosis (SSc) patients and found that PLOD2 mRNA is highly increased indeed. Furthermore, increased pyridinoline cross-link levels were found in the matrix deposited by SSc fibroblasts, demonstrating a clear link between mRNA levels of the putative TLH gene (PLOD2) and the hydroxylation of lysine residues within the telopeptides. These data underscore the significance of PLOD2 in fibrotic processes.

Vanden Abeele, F., M. Roudbaraki, et al. (2003). "Store-operated Ca2+ Current in Prostate Cancer Epithelial Cells. ROLE OF ENDOGENOUS Ca2+ TRANSPORTER TYPE 1." J. Biol. Chem. **278**(17): 15381-15389.

http://www.jbc.org/cgi/content/abstract/278/17/15381

Ca2+ influx via store-operated channels (SOCs) following stimulation of the plasma membrane receptors is the key event controlling numerous processes in nonexcitable cells. The human transient receptor potential vanilloid type 6 channel, originally termed Ca2+ transporter type 1 (CaT1) protein, is one of the promising candidates for the role of endogenous SOC, although investigations of its functions have generated considerable controversy. In order to assess the role of CaT1 in generating endogenous store-operated Ca2+ current (ISOC) in the lymph node carcinoma of the prostate (LNCaP) human prostate cancer epithelial cell line, we manipulated its endogenous levels by means of antisense hybrid depletion or pharmacological up-regulation (antiandrogen treatment) combined with functional evaluation of ISOC. Antisense hybrid depletion of CaT1 decreased ISOC in LNCaP cells by ~50%, whereas enhancement of CaT1 levels by 60% in response to Casodex treatment potentiated ISOC by 30%. The functional characteristics of ISOC in LNCaP cells were similar in many respects to those reported for heterologously expressed CaT1, although 2-aminoethoxydiphenyl borate sensitivity and lack of constitutive current highlighted notable departures. Our results suggest that CaT1 is definitely involved in ISOC, but it may constitute only a part of the endogenous SOC, which in general may be a heteromultimeric channel composed of homologous CaT1 and other transient receptor potential subunits.

Vannahme, C., N. Smyth, et al. (2002). "Characterization of SMOC-1, a Novel Modular Calcium-binding Protein in Basement Membranes." J. Biol. Chem. **277**(41): 37977-37986.

http://www.jbc.org/cgi/content/abstract/277/41/37977

We have isolated the novel gene SMOC-1 that encodes a secreted modular protein containing an EF-hand calcium-binding domain homologous to that in BM-40. It further consists of two thyroglobulin-like domains, a follistatin-like domain and a novel domain. Recombinant expression in human cells showed that SMOC-1 is a glycoprotein with a calcium-dependent conformation. Results from Northern blots, reverse transcriptase-PCR, and immunoblots revealed a widespread expression in many tissues. Immunofluorescence studies with an antiserum directed against recombinant human SMOC-1 demonstrated a basement membrane localization of the protein and additionally its presence in other extracellular matrices. Immunogold electron microscopy confirmed the localization of SMOC-1 within basement membranes in kidney and skeletal muscle as well as its expression in the zona pellucida surrounding the oocyte.

Vargas, M. R., M. Pehar, et al. (2005). "Fibroblast growth factor-1 induces heme oxygenase-1 via nuclear factor erythroid 2-related factor 2 (Nrf2) in spinal cord astrocytes: Consequences for motor neuron survival." J. Biol. Chem.: M501920200.

http://www.jbc.org/cgi/content/abstract/M501920200v1

Fibroblast growth factor-1 (FGF-1) is highly expressed in motor neurons and can be released in response to sub-lethal cell injury. Because FGF-1 potently activates astroglia and exerts a direct neuroprotection after spinal cord injury or axotomy, we examined whether it regulated the expression of inducible and cytoprotective heme oxygenase-1 (HO-1) enzyme in astrocytes. FGF-1 induced the expression of HO-1 in cultured rat spinal cord astrocytes, which was dependent on FGF receptor activation and prevented by cycloheximide. FGF-1 also induced Nrf2 mRNA and protein levels and prompted its nuclear translocation. HO-1 induction was abolished by transfection of astrocytes with a dominant-negative mutant Nrf2, indicating that FGF-1 regulates HO-1 expression through Nrf2. FGF-1 also modified the expression of other antioxidant genes regulated by Nrf2. Both Nrf2 and HO-1 levels were increased and co-localized with reactive astrocytes in the degenerating lumbar spinal cord of rats expressing the amyotrophic lateral sclerosis (ALS)-linked SOD1 G93A mutation. Overexpression of Nrf2 in astrocytes increased survival of co-cultured embryonic motor neurons and prevented motor neuron apoptosis mediated by nerve growth factor through p75 neurotrophin receptor. Taken together, these results emphasize the key role of astrocytes in determining motor neuron fate in ALS.

Wang, G., A. Woods, et al. (2004). "RhoA/ROCK Signaling Suppresses Hypertrophic Chondrocyte Differentiation." J. Biol. Chem. **279**(13): 13205-13214.

http://www.jbc.org/cgi/content/abstract/279/13/13205

Coordinated proliferation and differentiation of growth plate chondrocytes is required for normal growth and development of the endochondral skeleton, but little is known about the intracellular signal transduction pathways regulating these processes. We have investigated the roles of the GTPase RhoA and its effector kinases ROCK1/2 in hypertrophic chondrocyte differentiation. RhoA, ROCK1, and ROCK2 are expressed throughout chondrogenic differentiation. RhoA overexpression in chondrogenic ATDC5 cells results in increased proliferation and a marked delay of hypertrophic differentiation, as shown by decreased induction of alkaline phosphatase activity, mineralization, and expression of the hypertrophic markers collagen X, bone sialoprotein, and matrix metalloproteinase 13. These effects are accompanied by activation of cyclin D1 transcription and repression of the collagen X promoter by RhoA. In contrast, inhibition of Rho/ROCK signaling by the pharmacological inhibitor Y27632 inhibits chondrocyte proliferation and accelerates hypertrophic differentiation. Dominant-negative RhoA also inhibits induction of the cyclin D1 promoter by parathyroid hormone-related peptide. Finally, Y27632 treatment partially rescues the effects of RhoA overexpression. In summary, we identify the RhoA/ROCK signaling pathway as a novel and important regulator of chondrocyte proliferation and differentiation.

Wang, X.-D., J. Shou, et al. (2004). "Notch1-expressing Cells Are Indispensable for Prostatic Branching Morphogenesis during Development and Re-growth Following Castration and Androgen Replacement." J. Biol. Chem. **279**(23): 24733-24744.

http://www.jbc.org/cgi/content/abstract/279/23/24733

Notch expression is frequently associated with progenitor cells, and its function is crucial for

development. Our recent work showing that Notch1 is selectively expressed in basal epithelial cells of the prostate and higher Notch1 expression during development suggests that Notch1-expressing cells may define progenitor cells in the prostate. To test this hypothesis, we have generated a transgenic mouse line in which the Notch1-expressing cells can be ablated in a controlled manner. Specific targeting was achieved by expressing the bacterial nitroreductase, an enzyme that catalyzes its substrate into a cytotoxin capable of inducing apoptosis, under the Notch1 promoter. Cell death in transgenic prostate was confirmed by histological analyses including terminal dUTP nick-end labeling and caspase 3 immunocytochemical staining. We evaluated the consequences of ablation of Notch1-expressing cells in two systems, organ culture of early postnatal prostates and re-growth of prostate in castrated mice triggered by hormone replacement. Our data show that elimination of Notch1-expressing cells inhibited the branching morphogenesis, growth, and differentiation of early postnatal prostate in culture and impaired prostate re-growth triggered by hormone replacement in castrated mice. Furthermore, we found that Notch1 expression following castration and hormone replacement was concomitant with known basal cell markers p63 and cytokeratin 14 and was high in the proliferative human prostate epithelial cells. Taken together, these data suggest that Notch1-expressing cells define the progenitor cells in the prostatic epithelial cell lineage, which are indispensable for prostatic development and re-growth.

Wang, Y. L., K. A. Frauwirth, et al. (2002). "Thiazolidinedione Activation of Peroxisome Proliferator-activated Receptor gamma Can Enhance Mitochondrial Potential and Promote Cell Survival." J. Biol. Chem. **277**(35): 31781-31788.

http://www.jbc.org/cgi/content/abstract/277/35/31781

Thiazolidinediones (TZDs) are widely used for treatment of type 2 diabetes mellitus. Peroxisome proliferator-activated receptor [gamma] (PPAR[gamma]) is the molecular target of TZDs and is believed to mediate the apoptotic effects of this class of drugs in a variety of cell types, including B and T lymphocytes. The finding that TZDs induce lymphocyte death has raised concerns regarding whether TZDs might further impair immune functions in diabetics. To address this issue, we investigated the roles of PPAR[gamma] and TZDs in lymphocyte survival. PPAR[gamma] was up-regulated upon T cell activation. As previously reported, PPAR[gamma] agonists induced T cell death in a dose-dependent manner. However, the concentrations of TZD needed to cause T cell death were above those needed to induce PPAR[gamma]-dependent transcription. Surprisingly, at concentrations that induce optimal transcriptional activation, TZD activation of PPAR[gamma] protected cells from apoptosis following growth factor withdrawal. The survival-enhancing effects depended on both the presence and activation of PPAR[gamma]. Measurements of mitochondrial potential revealed that PPAR[gamma] activation enhanced the ability of cells to maintain their mitochondrial potential. These data indicate that activation of PPAR[gamma] with TZDs can promote cell survival and suggest that PPAR[gamma] activation may potentially augment the immune responses of diabetic patients.

Warren, C. E., A. Krizus, et al. (2002). "The Caenorhabditis elegans Gene, gly-2, Can Rescue the N-Acetylglucosaminyltransferase V Mutation of Lec4 Cells." J. Biol. Chem. **277**(25): 22829-22838.

http://www.jbc.org/cgi/content/abstract/277/25/22829

UDP-N-acetylglucosamine:[alpha]-6-D-mannoside [beta]-1,6-N-acetylglucosaminyltransferase V (GlcNAc-TV) is a regulator of polylactosamine-containing N-glycans and is causally involved in T cell regulation and tumor metastasis. The Caenorhabditis elegans genome contains a single orthologous gene, gly-2, that is transcribed and encodes a 669-residue type II membrane protein that is 36.7% identical to mammalian GlcNAc-TV (Mgat-5). Recombinant GLY-2 possessed

GlcNAc-TV activity when assayed in vitro, and protein truncations demonstrated that the N-terminal boundary of the catalytic domain is Ile-138. gly-2 complemented the Phaseolus vulgaris leucoagglutinin binding defect of Chinese hamster ovary Lec4 cells, whereas GLY-2(L116R), an equivalent mutation to that which causes the Lec4A phenotype, could not. We conclude that the worm gene is functionally interchangeable with the mammalian form. GlcNAc-TV activity was detected in wild-type animals but not those homozygous for a deletion allele of gly-2. Activity was restored in mutant animals by an extrachromosomal array that encompassed the gly-2 gene. Green fluorescent protein reporter transgenes driven by the gly-2 promoter were expressed by developing embryos from the late comma stage onward, present in a complex subset of neurons in larvae and, in addition, the spermathecal and pharyngeal-intestinal valves and certain vulval cells of adults. However, no overt phenotypes were observed in animals homozygous for deletion alleles of gly-2.

Wehage, E., J. Eisfeld, et al. (2002). "Activation of the Cation Channel Long Transient Receptor Potential Channel 2 (LTRPC2) by Hydrogen Peroxide. A SPLICE VARIANT REVEALS A MODE OF ACTIVATION INDEPENDENT OF ADP-RIBOSE." J. Biol. Chem. **277**(26): 23150-23156.

http://www.jbc.org/cgi/content/abstract/277/26/23150

LTRPC2 is a cation channel recently reported to be activated by adenosine diphosphate-ribose (ADP-ribose) and NAD. Since ADP-ribose can be formed from NAD and NAD is elevated during oxidative stress, we studied whole cell currents and increases in the intercellular free calcium concentration ([Ca2+]i) in long transient receptor potential channel 2 (LTRPC2)-transfected HEK 293 cells after stimulation with hydrogen peroxide (H2O2). Cation currents carried by monovalent cations and Ca2+ were induced by H2O2 (5 mM in the bath solution) as well as by intracellular ADP-ribose (0.3 mM in the pipette solution) but not by NAD (1 mM). H2O2-induced currents developed slowly after a characteristic delay of 3-6 min and receded after wash-out of H2O2. [Ca2+]i was rapidly increased by H2O2 in LTRPC2-transfected cells as well as in control cells; however, in LTRPC2-transfected cells, H2O2 evoked a second delayed rise in [Ca2+]i. A splice variant of LTRPC2 with a deletion in the C terminus (amino acids 1292-1325) was identified in neutrophil granulocytes. This variant was stimulated by H2O2 as the wild type. However, it did not respond to ADP-ribose. We conclude that activation of LTRPC2 by H2O2 is independent of ADP-ribose and that LTRPC2 may mediate the influx of Na+ and Ca2+ during oxidative stress, such as the respiratory burst in granulocytes.

Whiteman, S. C., A. Bianco, et al. (2003). "Human Rhinovirus Selectively Modulates Membranous and Soluble Forms of Its Intercellular Adhesion Molecule-1 (ICAM-1) Receptor to Promote Epithelial Cell Infectivity." J. Biol. Chem. **278**(14): 11954-11961.

http://www.jbc.org/cgi/content/abstract/278/14/11954

Human rhinoviruses are responsible for many upper respiratory tract infections. 90% of rhinoviruses utilize intercellular adhesion molecule-1 (ICAM-1) as their cellular receptor, which also plays a critical role in recruitment of immune effector cells. Two forms of this receptor exist; membrane-bound (mICAM-1) and soluble ICAM-1 (sICAM-1). The soluble receptor may be produced independently from the membrane-bound form or it may be the product of proteolytic cleavage of mICAM-1. The ratio of airway epithelial cell expression of mICAM-1 to the sICAM-1 form may influence cell infectivity and outcome of rhinovirus infection. We therefore investigated the effect of rhinovirus on expression of both ICAM-1 receptors in normal human bronchial epithelial cells. We observed separate distinct messenger RNA transcripts coding for mICAM-1 and sICAM-1 in these cells, which were modulated by virus. Rhinovirus induced mICAM-1 expression on epithelial cells while simultaneously down-regulating sICAM-1 release, with

consequent increase in target cell infectivity. The role of protein tyrosine kinases was investigated as a potential mechanistic pathway. Rhinovirus infection induced rapid phosphorylation of intracellular tyrosine kinase, which may be critical in up-regulation of mICAM-1. Elucidation of the underlying molecular mechanisms involved in differential modulation of both ICAM-1 receptors may lead to novel therapeutic strategies.

Winn, R. A., L. Marek, et al. (2005). "Restoration of Wnt-7a expression reverses non-small cell lung cancer cell transformation through frizzled-9 mediated growth inhibition and promotion of cellular differentiation." J. Biol. Chem.: M409392200.

http://www.jbc.org/cgi/content/abstract/M409392200v1

The Wnt signaling pathway is critical in normal development and mutation of specific components is frequently observed in carcinomas of diverse origins. The potential involvement of this pathway in lung tumorigenesis, however, has not been established. In this study, analysis of multiple Wnt mRNAs in non-small cell lung cancer (NSCLC) cell lines and primary lung tumors revealed markedly decreased Wnt-7a expression when compared to normal short term bronchial epithelial cell lines and normal uninvolved lung tissue. Wnt-7a transfection in NSCLC cell lines reversed cellular transformation, decreased anchorage independent growth, and induced epithelial differentiation as demonstrated by soft agar and three dimensional cell culture assays in a subset of the NSCLC cell lines. The action of Wnt-7a correlated with the expression of the specific Wnt receptor Fzd-9, and transfection of Fzd-9 into a Wnt-7a-insensitive NSCLC cell line established Wnt-7a sensitivity. Moreover, Wnt-7a was present in Fzd-9 immunoprecipitates, indicating a direct interaction of Wnt-7a and Fzd-9. In NSCLC cells, Wnt-7a and Fzd-9 induced both cadherin and Sprouty 4 expression, and stimulated the JNK pathway, but not b-catenin/TCF-activity. In addition, transfection of gain-of-function JNK strongly inhibited anchorage-independent growth. Thus, this study demonstrates that Wnt-7a and Fzd-9 signaling through activation of the JNK pathway induces cadherin proteins and the receptor tyrosine kinase inhibitor, Sprouty 4, and represents a novel tumor suppressor pathway in lung cancer that is required for maintenance of epithelial differentiation and inhibition of transformed cell growth in a subset of human NSCLC cancers.

Wright, G., J. J. Higgin, et al. (2003). "Activation of the Prolyl Hydroxylase Oxygen-sensor Results in Induction of GLUT1, Heme Oxygenase-1, and Nitric-oxide Synthase Proteins and Confers Protection from Metabolic Inhibition to Cardiomyocytes." J. Biol. Chem. **278**(22): 20235-20239.

http://www.jbc.org/cgi/content/abstract/278/22/20235

Recently an oxygen-sensing/transducing mechanism has been identified as a family of O2-dependent prolyl hydroxylase domain-containing enzymes (PHD). In normoxia, PHD hydroxylates a specific proline residue that directs the degradation of constitutively synthesized hypoxia-inducible factor-1{alpha}. During hypoxia, the cessation of hydroxylation of this proline results in less degradation and thus increases hypoxia-inducible factor-1{alpha} protein levels. In this study we have examined the consequences of activating the PHD oxygen-sensing pathway in cultured neonatal myocytes using ethyl-3,4 dihydroxybenzoate and dimethyloxalylglycine, inhibitors that, similar to hypoxia, inhibit this family of O2-dependent PHD enzymes. Increased glucose uptake and enhanced glycolytic metabolism are classical cellular responses to hypoxia. Ethyl-3,4 dihydroxybenzoate treatment of cardiomyocyte cultures for 24 h increased [3H]deoxy-4-glucose uptake concurrent with an induction of GLUT1 protein. In addition, ethyl-3,4 dihydroxybenzoate, dimethyloxalylglycine, and hypoxia treatments were found to induce protein levels of nitricoxide synthase-2 and heme oxygenase-1, two important cardioregulatory proteins whose expression in response to hypoxic conditions is poorly understood. In conjunction with these changes in gene

expression, activation of the PHD oxygen-sensing mechanism was found to preserve myocyte viability in the face of metabolic inhibition with cyanide and 2-deoxyglucose. These results point to a key role for the PHD pathway in the phenotypic changes that are observed in a hypoxic myocyte and may suggest a strategy to pharmacologically induce protection in heart.

Xiao, J., P. Jethanandani, et al. (2003). "Regulation of {alpha}7 Integrin Expression during Muscle Differentiation." J. Biol. Chem. **278**(50): 49780-49788.

http://www.jbc.org/cgi/content/abstract/278/50/49780

Expression of the laminin-binding {alpha}7 integrin is tightly regulated during myogenic differentiation, reflecting required functions that range from cell motility to formation of stable myotendinous junctions. However, the exact mechanism controlling {alpha}7 expression in a tissue- and differentiation-specific manner is poorly understood. This report provides evidence that {alpha}7 gene expression during muscle differentiation is regulated by the c-Myc transcription factor. In myoblasts, {alpha}7 is expressed at basal levels, but following conversion to myotubes the expression of the integrin is strongly elevated. The increased {alpha}7 mRNA and protein levels following myogenic differentiation are inversely correlated with c-Myc expression. Transfection of myoblasts with the c-Myc transcription factor down-regulated {alpha}7 expression, whereas overexpression of Madmyc, a dominant-negative c-Myc chimera, induced elevated {alpha}7 expression. Functional analysis with site-specific deletions identified a specific double E-box sequence in the upstream promoter region (-2.0 to -2.6 kb) that is responsible for c-Myc-induced suppression of {alpha}7 expression. DNA-protein binding assays and supershift analysis revealed that c-Myc forms a complex with this double E-box sequence. Our results suggest that the interaction of c-Myc with this promoter region is an important regulatory element controlling {alpha}7 integrin expression during muscle development and myotendinous junction formation.

Xu, G., L.-x. Pan, et al. (2002). "Regulation of the Farnesoid X Receptor (FXR) by Bile Acid Flux in Rabbits." J. Biol. Chem. **277**(52): 50491-50496.

http://www.jbc.org/cgi/content/abstract/277/52/50491

We investigated the roles of hydrophobic deoxycholic acid (DCA) and hydrophilic ursocholic acid (UCA) in the regulation of the orphan nuclear farnesoid X receptor (FXR) in vivo. Rabbits with bile fistula drainage (removal of the endogenous bile acid pool), rabbits with bile fistula drainage and replacement with either DCA or UCA, and intact rabbits fed 0.5% cholic acid (CA) (enlarged endogenous bile acid pool) were studied. After bile fistula drainage, cholesterol 7[alpha]-hydroxylase (CYP7A1) mRNA and activity levels increased, FXR-mediated transcription was decreased, and FXR mRNA and nuclear protein levels declined. Replacing the enterohepatic bile acid pool with DCA restored FXR mRNA and nuclear protein levels and activated FXR-mediated transcription as evidenced by the increased expression of its target genes, SHP and BSEP, and decreased CYP7A1 mRNA level and activity. Replacing the bile acid pool with UCA also restored FXR mRNA and nuclear protein levels but did not activate FXR-mediated transcription, because the SHP mRNA level and CYP7A1 mRNA level and activity were unchanged. Feeding CA to intact rabbits expanded the bile acid pool enriched with the FXR high affinity ligand, DCA. FXR-mediated transcription became activated as shown by increased SHP and BSEP mRNA levels and decreased CYP7A1 mRNA level and activity but did not change FXR mRNA or nuclear protein levels. Thus, both hydrophobic and hydrophilic bile acids are effective in maintaining FXR mRNA and nuclear protein levels. However, the activating ligand (DCA) in the enterohepatic flux is necessary for FXR-mediated transcriptional regulation, which leads to down-regulation of CYP7A1.

Yamagata, H., K. Yonesu, et al. (2002). "TGTCACA Motif Is a Novel cis-Regulatory Enhancer Element Involved in Fruit-specific Expression of the cucumisin Gene." J. Biol. Chem. **277**(13): 11582-11590.

http://www.jbc.org/cgi/content/abstract/277/13/11582

Cucumisin, a subtilisin-like serine protease, is expressed at high levels in the fruit of melon (Cucumis melo L.) and accumulates in the juice. We investigated roles of the promoter regions and DNA-protein interactions in fruit-specific expression of the cucumisin gene. In transient expression analysis, a chimeric gene construct containing a 1.2-kb cucumisin promoter fused to a [beta]-glucuronidase (GUS) reporter gene was expressed in fruit tissues at high levels, but the promoter activities in leaves and stems were very low. Deletion analysis indicated that a positive regulatory region is located between nucleotides [-]234 and [-]214 relative to the transcriptional initiation site. Gain-of-function experiments revealed that this 20-bp sequence conferred fruit specificity and contained a regulatory enhancer. Gel mobility shift experiments demonstrated the presence of fruit nuclear factors that interact with the cucumisin promoter. A typical G-box (GACACGTGTC) present in the 20-bp sequence did not bind fruit protein, but two possible cis-elements, an I-box-like sequence (AGATATGATAAAA) and an odd base palindromic TGTCACA motif, were identified in the promoter region between positions [-]254 and [-]215. The I-box-like sequence bound more tightly to fruit nuclear protein than the TGTCACA motif. The I-box-like sequence functions as a negative regulatory element, and the TGTCACA motif is a novel enhancer element necessary for fruit-specific expression of the cucumisin gene. Specific nucleotides responsible for the binding of fruit nuclear protein in these two elements were also determined.

Yamamura, H., S. Ugawa, et al. (2004). "Capsazepine Is a Novel Activator of the {delta} Subunit of the Human Epithelial Na+ Channel." J. Biol. Chem. **279**(43): 44483-44489.

http://www.jbc.org/cgi/content/abstract/279/43/44483

The amiloride-sensitive epithelial Na+ channel (ENaC) regulates Na+ homeostasis into cells and across epithelia. So far, four homologous subunits of mammalian ENaC have been isolated and are denoted as {alpha}, {beta}, {gamma}, and {delta}. The chemical agents acting on ENaC are, however, largely unknown, except for amiloride and benzamil as ENaC inhibitors. In particular, there are no agonists currently known that are selective for ENaC{delta}, which is mainly expressed in the brain. Here we demonstrate that capsazepine, a competitive antagonist for transient receptor potential vanilloid subfamily 1, potentiates the activity of human ENaC{delta}{beta}{gamma} (hENaC{delta}{beta}{gamma}) heteromultimer expressed in Xenopus oocytes. The inward currents at a holding potential of -60 mV in hENaC{delta}{beta}{gamma}-expressing oocytes were markedly enhanced by the application of capsazepine ([&ge]1 {micro}M), and the capsazepine-induced current was mostly abolished by the addition of 100 {micro}M amiloride. The stimulatory effects of capsazepine on the inward current were concentration-dependent with an EC50 value of 8 {micro}M. Neither the application of other vanilloid compounds (capsaicin, resiniferatoxin, and olvanil) nor a structurally related compound (dopamine) modulated the inward current. Although hENaC{delta} homomer was also significantly activated by capsazepine, unexpectedly, capsazepine had no effect on hENaC{alpha} and caused a slight decrease on the hENaC{alpha}{beta}{gamma} current. In conclusion, capsazepine acts on ENaC{delta} and acts together with protons. Other vanilloids tested do not have any effect. These findings identify capsazepine as the first known chemical activator of ENaC{delta}.

Yan, C., H. Wang, et al. (2003). "Repression of 92-kDa Type IV Collagenase Expression by MTA1 Is Mediated through Direct Interactions with the Promoter via a Mechanism, Which Is Both Dependent on and Independent of Histone Deacetylation." J. Biol. Chem. **278**(4): 2309-2316.

http://www.jbc.org/cgi/content/abstract/278/4/2309

Although the expression of the metastases-associated gene MTA1 correlates with tumor metastases, its role in regulating type IV collagenase expression is unknown. Enforced MTA1 expression in HT1080 cells reduced basal and 12-myristate 13-acetate-induced 92-kDa type IV collagenase (MMP-9) protein/mRNA levels. DNase I hypersensitivity and PstI accessibility assays revealed multiple regions of the MMP-9 promoter ([-]650/[-]450 and [-]120/+1), showing reduced hypersensitivity in the MTA1-expressing cells. Chromatin immunoprecipitation assays demonstrated MTA1 binding to the distal region, which spans several regulatory cis elements. Co-immunoprecipitation and chromatin immunoprecipitation assay experiments revealed histone deacetylase 2 (HDAC2)-MTA1 protein-protein interactions and the MTA1-dependent recruitment of HDAC2 to the distal MMP-9 promoter region, yielding diminished histone H3/H4 acetylation. However, HDAC2 binding and H3/H4 acetylation at the proximal MMP-9 region were unaffected by MTA1 expression. Furthermore, trichostatin treatment only partially relieved MTA1-repressed MMP-9 expression, indicating a HDAC-insensitive component possibly involv ing the nucleosome-remodeling Mi2 activity, which was recruited to the promoter by MTA1. In summary, (a) MMP-9 adds to a short list of MTA1-regulated genes, which so far only includes c-myc and pS2, and (b) MTA1 binds to the MMP-9 promoter, thereby repressing expression of this type IV collagenase via histone-dependent and independent mechanisms.

Yang, T. and B. W. Poovaiah (2002). "A Calmodulin-binding/CGCG Box DNA-binding Protein Family Involved in Multiple Signaling Pathways in Plants." J. Biol. Chem. **277**(47): 45049-45058.

http://www.jbc.org/cgi/content/abstract/277/47/45049

We reported earlier that the tobacco early ethylene-responsive gene NtER1 encodes a calmodulin-binding protein (Yang, T., and Poovaiah, B. W. (2000) J. Biol. Chem. 275, 38467-38473). Here we demonstrate that there is one NtER1 homolog as well as five related genes in Arabidopsis. These six genes are rapidly and differentially induced by environmental signals such as temperature extremes, UVB, salt, and wounding; hormones such as ethylene and abscisic acid; and signal molecules such as methyl jasmonate, H2O2, and salicylic acid. Hence, they were designated as AtSR1-6 (Arabidopsis thaliana signal-responsive genes). Ca2+/calmodulin binds to all AtSRs, and their calmodulin-binding regions are located on a conserved basic amphiphilic [alpha]-helical motif in the C terminus. AtSR1 targets the nucleus and specifically recognizes a novel 6-bp CGCG box (A/C/G)CGCG(G/T/C). The multiple CGCG cis-elements are found in promoters of genes such as those involved in ethylene signaling, abscisic acid signaling, and light signal perception. The DNA-binding domain in AtSR1 is located on the N-terminal 146 bp where all AtSR1-related proteins share high similarity but have no similarity to other known DNA-binding proteins. The calmodulin-binding nuclear proteins isolated from wounded leaves exhibit specific CGCG box DNA binding activities. These results suggest that the AtSR gene family encodes a family of calmodulin-binding/DNA-binding proteins involved in multiple signal transduction pathways in plants.

Yasukawa, K., D. Sawamura, et al. (2002). "Dominant and Recessive Compound Heterozygous Mutations in Epidermolysis Bullosa Simplex Demonstrate the Role of the Stutter Region in Keratin Intermediate Filament Assembly." J. Biol. Chem. **277**(26): 23670-23674.

http://www.jbc.org/cgi/content/abstract/277/26/23670

Keratin intermediate filaments are important cytoskeletal structural proteins involved in maintaining cell shape and function. Mutations in the epidermal keratin genes, keratin 5 or keratin 14 lead to the disruption of keratin filament assembly, resulting in an autosomal dominant inherited blistering skin disease, epidermolysis bullosa simplex (EBS). We investigated a large EBS kindred who exhibited a markedly heterogeneous clinical presentation and detected two distinct keratin 5 mutations in the proband, the most severely affected. One missense mutation (E170K) in the highly conserved helix initiation peptide sequence of the 1A rod domain was found in all the affected family members. In contrast, the other missense mutation (E418K) was found only in the proband. The E418K mutation was located in the stutter region, an interruption in the heptad repeat regularity, whose function as yet remains unclear. We hypothesized that this mutated stutter allele was clinically silent when combined with the wild type allele but aggravates the clinical severity of EBS caused by the E170K mutation on the other allele. To confirm this in vitro, we transfected mutant keratin 5 cDNA into cultured cells. Although only 12.7% of the cells transfected with the E170K mutation alone showed disrupted keratin filament aggregations, significantly more cells (30.0%) cotransfected with both E170K and E418K mutations demonstrated keratin aggregation ($p < 0.05$). These transfection assay results corresponded to the heterogeneous clinical findings of the EBS patient in this kindred. We have identified the first case of both compound heterozygous dominant (E170K) and recessive (E418K) mutations in any keratin gene and confirmed the significant involvement of the stutter region in the assembly and organization of the keratin intermediate filament network in vitro.

Yoshimura, Y., M. Tani, et al. (2004). "Molecular Cloning and Functional Analysis of Zebrafish Neutral Ceramidase." J. Biol. Chem. **279**(42): 44012-44022.

http://www.jbc.org/cgi/content/abstract/279/42/44012

Almost all observations on the functions of neutral ceramidase have been carried out at cellular levels but not at an individual level. Here, we report the molecular cloning of zebrafish neutral ceramidase (znCD) and its functional analysis during embryogenesis. We isolated a cDNA clone encoding znCD by 5' and 3' rapid amplification of cDNA ends-PCR. It possessed an open reading frame of 2,229 base pairs encoding 743 amino acids. A possible signal/anchor sequence near the N terminus and four potential O-glycosylation and eight potential N-glycosylation sites were found in the putative sequence. The enzyme activity at neutral pH increased markedly after transformation of Chinese hamster CHOP and zebrafish BRF41 cells with the cDNA. The overexpressed enzyme was found to be distributed in endoplasmic reticulum/Golgi compartments as well as the plasma membranes. The antisense morpholino oligonucleotide (AMO), which was designed based on the sequence of znCD mRNA, successfully blocked the translation of znCD in a wheat germ in vitro translation system. The knockdown of znCD with AMO led to an increase in the number of zebrafish embryos with severe morphological and cellular abnormalities such as abnormal morphogenesis in the head and tail, pericardiac edema, defect of blood cell circulation, and an increase of apoptotic cells, especially in the head and neural tube regions, at 36 h post-fertilization. The ceramide level in AMO-injected embryos increased significantly compared with that in control embryos. Simultaneous injection of both AMO and synthetic znCD mRNA into one-cell-stage embryos rescued znCD activity and blood cell circulation. These results indicate that znCD is essential for the metabolism of ceramide and the early development of zebrafish.

Young, N. M., J.-R. Brisson, et al. (2002). "Structure of the N-Linked Glycan Present on Multiple Glycoproteins in the Gram-negative Bacterium, Campylobacter jejuni." J. Biol. Chem. **277**(45): 42530-42539.

http://www.jbc.org/cgi/content/abstract/277/45/42530

Mass spectrometry investigations of partially purified Campylobacter jejuni protein PEB3 showed it to be partially modified with an Asn-linked glycan with a mass of 1406 Da and composed of one hexose, five N-acetylhexosamines and a species of mass 228 Da, consistent with a trideoxydiacetamidohexose. By means of soybean lectin affinity chromatography, a mixture of glycoproteins was obtained from a glycine extract, and two-dimensional gel proteomics analysis led to the identification of at least 22 glycoproteins, predominantly annotated as periplasmic proteins. Glycopeptides were prepared from the glycoprotein mixture by Pronase digestion and gel filtration. The structure of the glycan was determined by using nano-NMR techniques to be GalNAc-[alpha]1,4-GalNAc-[alpha]1,4-[Glc[beta]1,3-]GalNAc-[alpha]1,4-GalNAc-[alpha]1,4-GalNAc-[alpha]1,3-Bac-[beta]1,N-Asn-Xaa, where Bac is bacillosamine, 2,4-diacetamido-2,4,6-trideoxyglucopyranose. Protein glycosylation was abolished when the pglB gene was mutated, providing further evidence that the enzyme encoded by this gene is responsible for formation of the glycopeptide N-linkage. Comparison of the pgl locus with that of Neisseria meningitidis suggested that most of the homologous genes are probably involved in the biosynthesis of bacillosamine.

Yu, W., N. R. Murray, et al. (2003). "Role of Cyclooxygenase 2 in Protein Kinase C beta II-mediated Colon Carcinogenesis." J. Biol. Chem. **278**(13): 11167-11174.

http://www.jbc.org/cgi/content/abstract/278/13/11167

Elevated expression of protein kinase C [beta]II (PKC[beta]II) is an early promotive event in colon carcinogenesis (Gokmen-Polar, Y., Murray, N. R., Velasco, M. A., Gatalica, Z., and Fields, A. P. (2001) Cancer Res. 61, 1375-1381). Expression of PKC[beta]II in the colon of transgenic mice leads to hyperproliferation and increased susceptibility to colon carcinogenesis due, at least in part, to repression of transforming growth factor beta type II receptor (TGF-[beta]RII) expression (Murray, N. R., Davidson, L. A., Chapkin, R. S., Gustafson, W. C., Schattenberg, D. G., and Fields, A. P. (1999) J. Cell Biol., 145, 699-711). Here we report that PKC[beta]II induces the expression of cyclooxygenase type 2 (Cox-2) in rat intestinal epithelial (RIE) cells in vitro and in transgenic PKC[beta]II mice in vivo. Cox-2 mRNA increases more than 10-fold with corresponding increases in Cox-2 protein and PGE2 production in RIE/PKC[beta]II cells. PKC[beta]II activates the Cox-2 promoter by 2- to 3-fold and stabilizes Cox-2 mRNA by at least 4-fold. The selective Cox-2 inhibitor Celecoxib restores expression of TGF-[beta]RII both in vitro and in vivo and restores TGF[beta]-mediated transcription in RIE/PKC[beta]II cells. Likewise, the [omega]-3 fatty acid eicosapentaenoic acid (EPA), which inhibits PKC[beta]II activity and colon carcinogenesis, causes inhibition of Cox-2 protein expression, re-expression of TGF-[beta]RII, and restoration of TGF-[beta]1-mediated transcription in RIE/PKC[beta]II cells. Our data demonstrate that PKC[beta]II promotes colon cancer, at least in part, through induction of Cox-2, suppression of TGF-[beta] signaling, and establishment of a TGF-[beta]-resistant, hyperproliferative state in the colonic epithelium. Our data define a procarcinogenic PKC[beta]II [right-arrow] Cox-2 [right-arrow] TGF-[beta] signaling axis within the colonic epithelium, and provide a molecular mechanism by which dietary [omega]-3 fatty acids and nonsteroidal antiinflammatory agents such as Celecoxib suppress colon carcinogenesis.

Zhang, D., R. C. M. Simmen, et al. (2002). "Secretory Leukocyte Protease Inhibitor Mediates Proliferation of Human Endometrial Epithelial Cells by Positive and Negative Regulation of Growth-associated Genes." J. Biol. Chem. **277**(33): 29999-30009.

http://www.jbc.org/cgi/content/abstract/277/33/29999

Secretory leukocyte protease inhibitor (SLPI) inhibits chymotrypsin, trypsin, elastase, and cathepsin G. This protein also exhibits proliferative effects, although little is known about the molecular mechanisms underlying this activity. We have generated SLPI-ablated epithelial sublines by stably transfecting the Ishikawa human endometrial cell line with an antisense human SLPI RNA expression vector. We demonstrate a positive correlation between cellular SLPI production and proliferation. We further show that Ishikawa sublines expressing low to undetectable SLPI have correspondingly increased and decreased expression, respectively, of transforming growth factor-[beta]1 and cyclin D1 genes, relative to parental cells. SLPI selectively increased cyclin D1 gene expression, with the effect occurring in part at the level of promoter activity. Cellular SLPI levels negatively influenced the anti-proliferative and pro-apoptotic insulin-like growth factor-binding protein-3 expression. We also identified lysyl oxidase, a phenotypic inhibitor of the ras oncogenic pathway and a tumor suppressor, as SLPI-repressed gene, whose expression is up-regulated by transforming growth factor-[beta]1. Our results suggest that SLPI acts at the node(s) of at least three major interacting growth inhibitory pathways. Because expression of SLPI is generally high in epithelial cells exhibiting abnormal proliferation such as in carcinomas, SLPI may define a novel pathway by which cellular growth is modulated.

Zhang, R.-Z., P. Sabatelli, et al. (2002). "Effects on Collagen VI mRNA Stability and Microfibrillar Assembly of Three COL6A2 Mutations in Two Families with Ullrich Congenital Muscular Dystrophy." J. Biol. Chem. **277**(46): 43557-43564.

http://www.jbc.org/cgi/content/abstract/277/46/43557

We recently reported a severe deficiency in collagen type VI, resulting from recessive mutations of the COL6A2 gene, in patients with Ullrich congenital muscular dystrophy. Their parents, who are all carriers of one mutant allele, are unaffected, although heterozygous mutations in collagen VI caused Bethlem myopathy. Here we investigated the consequences of three COL6A2 mutations in fibroblasts from patients and their parents in two Ullrich families. All three mutations lead to nonsense-mediated mRNA decay. However, very low levels of undegraded mutant mRNA remained in patient B with compound heterozygous mutations at the distal part of the triple-helical domain, resulting in deposition of abnormal microfibrils that cannot form extensive networks. This observation suggests that the C-terminal globular domain is not essential for triple-helix formation but is critical for microfibrillar assembly. In all parents, the COL6A2 mRNA levels are reduced to 57-73% of the control, but long term collagen VI matrix depositions are comparable with that of the control. The almost complete absence of abnormal protein and near-normal accumulation of microfibrils in the parents may account for their lack of myopathic symptoms.

Zhang, Y. J., W. K. O'Neal, et al. (2002). "Identification of Dynein Heavy Chain 7 as an Inner Arm Component of Human Cilia That Is Synthesized but Not Assembled in a Case of Primary Ciliary Dyskinesia." J. Biol. Chem. **277**(20): 17906-17915.

http://www.jbc.org/cgi/content/abstract/277/20/17906

Although the basic structure of the axoneme has been highly conserved throughout evolution, the varied functions of specialized axonemes require differences in structure and regulation. Cilia lining the respiratory tract propel mucus along airway surfaces, providing a critical function to the defense mechanisms of the pulmonary system, yet little is known of their molecular structure. We have identified and cloned a dynein heavy chain that is a component of the inner dynein arm. Bronchial epithelial cells were obtained from normal donors and from a patient with primary ciliary dyskinesia (PCD) whose cilia demonstrated an absence of inner dynein arms by electron microscopy. Cilia from normal and PCD cells were compared by gel electrophoresis, and mass spectrometry was used to identify DNAH7 as a protein absent in PCD cilia. The full-length

DNAH7 cDNA was cloned and shares 68% similarity with an inner arm dynein heavy chain from Drosophila. DNAH7 was induced during ciliated cell differentiation, and immunohistochemistry demonstrated the presence of DNAH7 in normal cilia. In cilia from PCD cells, DNAH7 was undetectable, whereas intracellular DNAH7 was clearly present. These studies identify DNAH7 as an inner arm component of human cilia that is synthesized but not assembled in a case of PCD.

Zhao, K., M. Liu, et al. (2005). "The Global Transcriptional Response of Escherichia coli to Induced {sigma}32 Protein Involves {sigma}32 Regulon Activation Followed by Inactivation and Degradation of {sigma}32 in Vivo." J. Biol. Chem. **280**(18): 17758-17768.

http://www.jbc.org/cgi/content/abstract/280/18/17758

{sigma} 32 is the first alternative {sigma} factor discovered in Escherichia coli and can direct transcription of many genes in response to heat shock stress. To define the physiological role of {sigma}32, we have used transcription profiling experiments to identify, on a genome-wide basis, genes under the control of {sigma}32 in E. coli by moderate induction of a plasmid-borne rpoH gene under defined, steady-state growth conditions. Together with a bioinformatics approach, we successfully confirmed genes known previously to be directly under the control of {sigma}32 and also assigned many additional genes to the {sigma}32 regulon. In addition, to understand better the functional relevance of the increased amount of {sigma}32 to changes in the transcriptional level of {sigma}32-dependent genes, we measured the protein level of {sigma}32 both before and after induction by a newly developed quantitative Western blot method. At a normal constant growth temperature (37 {degrees}C), we found that the {sigma}32 protein level rapidly increased, plateaued, and then gradually decreased after induction, indicating {sigma}32 can be regulated by genes in its regulon and that the mechanisms of {sigma}32 synthesis, inactivation, and degradation are not strictly temperature-dependent. The decrease in the transcriptional level of {sigma}32-dependent genes occurs earlier than the decrease in full-length {sigma}32 in the wild type strain, and the decrease in the transcriptional level of {sigma}32-dependent genes is greatly diminished in a {Delta}DnaK strain, suggesting that DnaK can act as an anti-{sigma} factor to functionally inactivate {sigma}32 and thus reduce {sigma}32-dependent transcription in vivo.

Zheng, M. and P. J. McKeown-Longo (2002). "Regulation of HEF1 Expression and Phosphorylation by TGF-beta 1 and Cell Adhesion." J. Biol. Chem. **277**(42): 39599-39608.

http://www.jbc.org/cgi/content/abstract/277/42/39599

Transforming growth factor-[beta]1 (TGF-[beta]1) is a multipotential cytokine, which regulates remodeling of tissue extracellular matrix during early tumorigenesis and wound healing. Human enhancer of filamentation-1 (HEF1), a multifunctional docking protein, is involved in integrin-based signaling, which affects cell motility, growth, and apoptosis. Our studies reveal that TGF-[beta]1 is a potent inducer of HEF1 gene transcription in human dermal fibroblasts. TGF-[beta]1 promoted HEF1 expression in a dose-dependent manner and resulted in a 16-fold increase in HEF1 protein level. TGF-[beta]1 had no effect on the stability of either HEF1 protein or mRNA. The TGF-[beta]1-induced HEF1 expression was independent of cell adhesion and resistant to cytoskeleton disruption. TGF-[beta]1 increased levels of both p105 and p115 HEF1 in adherent fibroblasts. Digestion with specific phosphatases indicated that the p115HEF1 resulted from serine/threonine phosphorylation of p105HEF1. The appearance of the p115HEF1 as well as tyrosine phosphorylation of p105HEF1 required cell adhesion and/or an organized cytoskeleton. An in vitro kinase assay indicated that p105HEF1 was a substrate for Src. PP1, a specific Src kinase inhibitor, was able to block adhesion-dependent tyrosine phosphorylation of p105HEF1. These findings suggest that TGF-[beta]1 regulates HEF1 gene expression and that HEF1 phosphorylation is dependent on cell adhesion and Src kinase activity.

Zimmermann, K., K. Ahrens, et al. (2002). "Targeted Disruption of the GAS41 Gene Encoding a Putative Transcription Factor Indicates That GAS41 Is Essential for Cell Viability." J. Biol. Chem. **277**(21): 18626-18631.

http://www.jbc.org/cgi/content/abstract/277/21/18626

The glioma-amplified sequence (GAS) 41 protein has been proposed to be a transcription factor. To investigate its functional role in vivo, we attempted to knock out the GAS41 gene by targeted disruption in the chicken pre-lymphoid cell line DT40. Heterozygous GAS41+/[-] cell lines generated by the first round of homologous recombination express approximately half the normal level of GAS41 mRNA. However, a homozygous GAS41[-]/[-] cell line with both GAS41 alleles disrupted was not obtained following the second round of transfection, indicating that the GAS41 gene is essential for cell viability. Indeed, homozygous GAS41[-]/[-] cell lines with two disrupted GAS41 alleles can be generated following substitution of the endogenous gene by stable integration of GAS41 cDNA controlled by a tetracycline-regulated CMV promoter. Inactivation of this promoter by tetracycline withdrawal results in rapid depletion of GAS41, causing a significant decrease in RNA synthesis and subsequently cell death. Thus, our results indicate that GAS41 is required for RNA transcription.

J. Biomol. Tech. (3)

Haqqi, T., X. Zhao, et al. (2002). "Sequencing in the presence of betaine: Improvement in sequencing of the localized repeat sequence regions." J. Biomol. Tech. **13**(4): 265-271.

http://jbt.highwire.org/cgi/content/abstract/13/4/265

The presence of several copies of the same class of repetitive element in DNA templates increases the probability of ambiguous base calling caused by band compression artifacts in the BigDye (Applied Biosystems, Foster City, CA) terminator cycle sequencing method. The presence of di-, tri-, and tetranucleotide repeats and short tandem repeats, which is widespread in the genome, poses a daunting task in sequencing laboratories, where a variety of DNA templates are submitted for sequencing. These base anomalies arise mainly as a result of the formation of secondary structures, including hairpins, and intramolecular base pairing between guanine and cytosine bases on the template strand. A common approach to the optimization of such sequencing reactions is either to replace the guanine with a base analog (such as deoxyinosine 5'-triphosphate [dITP] or 7-deaza-deoxyguanosine 5'-triphosphate [dGTP]) or to add a denaturant (such as dimethylsulfoxide [DMSO]) to the reaction mixture to overcome the undesired sequencing artifacts. Additives sometimes are ineffective for sequencing templates with GC-rich regions and repeat sequences. Herein we describe the effectiveness of (carboxymethyl)trimethylammonium (betaine) inner salt as an additive in the reaction mixture for reducing band compressions. The results presented show that betaine outperformed DMSO in sequencing through the localized regions containing GC-rich base pairs, guanine stretches, or TGC-type repeats in several DNA templates.

Vandenbergh, D. J., K. Heron, et al. (2003). "Simple Tests to Detect Errors in High-Throughput Genotype

Data in the Molecular Laboratory." J. Biomol. Tech. **14**(1): 9-16.

http://jbt.highwire.org/cgi/content/abstract/14/1/9

With the advent of high-density DNA marker data sets for the mouse and other model systems, 100 or more genotypes are routinely generated from large groups of mice. Issues of the accuracy and reliability of the genotyping are extremely important but often not addressed until genetic analysis is conducted. Simple tests that rely on the robust predictions arising from Mendelian genetics can be made quickly in the molecular laboratory as the data are generated, and require only a spreadsheet program. In this report, genotype data from 392 mice tested at 96 marker sites were analyzed for errors that are typical when handling large volumes of data generated in a repetitive process. The testing consisted of: (1) repeating the genotyping of approximately 1% of the samples; (2) examining the deviation from the expected segregation ratio (1:2:1) on a marker-by-marker basis; and (3) testing the correlation of the genotype at one marker with that at neighboring genetic markers on a chromosome. These three steps allowed analysis at the level of the microtiter plate, where errors are most likely to occur. A set of 96 dinucleotide repeat markers that are polymorphic between the C57BL/6J and DBA/2J mouse strains and can be multiplexed is reported for use in other genotyping projects.

Wiebe, G. J., R. Pershad, et al. (2003). "DNA SEQUENCING RESEARCH GROUP (DSRG) 2003--A GENERAL SURVEY OF CORE DNA SEQUENCING FACILITIES." J. Biomol. Tech. **14**(3): 231-235.

http://jbt.highwire.org/cgi/content/abstract/14/3/231

DNA sequencing core facilities serve as centralized resources within both academic and commercial institutions, providing expertise in the area of DNA analysis. The composition and configuration of these facilities continue to evolve in response to new developments in instrumentation and methodology. The goal of the 2003 DNA Sequencing Research Group (DSRG) survey was to identify recent changes in staffing, funding, instrumentation, services, and customer relations. Responses to 58 survey questions from 30 participants are presented to offer a look at the current typical DNA core sequencing facility. The results from this study will serve as a resource for institutions to benchmark their shared core laboratories, and to give facility directors an opportunity to compare and contrast their respective services and experiences.

J. Clin. Microbiol. (237)

Ahmad, S., Z. Khan, et al. (2002). "Seminested PCR for Diagnosis of Candidemia: Comparison with Culture, Antigen Detection, and Biochemical Methods for Species Identification." J. Clin. Microbiol. **40**(7): 2483-2489.

http://jcm.asm.org/cgi/content/abstract/40/7/2483

The rapid detection and identification of Candida species in clinical laboratories are extremely important for the management of patients with hematogenous candidiasis. The presently available culture and biochemical methods for detection and species identification of Candida are time-consuming and lack the required sensitivity and specificity. In this study, we have

established a seminested PCR (snPCR) using universal and species-specific primers for detection of Candida species in serum specimens. The universal outer primers amplified the 3' end of 5.8S ribosomal DNA (rDNA) and the 5' end of 28S rDNA, including the internally transcribed spacer 2 (ITS2), generating 350- to 410-bp fragments from the four commonly encountered Candida species, viz., C. albicans, C. tropicalis, C. glabrata, and C. parapsilosis. The species-specific primers, complementary to unique sequences within the ITS2 of each test species, amplified species-specific DNA in the reamplification step of the snPCR. The sensitivity of Candida detection by snPCR in spiked serum specimens was close to 1 organism/ml. Evaluation of snPCR for specific identification of Candida species with 76 clinical Candida isolates showed 99% concordant results with the Vitek and/or ID32C yeast identification system. Further evaluation of snPCR for detection of Candida species in sera from culture-proven (n = 12), suspected (n = 16), and superficially colonized (n = 10) patients and healthy subjects (n = 12) showed that snPCR results were consistently negative with sera from healthy individuals and colonized patients. In culture-proven candidemia patients, the snPCR results were in full agreement with blood culture results with respect to both positivity and species identity. In addition, snPCR detected candidemia due to two Candida species in five patients, compared to three by blood culture. In the category of suspected candidemia with negative blood cultures for Candida, nine patients (56%) were positive by snPCR; two of them had dual infection with C. albicans and either C. tropicalis or C. glabrata. In conclusion, the snPCR developed in this study is specific and more sensitive than culture for the detection of Candida species in serum specimens. Moreover, the improved detection of cases of candidemia caused by more than one Candida species is an additional advantage.

Amonsin, A., L. L. Li, et al. (2004). "Multilocus Short Sequence Repeat Sequencing Approach for Differentiating among Mycobacterium avium subsp. paratuberculosis Strains." J. Clin. Microbiol. **42**(4): 1694-1702.

http://jcm.asm.org/cgi/content/abstract/42/4/1694

We describe a multilocus short sequence repeat (MLSSR) sequencing approach for the genotyping of Mycobacterium avium subsp. paratuberculosis (M. paratuberculosis) strains. Preliminary analysis identified 185 mono-, di-, and trinucleotide repeat sequences dispersed throughout the M. paratuberculosis genome, of which 78 were perfect repeats. Comparative nucleotide sequencing of the 78 loci of six M. paratuberculosis isolates from different host species and geographic locations identified a subset of 11 polymorphic short sequence repeats (SSRs), with an average of 3.2 alleles per locus. Comparative sequencing of these 11 loci was used to genotype a collection of 33 M. paratuberculosis isolates representing different multiplex PCR for IS900 loci (MPIL) or amplified fragment length polymorphism (AFLP) types. The analysis differentiated the 33 M. paratuberculosis isolates into 20 distinct MLSSR types, consistent with geographic and epidemiologic correlates and with an index of discrimination of 0.96. MLSSR analysis was also clearly able to distinguish between sheep and cattle isolates of M. paratuberculosis and easily and reproducibly differentiated strains representing the predominant MPIL genotype (genotype A18) and AFLP genotypes (genotypes Z1 and Z2) of M. paratuberculosis described previously. Taken together, the results of our studies suggest that MLSSR sequencing enables facile and reproducible high-resolution subtyping of M. paratuberculosis isolates for molecular epidemiologic and population genetic analyses.

Antonsson, A., S. Karanfilovska, et al. (2003). "General Acquisition of Human Papillomavirus Infections of Skin Occurs in Early Infancy." J. Clin. Microbiol. **41**(6): 2509-2514.

http://jcm.asm.org/cgi/content/abstract/41/6/2509

The human skin papillomaviruses (HPVs) represent a group of ubiquitous viruses detected at a high prevalence in the normal skin of healthy adults. In the present study, we analyzed skin swab samples from babies during their first days of life and from infants at various ages up to age 4 years. Specimens from their parents and, for the newborn babies, environmental samples were also investigated. HPV DNA was already detected on the day of birth in samples from 2 of the 16 babies, and 45% of the samples from the babies were positive for HPV in the days following birth. Seventy-seven percent of the skin samples collected from the mothers were HPV DNA positive. The prevalence of HPV DNA among children from the ages of 1 month to 4 years varied between 50 and 70%. The HPV DNA sequences detected revealed a great diversity of genotypes and putative genotypes. Among 115 samples from 38 infants and 31 parents and 7 environmental samples, a total of 73 HPV types or putative types were isolated. Of these, 26 putative HPV types have not been described before. Our data suggest that asymptomatic HPV infections of normal skin are acquired very early in infancy and are caused by a great multiplicity of HPV types.

Bash, M. C., P. Zhu, et al. (2005). "por Variable-Region Typing by DNA Probe Hybridization Is Broadly Applicable to Epidemiologic Studies of Neisseria gonorrhoeae." J. Clin. Microbiol. **43**(4): 1522-1530.

http://jcm.asm.org/cgi/content/abstract/43/4/1522

The porin gene (porB) of Neisseria gonorrhoeae encodes the major outer membrane protein identified as PI or Por. To examine the utility of por variable-region (VR) typing, porB from 206 isolates was characterized by using oligonucleotide probes in a checkerboard hybridization assay that identifies the sequence types of five VRs of both PIA and PIB porB alleles. The strains represented temporally and geographically distinct isolates, isolates from a large cluster, epidemiologically linked partner isolates, and a collection of strains from disseminated gonococcal infections. By using rigorous epidemiologic criteria for transmission of infection between sex partners, por VR typing was more discriminatory than serovar typing in classifying isolates from both members of 43 epidemiologically linked pairs: 39 of 43 pairs were classified as coinciding by por VR typing compared to 43 of 43 by serovar determination (P = 0.058). porB sequence data confirmed the accuracy of the por VR method. Relationships between VR type and serovar typing monoclonal antibodies were observed for all six PIB and three of six PIA antibodies. por VR typing is a molecular tool that appears to have broad applicability. This method can be adapted to a wide range of technologies from simple hybridization to microarray and may allow for typing from noncultured clinical specimens.

Beck, I. A., M. Mahalanabis, et al. (2002). "Rapid and Sensitive Oligonucleotide Ligation Assay for Detection of Mutations in Human Immunodeficiency Virus Type 1 Associated with High-Level Resistance to Protease Inhibitors." J. Clin. Microbiol. **40**(4): 1413-1419.

http://jcm.asm.org/cgi/content/abstract/40/4/1413

A sensitive, specific, and high-throughput oligonucleotide ligation assay (OLA) for the detection of genotypic human immunodeficiency virus type 1 (HIV-1) resistance to Food and Drug Administration-approved protease inhibitors was developed and evaluated. This ligation-based assay uses differentially modified oligonucleotides specific for wild-type or mutant sequences, allowing sensitive and simple detection of both genotypes in a single well of a microtiter plate. Oligonucleotides were designed to detect primary mutations associated with high-level resistance to amprenavir, nelfinavir, indinavir, ritonavir, saquinavir, and lopinavir, including amino acid substitutions D30N, I50V, V82A/S/T, I84V, N88D, and L90M. Plasma HIV-1 RNA from 54 infected patients was amplified by reverse transcription-PCR and sequenced by using dideoxynucleotide chain terminators for evaluation of mutations associated with drug resistance. These same

amplicons were genotyped by the OLA at positions 30, 50, 82, 88, 84, and 90 for a total of 312 codons. The sensitivity of detection of drug-resistant genotypes was 96.7% (87 of 90 mutant codons) in the OLA compared to 92.2% (83 of 90) in consensus sequencing, presumably due to the increased sensitivity of the OLA. The OLA detected genetic subpopulations more often than sequencing, detecting 30 mixtures of mutant and wild-type sequences and two mixtures of drug-resistant sequences compared to 15 detected by DNA sequencing. Reproducible and semiquantitative detection of the mutant and the wild-type genomes by the OLA was observed by analysis of wild-type and mutant plasmid mixtures containing as little as 5% of either genotype in a background of the opposite genome. This rapid, simple, economical, and highly sensitive assay provides a practical alternative to dideoxy sequencing for genotypic evaluation of HIV-1 resistance to antiretrovirals.

Becker, K., A. W. Friedrich, et al. (2003). "Prevalence of Genes Encoding Pyrogenic Toxin Superantigens and Exfoliative Toxins among Strains of Staphylococcus aureus Isolated from Blood and Nasal Specimens." J. Clin. Microbiol. **41**(4): 1434-1439.

http://jcm.asm.org/cgi/content/abstract/41/4/1434

A total of 429 different Staphylococcus aureus isolates encompassing 219 blood isolates and 210 isolates taken from anterior nares were systematically searched by two multiplex PCR-DNA enzyme immunoassays (PCR-DEIA) for exfoliative toxin (ET) genes eta and etb, as well as for the classical members of the pyrogenic toxin superantigen (PTSAg) gene family comprising the staphylococcal enterotoxin (SE) genes sea-see and the toxic shock syndrome toxin 1 gene tst. In addition, a third PCR-DEIA was established to investigate the possession of four recently described SE genes, viz. seg-sej. The most frequent PTSAg/ET genes amplified were seg and sei, which were found strictly in combination in 55.0% of the S. aureus isolates tested. Other frequently detected toxin genes were tst (20.3%), sea (15.9%), and sec (11.2%). Only five isolates harbored ET genes. Regarding the origin of the S. aureus isolates, a significant difference (P = 0.037) was found for the possession of the sed/sej gene combination (10.5% of blood isolates versus 3.3% of nasal strains). Overall, about half of S. aureus isolates tested harbored genes of the classical members of the PTSAg family and ETs (50.8%), whereas 73.0% of S. aureus isolates were toxin gene positive if the recently described SE genes were included. This notable higher prevalence indicates that the possession of PTSAg genes in particular seems to be a habitual feature of S. aureus. Moreover, mainly due to the fixed combinations of seg plus sei, as well as sed plus sej, the possession of multiple PTSAg genes (62.9%) is more frequent than assumed so far.

Becker, M. R., B. J. Paster, et al. (2002). "Molecular Analysis of Bacterial Species Associated with Childhood Caries." J. Clin. Microbiol. **40**(3): 1001-1009.

http://jcm.asm.org/cgi/content/abstract/40/3/1001

Although substantial epidemiologic evidence links Streptococcus mutans to caries, the pathobiology of caries may involve more complex communities of bacterial species. Molecular methods for bacterial identification and enumeration now make it possible to more precisely study the microbiota associated with dental caries. The purpose of this study was to compare the bacteria found in early childhood caries (ECC) to those found in caries-free children by using molecular identification methods. Cloning and sequencing of bacterial 16S ribosomal DNAs from a healthy subject and a subject with ECC were used for identification of novel species or uncultivated phylotypes and species not previously associated with dental caries. Ten novel phylotypes were identified. A number of species or phylotypes that may play a role in health or disease were identified and warrant further investigation. In addition, quantitative measurements

for 23 previously known bacterial species or species groups were obtained by a reverse capture checkerboard assay for 30 subjects with caries and 30 healthy controls. Significant differences were observed for nine species: S. sanguinis was associated with health and, in order of decreasing cell numbers, Actinomyces gerencseriae, Bifidobacterium, S. mutans, Veillonella, S. salivarius, S. constellatus, S. parasanguinis, and Lactobacillus fermentum were associated with caries. These data suggest that A. gerencseriae and other Actinomyces species may play an important role in caries initiation and that a novel Bifidobacterium may be a major pathogen in deep caries. Further investigation could lead to the identification of targets for biological interventions in the caries process and thereby contribute to improved prevention of and treatment for this significant public health problem.

Beld, M., R. Minnaar, et al. (2004). "Highly Sensitive Assay for Detection of Enterovirus in Clinical Specimens by Reverse Transcription-PCR with an Armored RNA Internal Control." J. Clin. Microbiol. **42**(7): 3059-3064.

http://jcm.asm.org/cgi/content/abstract/42/7/3059

The objective of the present study was the development of a diagnostic reverse transcription (RT)-PCR for the specific detection of enterovirus (EV) RNA in clinical specimens controlled by an internal control (IC) RNA. The IC RNA contains the same primer binding sites as EV RNA but has a different probe region. The IC RNA was packaged into an MS2 phage core particle (armored) and was added to the clinical sample to allow monitoring of both extraction efficiency and RT-PCR efficiency. Serial dilutions of the IC RNA were made, and the detection limit of the RT-PCR was tested in a background of EV RNA-negative cerebrospinal fluid. The sensitivity and specificity of the RT-PCR assay were tested by using all 64 known EV serotypes, several non-EV serotypes, and two Quality Control for Molecular Diagnostics (QCMD) Program EV proficiency panels from 2001 and 2002. In total, 322 clinical specimens were tested by RT-PCR, and to establish the clinical utility of the RT-PCR, a comparison of the results of viral culture and RT-PCR was done with 87 clinical specimens. The lower limit of sensitivity was reached at about 150 copies of IC RNA/ml. All 64 EV serotypes were positive, while all non-EV serotypes were negative. All culture-positive samples of the 2001 QCMD proficiency panel (according to the 50% tissue culture infective doses per milliliter) were positive by RT-PCR. Invalid results, i.e., negativity for both EV RNA and IC RNA, due to inhibition of RT-PCR were observed for 33.3% of the members of the 2002 QCMD proficiency panel and 3.1% of the clinical specimens. Inhibition of RT-PCR could be relieved by the addition of 400 ng of bovine {alpha}-casein per {micro}l to both the RT reaction mixture and the PCR mixture. With this optimized protocol, the results for all samples of the 2002 QCMD proficiency panel and all clinical specimens except one fecal sample (0.3%) were valid. Evaluation of the clinical samples demonstrated that EV infection could be detected in 12 of 87 samples (13.8%) by RT-PCR, while viral culture was negative. Our data show that the RT-PCR with armored IC RNA offers a very reliable and rapid diagnostic tool for the detection of EV in clinical specimens and that the addition of bovine {alpha}-casein relieved inhibition of the RT-PCR for 99.7% of clinical specimens.

Bergmann, A. R., B. L. Schmidt, et al. (2002). "Importance of Sample Preparation for Molecular Diagnosis of Lyme Borreliosis from Urine." J. Clin. Microbiol. **40**(12): 4581-4584.

http://jcm.asm.org/cgi/content/abstract/40/12/4581

Urine PCR has been used for the diagnosis of Borrelia burgdorferi infection in recent years but has been abandoned because of its low sensitivity and the irreproducibility of the results. Our study aimed to analyze technical details related to sample preparation and detection methods. Crucial for a successful urine PCR were (i) avoidance of the first morning urine sample; (ii)

centrifugation at 36,000 x g; and (iii) the extraction method, with only DNAzol of the seven different extraction methods used yielding positive results with patient urine specimens. Furthermore, storage of frozen urine samples at -80{degrees}C reduced the sensitivity of a positive urine PCR result obtained with samples from 72 untreated erythema migrans (EM) patients from 85% in the first 3 months to <30% after more than 3 months. Bands were detected at 276 bp on ethidium bromide-stained agarose gels after amplification by a nested PCR. The specificity of bands for 32 of 33 samples was proven by hybridization with a GEN-ETI-K-DEIA kit and for a 10 further positive amplicons by sequencing. By using all of these steps to optimize the urine PCR technique, B. burgdorferi infection could be diagnosed by using urine samples from EM patients with a sensitivity (85%) substantially better than that of serological methods (50%). This improved method could be of future importance as an additional laboratory technique for the diagnosis of unclear, unrecognized borrelia infections and diseases possibly related to Lyme borreliosis.

Bialek, R., A. Cascante Cirera, et al. (2003). "Nested PCR Assays for Detection of Blastomyces dermatitidis DNA in Paraffin-Embedded Canine Tissue." J. Clin. Microbiol. **41**(1): 205-208.

http://jcm.asm.org/cgi/content/abstract/41/1/205

A Blastomyces dermatitidis nested PCR assay targeting the gene encoding the Wisconsin 1 (WI-1) adhesin was developed and compared with a nested PCR targeting the 18S rRNA gene (rDNA) of members of the family Onygenaceae. We examined 73 paraffin-embedded tissue samples obtained from nine dogs which died of blastomycosis and nine dogs which succumbed to lymphosarcoma according to autopsy findings; amplifiable canine DNA was extracted from 25 and 33 specimens from the two groups, respectively. The B. dermatitidis PCR amplified DNA from 8 of 13 tissue samples in which yeast cells were detected by microscopy. Sequencing revealed that all PCR products were homologous to the B. dermatitidis WI-1 adhesin gene. No PCR product was amplified from 12 microscopically negative biopsy specimens from dogs with blastomycosis or from 33 biopsy specimens from dogs with lymphosarcoma. The 18S rDNA PCR amplified DNA from 10 and 9 tissue samples taken from dogs which died of blastomycosis and lymphosarcoma, respectively. Only six products were identified as being identical to B. dermatitidis 18S rDNA; they were exclusively obtained from specimens positive by the B. dermatitidis nested PCR. For specificity testing, 20 human biopsy specimens proven to have histoplasmosis were examined, and a specific H. capsulatum product was amplified by the 18S rDNA PCR from all specimens, whereas no product was obtained from any of the 20 samples by the B. dermatitidis PCR assay. In conclusion, the PCR targeting a gene encoding the unique WI-1 adhesin is as sensitive as but more specific than the PCR targeting the 18S rDNA for detection of B. dermatitidis in canine tissue.

Bialek, R., A. Feucht, et al. (2002). "Evaluation of Two Nested PCR Assays for Detection of Histoplasma capsulatum DNA in Human Tissue." J. Clin. Microbiol. **40**(5): 1644-1647.

http://jcm.asm.org/cgi/content/abstract/40/5/1644

In order to evaluate the diagnostic relevance of two nested PCR assays for diagnosis of histoplasmosis in clinical specimens, 100 paraffin-embedded biopsy specimens were examined. Upon microscopy of tissue, 50 biopsy specimens were histoplasma positive and 50 were negative. Due to destruction by formalin fixation, successful extraction of amplifiable human DNA was limited to 29 and 33 samples, respectively. A product of the Histoplasma capsulatum nested PCR assay targeting the gene encoding the unique fungal 100-kDa-like protein was detected in 20 histopathologically positive biopsy specimens but in none of the microscopically negative samples. Sequencing revealed that all 20 products of 210 bp were identical to the sequence of H.

capsulatum in the GenBank database. In contrast, the nested PCR assay targeting the fungal 18S rRNA genes amplified products in 26 histopathologically positive but also in 18 microscopically negative biopsy specimens. However, sequencing revealed that only 20 of these 44 PCR products (231 bp) were identical to the sequence of H. capsulatum. The remaining 24 sequences were homologous to those of several Euascomycetes. These PCR products were detected only in tissues possibly colonized by nonpathogenic fungi, possibly causing these nonspecific amplifications. The detection limit of both H. capsulatum nested PCR assays was 1 to 5 fungal cells per sample. The two assays were similarly sensitive in identifying H. capsulatum. In this preliminary study, the novel 100-kDa-like-protein gene nested PCR revealed a specificity of 100% without requiring sequencing, which was necessary for identification of the 18S ribosomal DNA nested PCR products in order to avoid a high rate of false-positive results.

Bialek, R., J. Kern, et al. (2004). "PCR Assays for Identification of Coccidioides posadasii Based on the Nucleotide Sequence of the Antigen 2/Proline-Rich Antigen." J. Clin. Microbiol. **42**(2): 778-783.

http://jcm.asm.org/cgi/content/abstract/42/2/778

A conventional nested PCR and a real-time LightCycler PCR assay for detection of Coccidioides posadasii DNA were designed and tested in 120 clinical strains. These had been isolated from 114 patients within 10 years in Monterrey, Nuevo Leon, Mexico, known to be endemic for coccidioidomycosis. The gene encoding the specific antigen 2/proline-rich antigen (Ag2/PRA) was used as a target. All strains were correctly identified, whereas DNA from related members of the family Onygenaceae remained negative. Melting curve analysis by LightCycler and sequencing of the 526-bp product of the first PCR demonstrated either 100% identity to the GenBank sequence of the Silveira strain, now known to be C. posadasii (accession number AF013256), or a single silent mutation at position 1228. Length determination of two microsatellite-containing loci (GAC and 621) identified all 120 isolates as C. posadasii. Specific DNA was amplified by conventional nested PCR from three microscopically spherule-positive paraffin-embedded tissue samples, whereas 20 human tissue samples positive for other dimorphic fungi remained negative. Additionally, the safety of each step of a modified commercially available DNA extraction procedure was evaluated by using 10 strains. At least three steps of the protocol were demonstrated to sufficiently kill arthroconidia. This safe procedure is applicable to cultures and to clinical specimens.

Birkenheuer, A. J., M. G. Levy, et al. (2003). "Development and Evaluation of a Seminested PCR for Detection and Differentiation of Babesia gibsoni (Asian Genotype) and B. canis DNA in Canine Blood Samples." J. Clin. Microbiol. **41**(9): 4172-4177.

http://jcm.asm.org/cgi/content/abstract/41/9/4172

Canine babesiosis has recently been recognized as an emerging infectious disease of dogs in North America. We sought to develop a seminested PCR to detect and differentiate Babesia gibsoni (Asian genotype), B. canis subsp. vogeli, B. canis subsp. canis, and B. canis subsp. rossi DNA in canine blood samples. An outer primer pair was designed to amplify an [~]340-bp fragment of the 18S rRNA genes from B. gibsoni (Asian genotype), B. canis subsp. vogeli, B. canis subsp. rossi, and B. canis subsp. canis but not mammalian DNA. Forward primers were designed that would specifically amplify a smaller fragment from each organism in a seminested PCR. The practical limit of detection was 50 organisms/ml of mock-infected EDTA anticoagulated whole blood. The primer pair also amplified an [~]370-bp fragment of the B. gibsoni (USA/California genotype) 18S rRNA gene from the blood of an experimentally infected dog with a high percentage of parasitemia. Amplicons were not detected when DNA extracted from the blood of a dog that was naturally infected with Theileria annae at a low percentage of parasitemia

was amplified. Due to limited sensitivity, this test is not recommended for the routine diagnosis of B. gibsoni (USA/California genotype) or T. annae. The PCR test did not amplify Toxoplasma gondii, Neospora caninum, Leishmania infantum, Cryptosporidium parvum, or canine DNA under any of the conditions tested. The seminested PCR test was able to detect and discriminate B. gibsoni (Asian genotype), B. canis subsp. vogeli, B. canis subsp. canis, and B. canis subsp. rossi DNA in blood samples from infected dogs.

Bischoff, K. M., D. G. White, et al. (2002). "Characterization of Chloramphenicol Resistance in Beta-Hemolytic Escherichia coli Associated with Diarrhea in Neonatal Swine." J. Clin. Microbiol. **40**(2): 389-394.

http://jcm.asm.org/cgi/content/abstract/40/2/389

Ninety beta-hemolytic Escherichia coli isolates associated with diarrhea in neonatal pigs from multiple farms in Oklahoma were investigated for known associated disease serotypes, virulence factors, ribotypes, and antimicrobial susceptibility phenotypes. Fifteen different serotypes were observed, with 58% of isolates belonging to groups that produce one of three major enterotoxins: O149, O147, and O139. Thirty percent of the swine E. coli isolates possessed a combination of F4 fimbriae and the heat-labile toxin and heat-stable toxin B enterotoxins. Seventy-three percent of the E. coli isolates were resistant to five or more antibiotics. Interestingly, 53% of swine E. coli isolates exhibited resistance to chloramphenicol (CHL), an antibiotic whose use in food animals has been prohibited in the United States since the mid-1980s. The cmlA gene, which encodes a putative CHL efflux pump, was detected by PCR in 47 of the 48 CHL-resistant isolates, and 4 of these also possessed the cat2 gene, which encodes a chloramphenicol acetyltransferase. The one CHL-resistant isolate that did not contain either cmlA or cat-2 possessed the flo gene, which confers resistance to both florfenicol and CHL. To determine whether CHL-resistant swine E. coli isolates represented dissemination of a clonal strain, all 90 isolates were analyzed by ribotyping. Seventeen distinct E. coli ribogroups were identified, with CHL resistance observed among the isolates in all except one of the major ribogroups. The identification of the cmlA gene among diverse hemolytic enterotoxigenic E. coli strains demonstrates its broad dissemination in the swine production environment and its persistence even in the absence of CHL selection pressure.

Bode, E., W. Hurtle, et al. (2004). "Real-Time PCR Assay for a Unique Chromosomal Sequence of Bacillus anthracis." J. Clin. Microbiol. **42**(12): 5825-5831.

http://jcm.asm.org/cgi/content/abstract/42/12/5825

Real-time PCR has become an important method for the rapid identification of Bacillus anthracis since the 2001 anthrax mailings. Most real-time PCR assays for B. anthracis have been developed to detect virulence genes located on the pXO1 and pXO2 plasmids. In contrast, only two published chromosomal targets exist, the rpoB gene and the gyrA gene. In the present study, subtraction-hybridization with a plasmid-cured B. anthracis tester strain and a Bacillus cereus driver was used to find a unique chromosomal sequence. By targeting this region, a real-time assay was developed with the Ruggedized Advanced Pathogen Identification Device. Further testing has revealed that the assay has 100% sensitivity and 100% specificity, with a limit of detection of 50 fg of DNA. The results of a search for sequences with homology with the BLAST program demonstrated significant alignment to the recently published B. anthracis Ames strain, while an inquiry for protein sequence similarities indicated homology with an abhydrolase from B. anthracis strain A2012. The importance of this chromosomal assay will be to verify the presence of B. anthracis independently of plasmid occurrence.

Boeckh, M., M. Huang, et al. (2004). "Optimization of Quantitative Detection of Cytomegalovirus DNA in Plasma by Real-Time PCR." J. Clin. Microbiol. **42**(3): 1142-1148.

http://jcm.asm.org/cgi/content/abstract/42/3/1142

Previous studies have shown that detection of cytomegalovirus (CMV) DNA in plasma is less sensitive than the antigenemia assay for CMV surveillance in blood. In 1,983 blood samples, plasma PCR assays with three different primer sets (UL125 alone, UL126 alone, and UL55/UL123-exon 4) were compared to the pp65 antigenemia assay and blood cultures. Plasma PCR detected CMV more frequently in blood specimens than either the antigenemia assay or cultures, but of the three PCR assays, the double-primer assay (UL55/UL123-exon 4) performed best with regard to sensitivity, specificity, and predictive values compared to antigenemia: 122 of 151 antigenemia-positive samples were detected (sensitivity, 80.1%), and there were 122 samples that were PCR positive-antigenemia negative (specificity, 93%). Samples with discrepant results had a low viral load (median, 0.5 cells per slide; 1,150 copies per ml) and were often obtained from patients receiving antiviral therapy. CMV could be detected by other methods in 15 of 29 antigenemia positive-PCR negative samples compared to 121 of 122 PCR positive-antigenemia negative samples ($P < 0.001$). On a per-subject basis, 21 of 25 patients (antigenemia positive-PCR negative) and all 57 (PCR positive-antigenemia negative) could be confirmed at different time points during follow-up. The higher sensitivity of the double-primer assay resulted in earlier detection compared to antigenemia in a time-to-event analysis of 42 CMV-seropositive stem cell transplant recipients, and two of three patients with CMV disease who were antigenemia negative were detected by plasma PCR prior to the onset of disease. Interassay variability was low, and the dynamic range was >5 log10. Automated DNA extraction resulted in high reproducibility, accurate CMV quantitation ($R = 0.87$, $P < 0.001$), improved sensitivity, and increased speed of sample processing. Thus, primer optimization and improved DNA extraction techniques resulted in a plasma-based PCR assay that is significantly more sensitive than pp65 antigenemia and blood cultures for detection of CMV in blood specimens.

Boel, C. H. E., C. M. C. van Herk, et al. (2005). "Evaluation of Conventional and Real-Time PCR Assays Using Two Targets for Confirmation of Results of the COBAS AMPLICOR Chlamydia trachomatis/Neisseria gonorrhoeae Test for Detection of Neisseria gonorrhoeae in Clinical Samples." J. Clin. Microbiol. **43**(5): 2231-2235.

http://jcm.asm.org/cgi/content/abstract/43/5/2231

Two conventional PCR-enzyme immunoassays (PCR-EIAs) and two real-time PCR assays (LightCycler system; Roche Diagnostics) were evaluated as confirmation assays with cppB and 16S rRNA genes as targets. Of 765 male and female genitourinary and nasopharyngeal specimens positive for Neisseria gonorrhoeae in the COBAS AMPLICOR Chlamydia trachomatis/Neisseria gonorrhoeae PCR test (Roche Diagnostics), 229 (30%) were confirmed positive; 13 of these (5.7%) were lacking the cppB gene. Of the 534 samples (70%) that could not be confirmed, 81 (15%) showed a positive crossing point. However, melting curve analysis revealed an aberrant melting temperature in the LightCycler 16S rRNA assay; therefore, these samples were considered non-N. gonorrhoeae Neisseria species. Both of the 16S rRNA assays performed well, with positive predictive values of 99.1% and 100% for the PCR-EIAs and the real-time assays, respectively, and a negative predictive value of 99.8% for both. The cppB assays were compromised by the absence of the cppB gene in 5.7% of the N. gonorrhoeae-positive samples, resulting in negative predictive values of 96.8% and 97.6% for the PCR-EIAs and the real-time assays, respectively. Therefore, the 16S rRNA gene is preferable to the cppB gene as a target for confirmation assays. The melting curve analysis of the real-time assays provides useful additional information.

Bopp, D. J., B. D. Sauders, et al. (2003). "Detection, Isolation, and Molecular Subtyping of Escherichia coli O157:H7 and Campylobacter jejuni Associated with a Large Waterborne Outbreak." J. Clin. Microbiol. **41**(1): 174-180.

http://jcm.asm.org/cgi/content/abstract/41/1/174

The largest reported outbreak of waterborne Escherichia coli O157:H7 in the United States occurred in upstate New York following a county fair in August 1999. Culture methods were used to isolate E. coli O157:H7 from specimens from 128 of 775 patients with suspected infections. Campylobacter jejuni was also isolated from stools of 44 persons who developed diarrheal illness after attending this fair. There was one case of a confirmed coinfection with E. coli O157:H7 and C. jejuni. Molecular detection of stx1 and stx2 Shiga toxin genes, immunomagnetic separation (IMS), and selective culture enrichment were utilized to detect and isolate E. coli O157:H7 from an unchlorinated well and its distribution points, a dry well, and a nearby septic tank. PCR for stx1 and stx2 was shown to provide a useful screen for toxin-producing E. coli O157:H7, and IMS subculture improved recovery. Pulsed-field gel electrophoresis (PFGE) was used to compare patient and environmental E. coli O157:H7 isolates. Among patient isolates, 117 of 128 (91.5%) were type 1 or 1a (three or fewer bands different). Among the water distribution system isolates, 13 of 19 (68%) were type 1 or 1a. Additionally, PFGE of C. jejuni isolates revealed that 29 of 35 (83%) had indistinguishable PFGE patterns. The PFGE results implicated the water distribution system as the main source of the E. coli O157:H7 outbreak. This investigation demonstrates the potential for outbreaks involving more than one pathogen and the importance of analyzing isolates from multiple patients and environmental samples to develop a better understanding of bacterial transmission during an outbreak.

Bosshard, P. P., S. Abels, et al. (2004). "Comparison of Conventional and Molecular Methods for Identification of Aerobic Catalase-Negative Gram-Positive Cocci in the Clinical Laboratory." J. Clin. Microbiol. **42**(5): 2065-2073.

http://jcm.asm.org/cgi/content/abstract/42/5/2065

Over a period of 18 months we have evaluated the use of 16S ribosomal DNA (rDNA) sequence analysis as a means of identifying aerobic catalase-negative gram-positive cocci in the clinical laboratory. A total of 171 clinically relevant strains were studied. The results of molecular analyses were compared with those obtained with a commercially available phenotypic identification system (API 20 Strep system; bioMerieux sa, Marcy l'Etoile, France). Phenotypic characterization identified 67 (39%) isolates to the species level and 32 (19%) to the genus level. Seventy-two (42%) isolates could not be discriminated at any taxonomic level. In comparison, 16S rDNA sequencing identified 138 (81%) isolates to the species level and 33 (19%) to the genus level. For 42 of 67 isolates assigned to a species with the API 20 Strep system, molecular analyses yielded discrepant results. Upon further analysis it was concluded that among the 42 isolates with discrepant results, 16S rDNA sequencing was correct for 32 isolates, the phenotypic identification was correct for 2 isolates, and the results for 8 isolates remained unresolved. We conclude that 16S rDNA sequencing is an effective means for the identification of aerobic catalase-negative gram-positive cocci. With the exception of Streptococcus pneumoniae and beta-hemolytic streptococci, we propose the use of 16S rDNA sequence analysis if adequate species identification is of concern.

Bosshard, P. P., S. Abels, et al. (2003). "Ribosomal DNA Sequencing for Identification of Aerobic Gram-

Positive Rods in the Clinical Laboratory (an 18-Month Evaluation)." J. Clin. Microbiol. **41**(9): 4134-4140.

http://jcm.asm.org/cgi/content/abstract/41/9/4134

We have evaluated over a period of 18 months the use of 16S ribosomal DNA (rDNA) sequence analysis as a means of identifying aerobic gram-positive rods in the clinical laboratory. Two collections of strains were studied: (i) 37 clinical strains of gram-positive rods well identified by phenotypic tests, and (ii) 136 clinical isolates difficult to identify by standard microbiological investigations, i.e., identification at the species level was impossible. Results of molecular analyses were compared with those of conventional phenotypic identification procedures. Good overall agreement between phenotypic and molecular identification procedures was found for the collection of 37 clinical strains well identified by conventional means. For the 136 clinical strains which were difficult to identify by standard microbiological investigations, phenotypic characterization identified 71 of 136 (52.2%) isolates at the genus level; 65 of 136 (47.8%) isolates could not be discriminated at any taxonomic level. In comparison, 16S rDNA sequencing identified 89 of 136 (65.4%) isolates at the species level, 43 of 136 (31.6%) isolates at the genus level, and 4 of 136 (2.9%) isolates at the family level. We conclude that (i) rDNA sequencing is an effective means for the identification of aerobic gram-positive rods which are difficult to identify by conventional techniques, and (ii) molecular identification procedures are not required for isolates well identified by phenotypic investigations.

Boutaga, K., A. J. van Winkelhoff, et al. (2003). "Comparison of Real-Time PCR and Culture for Detection of Porphyromonas gingivalis in Subgingival Plaque Samples." J. Clin. Microbiol. **41**(11): 4950-4954.

http://jcm.asm.org/cgi/content/abstract/41/11/4950

Porphyromonas gingivalis is a major pathogen in destructive periodontal disease in humans. Detection and quantification of this microorganism are relevant for diagnosis and treatment planning. The prevalence and quantity of P. gingivalis in subgingival plaque samples of periodontitis patients were determined by anaerobic culture and real-time PCR amplification of the 16S small-subunit rRNA gene. The PCR was performed with primers and a fluorescently labeled probe specific for the P. gingivalis 16S rRNA gene. By the real-time PCR assay, as few as 1 CFU of P. gingivalis could be detected. Subgingival plaque samples from 259 adult patients with severe periodontitis were analyzed. P. gingivalis was detected in 111 (43%) of the 259 subgingival plaque samples by culture and in 138 (53%) samples by PCR. The sensitivity, specificity, and positive and negative predictive values of the real-time PCR were 100, 94, 94, and 100%, respectively. We conclude that real-time PCR confirms the results of quantitative culture of P. gingivalis and offers significant advantages with respect to the rapidity and sensitivity of detection of P. gingivalis in subgingival plaque samples.

Brennan, R. E. and J. E. Samuel (2003). "Evaluation of Coxiella burnetii Antibiotic Susceptibilities by Real-Time PCR Assay." J. Clin. Microbiol. **41**(5): 1869-1874.

http://jcm.asm.org/cgi/content/abstract/41/5/1869

Coxiella burnetii is an obligate intracellular bacterium. The inability to cultivate this organism on axenic medium has made calculation of infectious units challenging and prevents the use of conventional antibiotic susceptibility assays. A rapid and reliable real-time PCR assay was developed to quantify C. burnetii cells from J774.16 mouse macrophage cells and was applied to

antibiotic susceptibility testing of C. burnetii Nine Mile, phase I. For calculation of bacterial replication, real-time PCR performed equally as well as immunofluorescent-antibody (IFA) assay when J774.16 cells were infected with 10-fold serial dilutions of C. burnetii and was significantly ($P < 0.05$) more repeatable than IFA when 2-fold dilutions were used. Newly infected murine macrophage-like J774.16 cells were treated with 8 {micro}g of chloramphenicol per ml, 4 {micro}g of tetracycline per ml, 4 {micro}g of rifampin per ml, 4 {micro}g of ampicillin per ml, or 1 {micro}g of ciprofloxacin per ml. After 6 days of treatment, tetracycline, rifampin, and ampicillin significantly ($P < 0.01$) inhibited the replication of C. burnetii, while chloramphenicol and ciprofloxacin did not. In general, these results are consistent with those from prior reports on the efficacy of these antibiotics against C. burnetii Nine Mile, phase I, and indicate that a real-time PCR-based assay is an appropriate alternative to the present methodology for evaluation of the antibiotic susceptibilities of C. burnetii.

Briedis, D. J., A. Khamessan, et al. (2002). "Isolation of Campylobacter fetus subsp. fetus from a Patient with Cellulitis." J. Clin. Microbiol. **40**(12): 4792-4796.

http://jcm.asm.org/cgi/content/abstract/40/12/4792

Campylobacter fetus subsp. fetus is a gram-negative, slender, spirally curved bacterial pathogen. It has been isolated from human blood, spinal fluid, and abscesses, but cellulitis associated with bacteremia is rare. We report its isolation from a blood culture of a human patient with cellulitis as well as difficulties encountered in determining the identity of the subspecies of C. fetus.

Brinkman, J. A., W. E. Jones, et al. (2002). "Detection of Human Papillomavirus DNA in Urine Specimens from Human Immunodeficiency Virus-Positive Women." J. Clin. Microbiol. **40**(9): 3155-3161.

http://jcm.asm.org/cgi/content/abstract/40/9/3155

Human immunodeficiency virus (HIV)-positive women may represent one of the fastest-growing populations at risk for acquiring cervical cancer and thus require frequent screening. The purpose of the present studies was to validate a PCR-based urine assay by comparing detection and genotyping of human papillomavirus (HPV) DNA in urine samples and matching cervical swab specimens of HIV-positive women. Despite a difference in amplifiability, the prevalence of any HPV genotype (58% for the cervical swab specimens and 48% for the urine specimens) was not significantly different in this population. The levels of concordance were 70, 71, and 78% for detection of any HPV type, any high-risk HPV type, or any low-risk HPV type in the two specimen types, respectively. While instances of discordant detection were greater for the cervical swab specimens than for the urine specimens, this was not statistically significant. The distributions of HPV genotypes were similar in the cervix and the urine for the majority of types examined. Importantly, detection of HPV DNA in urine was associated with an abnormal Papanicolaou smear to the same extent that detection of HPV DNA in a cervical swab specimen was. These data provide preliminary support for the proposal to use urine testing as a primary or secondary screening tool for cervical cancer in HIV-positive women or as an epidemiological tool. Additional studies with larger sample sizes must be conducted in order to further verify these findings.

Broccolo, F., G. Locatelli, et al. (2002). "Calibrated Real-Time PCR Assay for Quantitation of Human Herpesvirus 8 DNA in Biological Fluids." J. Clin. Microbiol. **40**(12): 4652-4658.

http://jcm.asm.org/cgi/content/abstract/40/12/4652

Accurate laboratory tests for the diagnosis of active human herpesvirus 8 (HHV-8) infection are becoming essential to study the pathogenesis of HHV-8-associated tumors and for the clinical management of HHV-8-infected individuals. We have developed a highly sensitive, calibrated quantitative real-time PCR assay for the measurement of cell-free HHV-8 DNA in body fluids, based on the addition of a synthetic DNA calibrator prior to DNA extraction. The calibrator controls each sample for the presence of PCR inhibitors, determines a cutoff value of sensitivity for negative samples, and normalizes positive samples for the efficiency of DNA recovery. The assay shows a wide dynamic range of detection (between 1 and 106 viral genome equivalents/reaction) and a high degree of accuracy even in the presence of high amounts (up to 1 {micro}g) of human genomic DNA. Moreover, the assay has a very high sensitivity (lower detection limit, 10 genome equivalents/ml) and a high degree of reproducibility and repeatability with a coefficient of variation (CV) of <15 and 23%, respectively. Furthermore, the use of the calibrator improves the accuracy of quantitation and decreases the intersample variability (CV, 9 and 6%, respectively). The sensitivity and specificity of the assay were tested with a series of clinical specimens obtained from patients affected by various HHV-8-related diseases, as well as from a wide number of controls. In conclusion, our calibrated real-time PCR assay provides a reliable high-throughput method for quantitation of HHV-8 DNA in clinical and laboratory specimens.

Buller, R. S. and G. Storch (2004). "Evaluation of a Real-Time PCR Assay Using the LightCycler System for Detection of Parvovirus B19 DNA." J. Clin. Microbiol. **42**(7): 3326-3328.

http://jcm.asm.org/cgi/content/abstract/42/7/3326

We evaluated the artus RealArt Parvovirus B19 LC PCR reagent (artus biotech USA, San Francisco, Calif.) for real-time PCR detection of parvovirus B19 DNA by retesting 71 specimens previously submitted to our laboratory. The artus assay, which produces a quantitative result and provides an internal PCR control, appeared to be slightly more sensitive than our conventional qualitative PCR assay.

Campsall, P. A., N. H. C. Au, et al. (2004). "Detection and Genotyping of Varicella-Zoster Virus by TaqMan Allelic Discrimination Real-Time PCR." J. Clin. Microbiol. **42**(4): 1409-1413.

http://jcm.asm.org/cgi/content/abstract/42/4/1409

A proportion of individuals vaccinated with live attenuated Oka varicella-zoster virus (VZV) vaccine subsequently develop attenuated chicken pox and/or herpes zoster. To determine whether postvaccination varicella infections are caused by vaccine or wild-type virus, a simple method for distinguishing the vaccine strain from wild-type virus is required. We have developed a TaqMan real-time PCR assay to detect and differentiate wild-type virus from Oka vaccine strains of VZV. The assay utilized two fluorogenic, minor groove binding probes targeted to a single nucleotide polymorphism in open reading frame 62 that distinguishes the Oka vaccine from wild-type strains. VZV DNA could be genotyped and quantified within minutes of thermocycling completion due to real-time monitoring of PCR product formation and allelic discrimination analysis. The allelic discrimination assay was performed in parallel with two standard PCR-restriction fragment length polymorphism (RFLP) methods on 136 clinical and laboratory VZV strains from Canada, Australia, and Japan. The TaqMan assay exhibited a genotyping accuracy of 100% and, when compared to both PCR-RFLP methods, was 100 times more sensitive. In addition, the method was technically simpler and more rapid. The TaqMan assay also allows for high-throughput genotyping, making it ideal for epidemiologic study of the live attenuated varicella vaccine.

Cano, J., J. Guarro, et al. (2004). "Molecular and Morphological Identification of Colletotrichum Species of Clinical Interest." J. Clin. Microbiol. **42**(6): 2450-2454.

http://jcm.asm.org/cgi/content/abstract/42/6/2450

Colletotrichum species have caused human infections in recent years. Because of the difficulties in recognizing them in vitro, we have designed a quick and unambiguous molecular test, based on the amplification of a specific fragment of the internal transcribed spacer 1 region, to distinguish any Colletotrichum isolate from other fungi, including the common pathogenic species. Analysis of the sequences of the ribosomal DNA (rDNA) fragment showed sufficient variability to clearly separate the five species of Colletotrichum that are of clinical interest, i.e., Colletotrichum coccodes, C. crassipes, C. dematium, C. gloeosporioides, and C. graminicola. Sequencing of the D1-D2 region of the large-subunit rDNA gene also supported these results. Additionally, we reviewed the most suitable morphological characteristics for the in vitro identification of these increasingly important opportunistic fungi.

Cardinali, G., A. Martini, et al. (2002). "Multicenter Comparison of Three Different Analytical Systems for Evaluation of DNA Banding Patterns from Cryptococcus neoformans." J. Clin. Microbiol. **40**(6): 2095-2100.

http://jcm.asm.org/cgi/content/abstract/40/6/2095

The enormous improvement of molecular typing techniques for epidemiological and clinical studies has not always been matched by an equivalent effort in applying optimal criteria for the analysis of both phenotypic and molecular data. In spite of the availability of a large collection of statistical and phylogenetic methods, the vast majority of commercial packages are limited by using only the unweighted pair group method with arithmetic mean algorithm to construct trees and by considering electrophoretic pattern only as migration distances. The latter method has serious drawbacks when different runs (separate gels) of the same molecular analysis are to be compared. This work presents a multicenter comparison of three different systems of banding pattern analysis on random amplified polymorphic DNA, (GACA)4, and contour-clamped homogeneous electric field patterns from strains of Cryptococcus neoformans var. neoformans isolated in different clinical and geographical situations and a standard Saccharomyces cerevisiae strain employed as an outgroup. The systems considered were evaluated for their actual ability to(i) recognize identities, (ii) define complete differences (i.e., the ability to place S. cerevisiae out of the C. neoformans cluster), and (iii) estimate the extent of similarity among different strains. The ability to cluster strains according to the patient from which they were isolated was also evaluated. The results indicate that different algorithms do indeed produce divergent trees, both in overall topology and in clustering of individual strains, thus suggesting that care must be taken by individual investigators to use the most appropriate procedure and by the scientific community in defining a consensus system.

Castle, P. E., A. T. Lorincz, et al. (2003). "Comparison between Prototype Hybrid Capture 3 and Hybrid Capture 2 Human Papillomavirus DNA Assays for Detection of High-Grade Cervical Intraepithelial Neoplasia and Cancer." J. Clin. Microbiol. **41**(9): 4022-4030.

http://jcm.asm.org/cgi/content/abstract/41/9/4022

We compared the performance of a prototype version of the Hybrid Capture 3 (HC3) human

papillomavirus (HPV) DNA assay to the current generation Hybrid Capture 2 (HC2) assay, both of which target 13 oncogenic HPV types, for the detection of cervical intraepithelial neoplasia grade 3 and cancer (CIN3+) with cervicovaginal lavage specimens collected at enrollment into a 10-year cohort study at Kaiser Permanente (Portland, Oreg.). HC3 results for a risk-stratified sample (n = 4,364) were compared to HC2 results for the entire cohort (n = 20,810) with receiver operating characteristics curves, and the optimal cut points for both tests (relative light units [RLU]/positive control [PC]) for the detection of CIN3+ were determined. Specimens were also tested for HPV16 and HPV18 with separate HC3 type-specific probes. The optimal cut point for detecting CIN3+ was 1.0 RLU/PC for HC2, as previously shown, and was 0.6 RLU/PC for HC3. At the optimal cut points, HC3 and HC2 had similar screening performance characteristics for CIN3+ diagnosed at the enrollment visit. In analyses that included cases CIN3+ at enrollment and those diagnosed during early follow-up, HC3 had nonsignificantly higher sensitivity and equal specificity for the detection of CIN3+ compared to HC2; this increase in sensitivity was primarily the result of increased detection of CIN3+ in women who were 30 years of age or older and were cytologically negative (P = 0.006). We also compared the performance of the hybrid capture tests to MY09/11 L1 consensus primer PCR results (n = 1,247). HC3 was less likely than HC2 to test positive for specimens that tested positive by PCR for any untargeted types (P < 0.001). HC3 was less likely than HC2 to test positive for untargeted PCR-detected single infections with HPV53 (P = 0.001) and HPV66 (P = 0.01). There was good agreement between test positivity by PCR and by single type-specific HC3 probes for HPV16 (kappa = 0.76; 95% confidence interval [CI] = 0.71 to 0.82) and for HPV18 (kappa = 0.73; 95% CI = 0.68 to 0.79). In conclusion, we suggest that HC3 ([IMG]=" BORDER="0">0.6 RLU/PC) may be slightly more sensitive than and equally specific test as HC2 ([IMG]=" BORDER="0">1.0 RLU/PC) for the detection of CIN3+ over the duration of typical screening intervals.

Cerna, J. F., J. P. Nataro, et al. (2003). "Multiplex PCR for Detection of Three Plasmid-Borne Genes of Enteroaggregative Escherichia coli Strains." J. Clin. Microbiol. **41**(5): 2138-2140.

http://jcm.asm.org/cgi/content/abstract/41/5/2138

We developed a novel multiplex PCR assay for enteroaggregative Escherichia coli (EAEC) detection, by using three plasmid-borne genes (the aggregative adherence [AA] probe, aap, and aggR). One or more of the loci were detected in 24 (86%) of 28 patient isolates analyzed. The multiplex PCR assay is a fast, convenient, and sensitive molecular test to detect EAEC.

Chi, X. S., A. Hu, et al. (2005). "Detection and Characterization of New Influenza B Virus Variants in 2002." J. Clin. Microbiol. **43**(5): 2345-2349.

http://jcm.asm.org/cgi/content/abstract/43/5/2345

One-hundred five influenza B-positive specimens obtained from southeast Asia in 2002 were categorized on the basis of DNA sequencing of HA1 gene as well as real-time PCR analysis of the NA gene. Phylogenetic analysis of the HA1 gene sequences showed that the majority of the viruses (96.2%) belonged to the B/Victoria/2/87 lineage, while a smaller percentage of the viruses (3.8%) belonged to the B/Yamagata/16/88 lineage. The B/Yamagata/16/88 viruses displayed significant antigenic drift in the deduced amino acid sequences of the HA1 protein, and the B/Victoria/2/87-like viruses consisted of B/Hong Kong/1351/02-like (72.3%) and B/Hong Kong/330/01-like (27.7%) viruses. The B/Hong Kong/1351/02-like viruses were reassortants with the HA gene belonging to the B/Victoria/2/87 lineage and the NA gene belonging to the B/Yamagata/16/88 lineage, whereas both the HA and NA genes of B/Hong Kong/330/01 virus belonged to the B/Victoria/2/87 lineage. In this study, however, all the B/Hong Kong/330/01-like isolates exhibited the B/Yamagata/16/88-like NA gene, which likely resulted from reassortment of

B/Hong Kong/330/01 and B/Hong Kong/1351/02 viruses during coinfection. Additional molecular characterization of the six internal genes showed that the M, NS, PA, and PB2 genes of the new variants were B/Hong Kong/1351/02 in origin, whereas the NP and PA genes retained the B/Hong Kong/330/01 origin. Interestingly, these new variants all appeared late in the year 2002. These results support the notion that influenza B viruses continued to evolve through antigenic drift and shift.

Chizhikov, V., M. Wagner, et al. (2002). "Detection and Genotyping of Human Group A Rotaviruses by Oligonucleotide Microarray Hybridization." J. Clin. Microbiol. **40**(7): 2398-2407.

http://jcm.asm.org/cgi/content/abstract/40/7/2398

A rapid and reliable method for the identification of five clinically relevant G genotypes (G1 to G4 and G9) of human rotaviruses based on oligonucleotide microarray hybridization has been developed. The genotype-specific oligonucleotides immobilized on the surface of glass slides were selected to bind to the multiple target regions within the VP7 gene that are highly conserved among individual rotavirus genotypes. Rotavirus cDNA was amplified in a PCR with primers common to all group A rotaviruses. A second round of nested PCR amplification was performed in the presence of indodicarbocyanine-dCTP and another pair of degenerate primers also broadly specific for all genotypes. The use of one primer containing 5'-biotin allowed us to prepare fluorescently labeled single-stranded hybridization probe by binding of another strand to magnetic beads. The identification of rotavirus genotype was based on hybridization with several individual genotype-specific oligonucleotides. This approach combines the high sensitivity of PCR with the selectivity of DNA-DNA hybridization. The specificity of oligonucleotide microchip hybridization was evaluated by testing 20 coded rotavirus isolates from different geographic areas for which genotypes were previously determined by conventional methods. Analysis of the coded specimens showed that this microarray-based method is capable of unambiguous identification of all rotavirus strains. Because of the presence of random mutations, each individual virus isolate produced a unique hybridization pattern capable of distinguishing different isolates of the same genotype and, therefore, subgenotype differentiation. This strain information indicates one of several advantages that microarray technology has over conventional PCR techniques.

Chomel, B. B., A. C. Wey, et al. (2003). "Isolation of Bartonella washoensis from a Dog with Mitral Valve Endocarditis." J. Clin. Microbiol. **41**(11): 5327-5332.

http://jcm.asm.org/cgi/content/abstract/41/11/5327

We report the first documented case of Bartonella washoensis bacteremia in a dog with mitral valve endocarditis. B. washoensis was isolated in 1995 from a human patient with cardiac disease. The main reservoir species appears to be ground squirrels (Spermophilus beecheyi) in the western United States. Based on echocardiographic findings, a diagnosis of infective vegetative valvular mitral endocarditis was made in a spayed 12-year-old female Doberman pinscher. A year prior to presentation, the referring veterinarian had detected a heart murmur, which led to progressive dyspnea and a diagnosis of congestive heart failure the week before examination. One month after initial presentation, symptoms worsened. An emergency therapy for congestive heart failure was unsuccessfully implemented, and necropsy evaluation of the dog was not permitted. Indirect immunofluorescence tests showed that the dog was strongly seropositive (titer of 1:4,096) for several Bartonella antigens (B. vinsonii subsp. berkhoffii, B. clarridgeiae, and B. henselae), highly suggestive of Bartonella endocarditis. Standard aerobic and aerobic-anaerobic cultures were negative. However, a specific blood culture for Bartonella isolation grew a fastidious, gram-negative organism 7 days after being plated. Phenotypic and genotypic characterizations of the isolate, including partial sequencing of the citrate synthase

(gltA), groEL, and 16S rRNA genes, indicated that this organism was identical to B. washoensis. The dog was seronegative for all tick-borne pathogens tested (Anaplasma phagocytophilum, Ehrlichia canis, and Rickettsia rickettsii), but the sample was highly positive for B. washoensis (titer of 1:8,192) and, according to indirect immunofluorescent-antibody assay, weakly positive for phase II Coxiella burnetii infection.

Collantes-Fernandez, E., A. Zaballos, et al. (2002). "Quantitative Detection of Neospora caninum in Bovine Aborted Fetuses and Experimentally Infected Mice by Real-Time PCR." J. Clin. Microbiol. **40**(4): 1194-1198.

http://jcm.asm.org/cgi/content/abstract/40/4/1194

We report the development of a real-time PCR assay for the quantitative detection of Neospora caninum in infected host tissues. The assay uses the double-stranded DNA-binding dye SYBR Green I to continuously monitor product formation. Oligonucleotide primers were designed to amplify a 76-bp DNA fragment corresponding to the Nc5 sequence of N. caninum. A similar method was developed to quantify the 28S rRNA host gene in order to compare the parasite load of different samples and to correct for the presence of potential PCR-inhibiting compounds in the DNA samples. A linear quantitative detection range of 6 logs with a calculated detection limit of 10-1 tachyzoite per assay was observed with excellent linearity (R2 = 0.998). Assay specificity was confirmed by using DNA from the closely related parasite Toxoplasma gondii. The applicability of the technique was successfully tested in a variety of host brain tissues: (i) aborted bovine fetuses classified into negative or positive Neospora-infected animals according to the observation of compatible lesions by histopathological study and (ii) experimentally infected BALB/c mice, divided into three groups, inoculated animals with or without compatible lesions and negative controls. All samples were also tested by ITS1 Neospora nested PCR and a high degree of agreement was shown between both PCR techniques ({kappa} = 0.86). This technique represents a useful quantitative diagnostic tool to be used in the study of the pathogenicity, immunoprophylaxis, and treatment of Neospora infection.

Collot, S., B. Petit, et al. (2002). "Real-Time PCR for Quantification of Human Herpesvirus 6 DNA from Lymph Nodes and Saliva." J. Clin. Microbiol. **40**(7): 2445-2451.

http://jcm.asm.org/cgi/content/abstract/40/7/2445

A real-time quantitative PCR assay has been developed to measure human herpesvirus 6 (HHV-6) DNA in biological specimens. The assay sensitivity was 10 copies of DNA per well, with a linear dynamic range of 10 to 107 copies of HHV-6 DNA. Intra- and interassay variations were, respectively, 0.88 and 0.8% for samples containing 102 DNA copies, 0.99 and 0.96% for samples containing 104 copies, and 0.76 and 0.9% for samples containing 106 copies. Among 34 saliva samples from healthy subjects, 26 were found to contain HHV-6 DNA (76.5%; median, 23,870 copies/ml), and following a single freeze-thaw cycle, 25 of the same samples were found to be positive for HHV-6 DNA, although at a statistically significantly lower concentration (median, 3,497 copies/ml). The assay enabled detection of HHV-6 DNA in lymph node biopsies from patients with Hodgkin's disease (HD) (13 of 37 patients [35.1%]), B-cell neoplasms (8 of 36 patients [22.2%]), and T- or NK-cell neoplasms (3 of 13 patients [23.1%]), with concentrations ranging from 100 to 864,640 HHV-6 copies per {micro}g of DNA (HHV-6B being found in every case except two). All HD patients infected with HHV-6 presented clinically with the nodular sclerosis subtype of HD. The real-time quantitative PCR assay developed here was simple to perform and was sensitive over a wide range of HHV-6 concentrations. It therefore appears to be of potential value in clinical investigation or diagnosis of HHV-6 infection.

Coutlee, F., P. Gravitt, et al. (2002). "Use of PGMY Primers in L1 Consensus PCR Improves Detection of Human Papillomavirus DNA in Genital Samples." J. Clin. Microbiol. **40**(3): 902-907.

http://jcm.asm.org/cgi/content/abstract/40/3/902

The novel PGMY L1 consensus primer pair is more sensitive than the MY09 and MY11 primer mix for detection and typing with PCR of human papillomavirus (HPV) DNA in genital specimens. We assessed the diagnostic yield of PGMY primers for the detection and typing of HPV by comparing the results obtained with PGMY09/PGMY11 and MY09/MY11/HMB01 on 299 genital samples. Amplicons generated with PGMY primers were typed with the line blot assay (PGMY-line blot), while HPV amplicons obtained with the degenerate primer pool MY09/MY11/HMB01 were detected with type-specific radiolabeled probes in a dot blot assay (standard consensus PCR test). Cervicovaginal lavage samples (N = 272) and cervical scrape samples (N = 27) were tested in parallel with both PCR tests. The PGMY-line blot test detected the presence of HPV DNA more frequently than the standard consensus PCR assay. The concordance for HPV typing between the two assays was 84.3% (214 of 255 samples), for a good kappa value of 0.69. Of the 177 samples containing HPV DNA by at least one method, 40 samples contained at least one HPV type detected only with PGMY-line blot, whereas positivity exclusively with the standard consensus PCR test was found for only 7 samples ($P < 0.001$). HPV types 45 and 52 were especially more frequently detected with PGMY than MY primers. However, most HPV types were better amplified with PGMY primers, including HPV-16. Samples with discordant results between the two PCR assays more frequently contained multiple HPV types. Studies using PGMY instead of MY primers have the potential to report higher detection rates of HPV infection not only for newer HPV types but also for well-known genital types.

Cowan, L. S., L. Mosher, et al. (2002). "Variable-Number Tandem Repeat Typing of Mycobacterium tuberculosis Isolates with Low Copy Numbers of IS6110 by Using Mycobacterial Interspersed Repetitive Units." J. Clin. Microbiol. **40**(5): 1592-1602.

http://jcm.asm.org/cgi/content/abstract/40/5/1592

A study set of 180 Mycobacterium tuberculosis and Mycobacterium bovis isolates having low copy numbers of IS6110 were genotyped using the recently introduced method based on the variable-number tandem repeats of mycobacterial interspersed repetitive units (MIRU-VNTR). The results were compared with results of the more commonly used methods, IS6110 restriction fragment length polymorphism (RFLP) and spoligotyping. The isolates were collected in Michigan from 1996 to 1999 as part of a project to genotype all isolates from new cases of tuberculosis in the state. Twelve MIRU loci were amplified, and the amplicons were analyzed by agarose gel electrophoresis to determine the copy number at each MIRU locus. MIRU-VNTR produced more distinct patterns (80 patterns) than did IS6110 RFLP (58 patterns), as would be expected in this study set. Spoligotyping identified 59 patterns. No single method defined all unique isolates, and the combination of all three typing methods generated 112 distinct patterns identifying 90 unique isolates and 90 isolates in 22 clusters. The results confirm the potential utility of MIRU-VNTR typing and show that typing with multiple methods is required to attain maximum specificity.

Dayan, G. H., M. S. Panero, et al. (2004). "Varicella Seroprevalence and Molecular Epidemiology of Varicella-Zoster Virus in Argentina, 2002." J. Clin. Microbiol. **42**(12): 5698-5704.

http://jcm.asm.org/cgi/content/abstract/42/12/5698

There is limited data on immunity against varicella-zoster virus (VZV) in adults in different parts of Argentina, and it is not known which VZV strains are circulating in Argentina. The objectives of this study were as follows: (i) to evaluate seroprevalence of varicella among adults, assessing the accuracy of clinical history and determining the sociodemographic factors associated with seropositivity; and (ii) to determine the VZV strains circulating in Argentina. A cross-sectional serological survey enrolling 2,807 women aged 15 to 49 years attending public health-care settings in four cities in Argentina (i.e., Buenos Aires, Salta, Mendoza, and Rosario) and one rural area was conducted from August to November 2002. Specimens for identification of VZV strains were obtained from vesicular lesions from 13 pediatric patients with varicella from different areas of the country. PCR amplification was used for genotyping. The overall seroprevalence of varicella antibodies was 98.5% (95% confidence interval, 98.0 to 98.9), ranging from 97.2% in central Buenos Aires to 99.3% in southern Buenos Aires and Salta. Varicella seroprevalence increased with age. Crowding and length of residence in the same place were associated with seropositivity. The positive predictive value of varicella history for immunity to varicella was 99.4%; however, the negative predictive value was 2.5%. The European genotype was identified in all viral specimens. In Argentina, seroprevalence in women more than 15 years old was high regardless of the area of residence. Negative or uncertain varicella history was not a good predictor of immunity. VZV genotype was stable in all areas of the country.

De Cock, H. E. V., S. L. Marks, et al. (2004). "Ileocolitis Associated with Anaerobiospirillum in Cats." J. Clin. Microbiol. **42**(6): 2752-2758.

http://jcm.asm.org/cgi/content/abstract/42/6/2752

Ileocolitis associated with spiral bacteria identified as an Anaerobiospirillum sp. was found in six cats. Two cats had acute onset of gastrointestinal signs characterized by vomiting and diarrhea in one cat and vomiting in another cat, one cat had chronic diarrhea that was refractory to medical therapy; one cat had acute onset of anorexia and lethargy, and two cats had clinical signs that were not related to the gastrointestinal tract. The presence of an Anaerobiospirillum sp. was demonstrated on the basis of ultrastructural morphology of spiral bacteria associated with intestinal lesions and PCR amplification of a genus-specific 16S rRNA gene from affected tissues from each cat. The colons of three clinically healthy cats without lesions and one cat with mild colitis not associated with spiral bacteria were negative for Anaerobiospirillum spp. in the same assay. Comparative nucleotide sequence analysis of cloned PCR products from three affected cats further suggested that the spiral bacteria were closely related to Anaerobiospirillum succiniciproducens.

del Mar Mosquera, M., F. de Ory, et al. (2002). "Simultaneous Detection of Measles Virus, Rubella Virus, and Parvovirus B19 by Using Multiplex PCR." J. Clin. Microbiol. **40**(1): 111-116.

http://jcm.asm.org/cgi/content/abstract/40/1/111

We describe here a multiplex reverse transcription-PCR (RTMNPCR) assay designed to detect and differentiate measles virus, rubella virus, and parvovirus B19. Serial dilution experiments with vaccine strains that compared cell culture isolation of measles in B95 cells and rubella in RK13 cells showed sensitivity rates of 0.004 50% tissue culture infective dose (TCID50) for measles virus and 0.04 TCID50 for rubella virus. This RTMNPCR can detect as few as 10 molecules for measles virus and rubella virus and one molecule for parvovirus B19 in dilution experiments with plasmids containing inserts of the primary reaction amplification products. Five pharyngeal exudates from measles patients and 2 of 15 cerebrospinal fluid samples from measles-related encephalitis were found to be positive for measles virus by this RTMNPCR. A total of 3 of 27 pharyngeal exudates from vaccinated children and 2 pharyngeal exudates, plus one urine sample

from a case of congenital rubella syndrome, were found to be positive for rubella virus by RTMNPCR, whereas 16 of 19 sera from patients with erythema infectiosum were determined to be positive for parvovirus B19 by RTMNPCR. In view of these results, we can assess that this method is a useful tool in the diagnosis of these three viruses and could be used as an effective surveillance tool in measles eradication programs.

Domann, E., G. Hong, et al. (2003). "Culture-Independent Identification of Pathogenic Bacteria and Polymicrobial Infections in the Genitourinary Tract of Renal Transplant Recipients." J. Clin. Microbiol. **41**(12): 5500-5510.

http://jcm.asm.org/cgi/content/abstract/41/12/5500

Renal transplant recipients are predisposed to urinary tract infections caused by both common uropathogens and opportunistic bacteria resulting frequently in significant polymicrobial infections. In this study, a culture-independent 16S rRNA-based approach was established to identify unusual, fastidious, or anaerobic bacteria and to investigate bacterial diversity in urinary tract specimens. Similarly sized amplicons encompassing the V6 to V8 region of the 16S rRNA were analyzed with denaturing high-performance liquid chromatography (DHPLC) (WAVE System). Artificial mixtures of single amplicons from commonly encountered uropathogenic bacteria produced distinct peak profiles whose identities were confirmed by sequencing individually collected peak products. We evaluated the application of the method on 109 urinary tract specimens from renal transplant recipients; 100% correlation was found for culture-positive specimens, and DHPLC generated peak profiles. However, for culture-negative specimens, DHPLC facilitated the detection of novel peak profiles. DNA sequencing of these individual peaks was used to identify the bacteria involved. Thus, in PCR-positive but culture-negative samples the method allowed detection of previously known uropathogens such as Corynebacterium urealyticum and Gardnerella vaginalis, but also unusual agents including Anaerococcus lactolyticus, Bacteroides vulgatus, Dialister invisus, Fusobacterium nucleatum, Lactobacillus iners, Leptotrichia amnionii, Prevotella buccalis, Prevotella ruminicola, Rahnella aquatilis, and Streptococcus intermedius were detected as single pathogens or as constituents of polymicrobial infections. The method described is reproducible and rapidly and enables both DHPLC-based profiling and sequence-based investigation of microbial communities and polymicrobial infections. A detailed understanding of infections found in recipients of renal transplants will guide antibiotic therapy regimens and provide new perspectives for decreasing the risk of graft rejection.

Eishi, Y., M. Suga, et al. (2002). "Quantitative Analysis of Mycobacterial and Propionibacterial DNA in Lymph Nodes of Japanese and European Patients with Sarcoidosis." J. Clin. Microbiol. **40**(1): 198-204.

http://jcm.asm.org/cgi/content/abstract/40/1/198

The cause(s) of sarcoidosis is unknown. Mycobacterium spp. are suspected in Europe and Propionibacterium spp. are suspected in Japan. The present international collaboration evaluated the possible etiological links between sarcoidosis and the suspected bacterial species. Formalin-fixed and paraffin-embedded sections of biopsy samples of lymph nodes, one from each of 108 patients with sarcoidosis and 65 patients with tuberculosis, together with 86 control samples, were collected from two institutes in Japan and three institutes in Italy, Germany, and England. Genomes of Propionibacterium acnes, Propionibacterium granulosum, Mycobacterium tuberculosis, Mycobacterium avium subsp. paratuberculosis, and Escherichia coli (as the control) were counted by quantitative real-time PCR. Either P. acnes or P. granulosum was found in all but two of the sarcoid samples. M. avium subsp. paratuberculosis was found in no sarcoid sample. M. tuberculosis was found in 0 to 9% of the sarcoid samples but in 65 to 100% of the

tuberculosis samples. In sarcoid lymph nodes, the total numbers of genomes of P. acnes or P. granulosum were far more than those of M. tuberculosis. P. acnes or P. granulosum was found in 0 to 60% of the tuberculosis and control samples, but the total numbers of genomes of P. acnes or P. granulosum in such samples were less than those in sarcoid samples. Propionibacterium spp. are more likely than Mycobacteria spp. to be involved in the etiology of sarcoidosis, not only in Japanese but also in European patients with sarcoidosis.

Emanuel, P. A., R. Bell, et al. (2003). "Detection of Francisella tularensis within Infected Mouse Tissues by Using a Hand-Held PCR Thermocycler." J. Clin. Microbiol. **41**(2): 689-693.

http://jcm.asm.org/cgi/content/abstract/41/2/689

The diagnosis of human cases of tularemia often relies upon the demonstration of an antibody response to Francisella tularensis or the direct culturing of the bacteria from the patient. Antibody response is not detectable until 2 weeks or more after infection, and culturing requires special media and suspicion of tularemia. In addition, handling live Francisella poses a risk to laboratory personnel due to the highly infectious nature of this pathogen. In an effort to develop a rapid diagnostic assay for tularemia, we investigated the use of TaqMan 5' hydrolysis fluorogenic PCR to detect the organism in tissues of infected mice. Mice were infected to produce respiratory tularemia. The fopA and tul4 genes of F. tularensis were amplified from infected spleen, lung, liver, and kidney tissues sampled over a 5-day period. The samples were analyzed using the laboratory-based Applied Biosystems International 7900 and the Smiths Detection-Edgewood BioSeeq, a hand-held portable fluorescence thermocycler designed for use in the field. A comparison of culturing and PCR for detection of bacteria in infected tissues shows that culturing was more sensitive than PCR. However, the results for culture take 72 h, whereas PCR results were available within 4 h. PCR was able to detect infection in all the tissues tested. Lung tissue showed the earliest response at 2 days when tested with the ABI 7900 and in 3 days when tested with the BioSeeq. The results were in agreement between the ABI 7900 and the BioSeeq when presented with the same sample. Template preparation may account for the loss of sensitivity compared to culturing techniques. The hand-held BioSeeq thermocycler shows promise as an expedient means of forward diagnosis of infection in the field.

Eshleman, S. H., G. Crutcher, et al. (2005). "Sensitivity and Specificity of the ViroSeq Human Immunodeficiency Virus Type 1 (HIV-1) Genotyping System for Detection of HIV-1 Drug Resistance Mutations by Use of an ABI PRISM 3100 Genetic Analyzer." J. Clin. Microbiol. **43**(2): 813-817.

http://jcm.asm.org/cgi/content/abstract/43/2/813

The ViroSeq human immunodeficiency virus type 1 (HIV-1) genotyping system is an integrated system for identification of drug resistance mutations in HIV-1 protease and reverse transcriptase (RT). Reagents are included for sample preparation, reverse transcription, PCR amplification, and sequencing. Software is provided to assemble and edit sequence data and to generate a drug resistance report. We determined the sensitivity and specificity of the ViroSeq system for mutation detection using an ABI PRISM 3100 genetic analyzer with a set of clinical samples and recombinant viruses. Twenty clinical plasma samples (viral loads, 1,800 to 10,500 copies/ml) were characterized by cloning and sequencing individual viral variants. Twelve recombinant-virus samples (viral loads, approximately 2,000 to 5,000 copies/ml) were also prepared. Eleven recombinant-virus samples contained drug resistance mutations as 40% mixtures. One recombinant-virus sample contained an insertion at codon 69 in RT (100% mutant). Plasma and recombinant-virus samples were analyzed using the ViroSeq system. Each sample was analyzed on three consecutive days at each of three testing laboratories. The sensitivity of mutation

detection was 99.65% for the clinical plasma samples and 99.7% for the recombinant-virus preparations. The specificity of mutation detection was 99.95% for the clinical samples and 100% for the recombinant-virus mixtures. The base calling accuracy of the 3100 instrument was 99.91%. Mutations in clinical plasma samples and recombinant-virus samples were detected with high sensitivity and specificity, including mutations present as mixtures. This report supports the use of the ViroSeq system for identification of drug resistance mutations in HIV-1 protease and RT genes.

Eshleman, S. H., J. Hackett, Jr., et al. (2004). "Performance of the Celera Diagnostics ViroSeq HIV-1 Genotyping System for Sequence-Based Analysis of Diverse Human Immunodeficiency Virus Type 1 Strains." J. Clin. Microbiol. **42**(6): 2711-2717.

http://jcm.asm.org/cgi/content/abstract/42/6/2711

The Celera Diagnostics ViroSeq HIV-1 Genotyping System is a Food and Drug Administration-cleared, integrated system for sequence-based analysis of drug resistance mutations in subtype B human immunodeficiency virus type 1 (HIV-1) protease and reverse transcriptase (RT). We evaluated the performance of this system for the analysis of diverse HIV-1 strains. Plasma samples were obtained from 126 individuals from Uganda, Cameroon, South Africa, Argentina, Brazil, and Thailand with viral loads ranging from 2.92 to >6.0 log10 copies/ml. HIV-1 genotyping was performed with the ViroSeq system. HIV-1 subtyping was performed by using phylogenetic methods. PCR products suitable for sequencing were obtained for 125 (99%) of the 126 samples. Genotypes including protease (amino acids 1 to 99) and RT (amino acids 1 to 321) were obtained for 124 (98%) of the samples. Full bidirectional sequence data were obtained for 95 of those samples. The sequences were categorized into the following subtypes: A1/A2 (16 samples), B (12 samples), C (13 samples), D (11 samples), CRF01_AE (9 samples), F/F2 (9 samples), G (7 samples), CRF02_AG (32 samples), H (1 sample), and intersubtype recombinant (14 samples). The performances of the individual sequencing primers were examined. Genotyping of duplicate samples in a second laboratory was successful for 124 of the 126 samples. The identity level for the sequence data from two laboratories ranged from 98 to 100% (median, 99.8%). The ViroSeq system performs well for the analysis of plasma samples with diverse non-B subtypes. The availability of this genotyping system should facilitate studies of HIV-1 drug resistance in non-subtype B strains of HIV-1.

Fiebelkorn, K. R., S. A. Crawford, et al. (2003). "Practical Disk Diffusion Method for Detection of Inducible Clindamycin Resistance in Staphylococcus aureus and Coagulase-Negative Staphylococci." J. Clin. Microbiol. **41**(10): 4740-4744.

http://jcm.asm.org/cgi/content/abstract/41/10/4740

Resistance to macrolides in staphylococci may be due to active efflux (encoded by msrA) or ribosomal target modification (macrolide-lincosamide-streptogramin B [MLSB] resistance; usually encoded by ermA or ermC). MLSB resistance is either constitutive or inducible following exposure to a macrolide. Induction tests utilize closely approximated erythromycin and clindamycin disks; the flattening of the clindamycin zone adjacent to the erythromycin disk indicates inducible MLSB resistance. The present study reassessed the reliability of placing erythromycin and clindamycin disks in adjacent positions (26 to 28 mm apart) in a standard disk dispenser, compared to distances of 15 or 20 mm. A group of 130 clinical isolates of Staphylococcus aureus and 100 isolates of erythromycin-resistant coagulase-negative staphylococci (CNS) were examined by disk approximation; all CNS isolates and a subset of S. aureus isolates were examined by PCR for ermA, ermC, and msrA. Of 114 erythromycin-resistant S. aureus isolates, 39 demonstrated constitutive resistance to clindamycin, while 33 showed inducible resistance by disk

approximation at all three distances. Only one isolate failed to clearly demonstrate induction at 26 mm. Of 82 erythromycin-resistant CNS isolates that contained ermA or ermC, 57 demonstrated constitutive clindamycin resistance, and 25 demonstrated inducible resistance, at 20 and 26 mm. None of the 42 S. aureus isolates or 18 CNS isolates containing only msrA and none of the erythromycin-susceptible isolates yielded positive disk approximation tests. Simple placement of erythromycin and clindamycin disks at a distance achieved with a standard disk dispenser allowed detection of 97% of S. aureus strains and 100% of CNS strains with inducible MLSB resistance in this study.

Fischer, A., C. Lejczak, et al. (2004). "Simple DNA Extraction Method for Dried Blood Spots and Comparison of Two PCR Assays for Diagnosis of Vertical Human Immunodeficiency Virus Type 1 Transmission in Rwanda." J. Clin. Microbiol. **42**(1): 16-20.

http://jcm.asm.org/cgi/content/abstract/42/1/16

Dried blood spots (DBS) on filter paper facilitate the collection, transport, and storage of blood samples for laboratory use. A rapid and simple DNA extraction procedure from DBS was developed and evaluated for the diagnosis of human immunodeficiency virus type 1 (HIV-1) infection in children by an in-house nested-PCR assay on three genome regions and by the Amplicor HIV-1 DNA prototype assay version 1.5 (Roche Molecular Systems). A total of 150 samples from children born to HIV-1-infected mothers were collected in Kigali, Rwanda, in parallel as DBS and as peripheral blood mononuclear cell (PBMC) pellets. The results obtained on DBS by the two PCR assays were compared to the results of nested PCR on PBMCs. Of 150 PBMC samples, 10 were positive, 117 were negative, and 23 were indeterminate for HIV-1 infection. In DNA extracted from filter papers and amplified by using the in-house nested PCR, 9 of these 10 positive samples (90%) were found to be positive, and 1 was found to be indeterminate (only the pol region could be amplified). All of the negative samples and all of the 23 indeterminate samples tested negative for HIV-1 infection. When we used the Amplicor DNA test on DBS, all of the 10 PBMC-positive samples were found to be positive and all of the 23 indeterminate samples were found to be negative. Of the PBMC-negative samples, 115 were found to be negative and 2 were found to be indeterminate. We conclude that this simple rapid DNA extraction method on DBS in combination with both detection methods gave a reliable molecular diagnosis of HIV-1 infection in children born to HIV-infected mothers.

Flaherty, J. D., P. N. Levett, et al. (2003). "Fatal Case of Endocarditis Due to Weissella confusa." J. Clin. Microbiol. **41**(5): 2237-2239.

http://jcm.asm.org/cgi/content/abstract/41/5/2237

This is the first reported case of endocarditis due to the Lactobacillus-like vancomycin-resistant gram-positive bacillus Weissella confusa. Full identification and susceptibility testing of Lactobacillus-like organisms recovered in blood culture should be performed for patients with clinical presentations that suggest endocarditis.

Fournier, P.-E., Y. Zhu, et al. (2004). "Use of Highly Variable Intergenic Spacer Sequences for Multispacer Typing of Rickettsia conorii Strains." J. Clin. Microbiol. **42**(12): 5757-5766.

http://jcm.asm.org/cgi/content/abstract/42/12/5757

By use of the nearly perfectly colinear genomes of Rickettsia conorii and Rickettsia prowazekii, we compared the usefulness of three types of sequences for typing of R. conorii isolates: (i) 5 variable coding genes comprising the 16S ribosomal DNA, gltA, ompB, and sca4 (gene D) genes, which are present in both genomes, and the ompA gene, which is degraded in R. prowazekii; (ii) 28 genes degraded in R. conorii but intact in R. prowazekii, including 23 split and 5 remnant genes; and (iii) 27 conserved and 25 variable intergenic spacers. The 4 conserved and 23 split genes as well as the 27 conserved intergenic spacers each had identical sequences in 34 human and 5 tick isolates of R. conorii. Analysis of the ompA sequences identified three genotypes of R. conorii. The variable intergenic spacers were significantly more variable than conserved genes, split genes, remnant genes, and conserved spacers (P < 10-2 in all cases). Four of the variable intergenic spacers (dksA-xerC, mppA-purC, rpmE-tRNAfMet, and tRNAGly-tRNATyr) had highly variable sequences; when they were combined for typing, multispacer typing (MST) identified 27 different genotypes in the 39 R. conorii isolates. Two batches from the same R. conorii strain, Malish (Seven), with different culture passage histories were found to exhibit the same MST type. MST was more discriminatory for strain genotyping than multiple gene sequencing (P < 10-2). Phylogenetic analysis based on MST sequences was concordant with the geographic origins of R. conorii isolates. Our study supports the usefulness of MST for strain genotyping. This tool may be useful for tracing a strain and identifying its source during outbreaks, including those resulting from bioterrorism.

Fukushima, M., K. Kakinuma, et al. (2003). "Detection and Identification of Mycobacterium Species Isolates by DNA Microarray." J. Clin. Microbiol. **41**(6): 2605-2615.

http://jcm.asm.org/cgi/content/abstract/41/6/2605

Rapid identification of Mycobacterium species isolates is necessary for the effective management of tuberculosis. Recently, analysis of DNA gyrase B subunit (gyrB) genes has been identified as a suitable means for the identification of bacterial species. We describe a microarray assay based on gyrB gene sequences that can be used for the identification of Mycobacteria species. Primers specific for a gyrB gene region common to all mycobacteria were synthesized and used for PCR amplification of DNA purified from clinical samples. A set of oligonucleotide probes for specific gyrB gene regions was developed for the identification of 14 Mycobacterium species. Each probe was spotted onto a silylated glass slide with an arrayer and used for hybridization with fluorescently labeled RNA derived from amplified sample DNA to yield a pattern of positive spots. This microarray produced unique hybridization patterns for each species of mycobacteria and could differentiate closely related bacterial species. Moreover, the results corresponded well with those obtained by the conventional culture method for the detection of mycobacteria. We conclude that a gyrB-based microarray can rapidly detect and identify closely related mycobacterial species and may be useful in the diagnosis and effective management of tuberculosis.

Fukushima, M., K. Kakinuma, et al. (2002). "Phylogenetic Analysis of Salmonella, Shigella, and Escherichia coli Strains on the Basis of the gyrB Gene Sequence." J. Clin. Microbiol. **40**(8): 2779-2785.

http://jcm.asm.org/cgi/content/abstract/40/8/2779

Phylogenetic analysis of about 200 strains of Salmonella, Shigella, and Escherichia coli was carried out using the nucleotide sequence of the gene for DNA gyrase B (gyrB), which was determined by directly sequencing PCR fragments. The results establish a new phylogenetic tree for the classification of Salmonella, Shigella, and Escherichia coli in which Salmonella forms a cluster separate from but closely related to Shigella and E. coli. In comparison with 16S rRNA

analysis, the gyrB sequences indicated a greater evolutionary divergence for the bacteria. Thus, in screening for the presence of bacteria, the gyrB gene might be a useful tool for differentiating between closely related species of bacteria such as Shigella spp. and E. coli. At present, 16S rRNA sequence analysis is an accurate and rapid method for identifying most unknown bacteria to the genus level because the highly conserved 16S rRNA region is easy to amplify; however, analysis of the more variable gyrB sequence region can identify unknown bacteria to the species level. In summary, we have shown that gyrB sequence analysis is a useful alternative to 16S rRNA analysis for constructing the phylogenetic relationships of bacteria, in particular for the classification of closely related bacterial species.

Gadea, I., M. Cuenca-Estrella, et al. (2004). "Genotyping and Antifungal Susceptibility Profile of Dipodascus capitatus Isolates Causing Disseminated Infection in Seven Hematological Patients of a Tertiary Hospital." J. Clin. Microbiol. **42**(4): 1832-1836.

http://jcm.asm.org/cgi/content/abstract/42/4/1832

Seven cases of disseminated infection due to Dipodascus capitatus are reported. Infections occurred in a hematological unit of a tertiary hospital during a period of 5 years. Five cases were refractory to antifungal therapy. Antifungal susceptibility testing of seven isolates was performed, and strains were typed by PCR fingerprinting with the core sequence of phage M13 and by random amplification of polymorphic DNA with two primers, Ap12h and W-80A. A very short range of MICs of each antifungal agent was observed. The MICs of amphotericin B ranged between 0.50 and 2 {micro}g/ml. Strains were susceptible in vitro to flucytosine and susceptible (dose-dependent) to fluconazole and itraconazole. Voriconazole exhibited an activity in vitro comparable to that of itraconazole. Typing techniques allowed seven additional isolates of D. capitatus neither geographically nor temporally related to be classified into two different genomic patterns. The genomic type of the seven strains from the hematological unit was identical regardless of typing technique utilized. It would indicate that the seven cases of disseminated infection could be related epidemiologically.

Gafan, G. P., V. S. Lucas, et al. (2004). "Prevalence of Periodontal Pathogens in Dental Plaque of Children." J. Clin. Microbiol. **42**(9): 4141-4146.

http://jcm.asm.org/cgi/content/abstract/42/9/4141

Porphyromonas gingivalis, Actinobacillus actinomycetemcomitans, and Tannerella forsythensis have been implicated as the main etiological agents of periodontal disease. The purpose of this work was to estimate the prevalence of these organisms in plaque from children without gingivitis (group 1; n = 65) and from those with gingivitis (group 2; n = 53). Extracted DNA from plaque was subjected to two rounds of PCR targeting the 16S rRNA gene using both universal primers and species-specific primers. The results were as follows: group 1, P. gingivalis, 49%; A. actinomycetemcomitans, 55%; and T. forsythensis, 65%; group 2, P. gingivalis, 47%; A. actinomycetemcomitans, 59%; and T. forsythensis, 45%. T. forsythensis was detected more frequently in children with no gingivitis than in those with gingivitis (P = 0.03). There was no significant difference between the two groups with respect to the presence of P. gingivalis or A. actinomycetemcomitans in either group (P > 0.05). Logistic regression analysis revealed that the odds of a patient having gingivitis were 2.3 times greater in the absence of T. forsythensis. In conclusion, the results of this study have shown that the three pathogens can be detected in the dental plaque of healthy children and of those with gingivitis and that T. forsythensis is associated with dental plaque at sites with no gingivitis.

Gaia, V., N. K. Fry, et al. (2003). "Sequence-Based Typing of Legionella pneumophila Serogroup 1 Offers the Potential for True Portability in Legionellosis Outbreak Investigation." J. Clin. Microbiol. **41**(7): 2932-2939.

http://jcm.asm.org/cgi/content/abstract/41/7/2932

Seven gene loci of Legionella pneumophila serogroup 1 were analyzed as potential epidemiological typing markers to aid in the investigation of legionella outbreaks. The genes chosen included four likely to be selectively neutral (acn, groES, groEL, and recA) and three likely to be under selective pressure (flaA, mompS, and proA). Oligonucleotide primers were designed to amplify 279- to 763-bp fragments from each gene. Initial sequence analysis of the seven loci from 10 well-characterized isolates of L. pneumophila serogroup 1 gave excellent reproducibility (R) and epidemiological concordance (E) values (R = 1.00; E = 1.00). The three loci showing greatest discrimination and nucleotide variation, flaA, mompS, and proA, were chosen for further study. Indices of discrimination (D) were calculated using a panel of 79 unrelated isolates. Single loci gave D values ranging from 0.767 to 0.857, and a combination of all three loci resulted in a D value of 0.924. When all three loci were combined with monoclonal antibody subgrouping, the D value was 0.971. Sequence-based typing of L. pneumophila serogroup 1 using only three loci is epidemiologically concordant and highly discriminatory and has the potential to become the new "gold standard" for the epidemiological typing of L. pneumophila.

Galli, R. A., B. Sattha, et al. (2003). "Sources and Magnitude of Intralaboratory Variability in a Sequence-Based Genotypic Assay for Human Immunodeficiency Virus Type 1 Drug Resistance." J. Clin. Microbiol. **41**(7): 2900-2907.

http://jcm.asm.org/cgi/content/abstract/41/7/2900

We assessed the intralaboratory reproducibility of a system for sequencing human immunodeficiency virus type 1 (HIV-1) protease (PR) and reverse transcriptase (RT) by using replicate subanalyses of 46 plasma samples collected from HIV-1-infected, antiretroviral-experienced patients in order to determine the relative contributions of the different procedural steps to final sequence variability. Complete sequence concordance between duplicates of each sample was 99.4%. Complete and partial mismatches occurred scattered throughout the PR-RT genome segment at >300 positions. Approximately 75% of the discordances involved mixtures, some of which appeared at key resistance sites. Most differences were the result of the first-round RT-PCR procedure. Inter-rater concordance for sequence analysis and assembly was >99.9%. There was no observed correlation between the number or frequency of mismatches and plasma viral loads. A separate longitudinal analysis of a single routine control sample sequenced 103 times over 9 months consistently gave highly reproducible sequences (median percentage of nucleotide discordances, 0.04%; range, 0 to 0.2%). Finally, sequence data from 168 sequential samples collected from 22 patients with long-term, predominantly wild type HIV showed that intrapatient nucleotide concordance with individual index sequences ranged from 96.5 to 100%. Together, these results confirm that sequence-based genotyping can be a precise and reliable tool for monitoring HIV drug resistance, and they suggest that efforts to reduce variability should focus on the first RT-PCR step. Consequently, the data suggest that the composition of external quality assessment panels should be based on clinical HIV isolates rather than DNA clones.

Gault, E., P. Soussan, et al. (2003). "Evaluation of a New Serotyping Assay for Detection of Anti-Hepatitis

C Virus Type-Specific Antibodies in Serum Samples." J. Clin. Microbiol. **41**(5): 2084-2087.

http://jcm.asm.org/cgi/content/abstract/41/5/2084

The performance of a new version (HC03) of the hepatitis C virus (HCV) serotyping 1-6 assay (Abbott Murex Laboratories), a specific test for serological determination of HCV types, was evaluated using a selected panel of 180 HCV RNA-positive sera. HC03 was more sensitive than the current HC02 version, typing 53 (37.6%) of 141 samples which were not typable with HC02. Furthermore, the HC03 specificity was 94.1% as evaluated with a panel of 22 genotyped samples. This new version of the test improves the quality of the serological approach to HCV type determination.

Gautier, A.-L., D. Dubois, et al. (2005). "Rapid and Accurate Identification of Human Isolates of Pasteurella and Related Species by Sequencing the sodA Gene." J. Clin. Microbiol. **43**(5): 2307-2314.

http://jcm.asm.org/cgi/content/abstract/43/5/2307

The identification of Pasteurella and related bacteria remains a challenge. Here, a 449- to 473-bp fragment (sodAint) internal to the sodA gene, encoding the manganese-dependent superoxide dismutase, was amplified and sequenced with a single pair of degenerate primers from the type strains of Pasteurella (18 strains), Gallibacterium (1 strain), and Mannheimia (5 strains) species. The sodAint-based phylogenetic tree was in general agreement with that inferred from the analysis of the corresponding 16S rRNA gene sequences, with members of the Pasteurella sensu stricto cluster (Pasteurella multocida, Pasteurella canis, Pasteurella dagmatis, and Pasteurella stomatis) forming a monophyletic group and Gallibacterium and Mannheimia being independent monophyletic genera. However, the sodAint sequences showed a markedly higher divergence than the corresponding 16S rRNA genes, confirming that sodA is a potent target to differentiate related species. Thirty-three independent human clinical isolates phenotypically assigned to 13 Pasteurella species by a reference laboratory were successfully identified by comparing their sodAint sequences to those of the type species. In the course of this work, we identified the first Gallibacterium anatis isolate ever reported from a human clinical specimen. The sodAint sequences of the clinical isolates displayed less than 2.5% divergence from those of the corresponding type strains, except for the Pasteurella pneumotropica isolates, which were closely related to each other (>98% sodAint sequence identity) but shared only 92% sodAint identity with the type strain. The method described here provides a rapid and accurate tool for species identification of Pasteurella isolates when access to a sequencing facility is available.

Ghosh, M. K., L. Kuhn, et al. (2003). "Quantitation of Human Immunodeficiency Virus Type 1 in Breast Milk." J. Clin. Microbiol. **41**(6): 2465-2470.

http://jcm.asm.org/cgi/content/abstract/41/6/2465

The distribution and stability of human immunodeficiency virus type 1 (HIV-1) in breast milk (BM) components remain largely unknown. Inhibitory effects, if any, of BM on HIV RNA and DNA PCR amplification are poorly understood. We have addressed these issues by using virus-spiked BM samples from HIV-negative women. BM samples from HIV-negative women were spiked with HIV-1 virions or cells containing a single integrated copy of HIV DNA (8E5/LAV). After incubation under different experimental conditions, viral RNA was detected by the Roche Amplicor UltraSensitive assay in whole-milk, skim milk, and lipid fractions. We found excellent correlation between HIV-1 input copy and recovery in whole milk ($r = 0.965$, $P < 0.0001$), skim milk ($r =$

0.972, P < 0.0001), and the lipid fraction (r = 0.905, P < 0.001). PCR inhibition was observed in less than 10% of the spiked samples. Similar levels of inhibition were noted in BM samples collected from HIV-infected women. HIV proviral DNA was detected in BM samples using real-time PCR (linear correlation between the threshold cycle versus log DNA copy number, >0.982). The effects of incubation duration and temperature and repeated freeze-thaw cycles on HIV RNA recovery were analyzed. HIV RNA levels were remarkably stable in whole milk after three freeze-thaw cycles and for up to 30 h at room temperature. Our findings improve the understanding of the dynamics of HIV detection in BM and the conditions for BM sample collection, storage, and processing.

Giovannelli, L., A. Lama, et al. (2004). "Detection of Human Papillomavirus DNA in Cervical Samples: Analysis of the New PGMY-PCR Compared To the Hybrid Capture II and MY-PCR Assays and a Two-Step Nested PCR Assay." J. Clin. Microbiol. **42**(8): 3861-3864.

http://jcm.asm.org/cgi/content/abstract/42/8/3861

The PGMY-PCR for human papillomavirus (HPV) was evaluated, in parallel with nested PCR (nPCR), in samples with noted Hybrid Capture II (HCII) and MY-PCR results. PGMY-PCR detected HPV DNA in 2.5% of HCII-negative-MY-PCR-negative samples and in 71.7% of HCII-positive-MY-PCR-negative samples; also, it detected the MY-PCR-negative-nPCR-negative types HPV-42, HPV-44, HPV-51, HPV-87, and HPV-89.

Gookin, J. L., A. J. Birkenheuer, et al. (2002). "Single-Tube Nested PCR for Detection of Tritrichomonasfoetus in Feline Feces." J. Clin. Microbiol. **40**(11): 4126-4130.

http://jcm.asm.org/cgi/content/abstract/40/11/4126

Tritrichomonas foetus, a venereal pathogen of cattle, was recently identified as an inhabitant of the large intestine in young domestic cats with chronic diarrhea. Recognition of the infection in cats has been mired by unfamiliarity with T. foetus in cats as well as misdiagnosis of the organisms as Pentatrichomonas hominis or Giardia sp. when visualized by light microscopy. The diagnosis of T. foetus presently depends on the demonstration of live organisms by direct microscopic examination of fresh feces or by fecal culturing. As T. foetus organisms are fastidious and fragile, routine flotation techniques and delayed examination and refrigeration of feces are anticipated to preclude the diagnosis in numerous cases. The objective of this study was to develop a sensitive and specific PCR test for the diagnosis of feline T. foetus infection. A single-tube nested PCR was designed and optimized for the detection of T. foetus in feline feces by using a combination of novel (TFITS-F and TFITS-R) and previously described (TFR3 and TFR4) primers. The PCR is based on the amplification of a conserved portion of the T. foetus internal transcribed spacer (ITS) region (ITS1 and ITS2) and the 5.8S rRNA gene. The absolute detection limit of the single-tube nested PCR was 1 organism, while the practical detection limit was 10 organisms per 200 mg of feces. Specificity was examined by using P. hominis, Giardia lamblia, and feline genomic DNA. Our results demonstrate that the single-tube nested PCR is ideally suited for (i) diagnostic testing of feline fecal samples that are found negative by direct microscopy and culturing and (ii) definitive identification of microscopically observable or cultivated organisms.

Gray, J., L. V. von Stedingk, et al. (2002). "Transmission Studies of Babesia microti in Ixodes ricinus Ticks and Gerbils." J. Clin. Microbiol. **40**(4): 1259-1263.

http://jcm.asm.org/cgi/content/abstract/40/4/1259

In order to investigate the possible role of Ixodes ricinus as a vector of zoonotic Babesia microti infection in Europe, a European rodent isolate (HK) and a zoonotic American isolate (GI) were studied in transmission experiments. PCR detected B. microti in the blood and spleens of infected gerbils (Meriones unguiculatus) and also in laboratory-induced infections of I. ricinus ticks. B. microti DNA was detected by PCR in all pooled samples of nymphs and the majority of adults that had fed as larvae and nymphs, respectively, on gerbils with acute infection of the European isolate, confirming that I. ricinus could serve as a vector in Europe. The American isolate, GI, proved to be equally infective for larval and nymphal I. ricinus as the HK strain, despite a very different appearance in gerbil erythrocytes. Nymphs infected with the HK and GI strains readily infected gerbils. In contrast to the finding in acute infections, ticks that fed on gerbils with chronic infections of HK and GI did not become infected. It was also found that the HK strain was not transmitted transovarially. The finding that a B. microti strain (GI) from a distant geographical region (United States) can infect and be transmitted by I. ricinus suggests that other European B. microti strains, in addition to the HK strain used here, are probably infective for I. ricinus, supporting the view that infection of humans with European B. microti may be a regular occurrence.

Greub, G. and D. Raoult (2002). ""Actinobaculum massiliae," a New Species Causing Chronic Urinary Tract Infection." J. Clin. Microbiol. **40**(11): 3938-3941.

http://jcm.asm.org/cgi/content/abstract/40/11/3938

We report on a new Actinobaculum species, "Actinobaculum massiliae," isolated from the urine of an elderly woman with recurrent cystitis. Its phenotypic pattern was similar to those of both of the other Actinobaculum species described to date. On 16S rRNA sequencing, the Marseille isolate shared 95% homology with Actinobaculum suis, 92 to 93% homology with Actinobaculum schaalii, 91 to 92% homology with Arcanobacterium spp., and 87 to 90% homology with Actinomyces species. A bootstrap value of 99% supports the node separating the Actinobaculum sp. from its closest neighbor (A. suis). In conclusion, on the basis of phenotypic, genotypic, and phylogenetic assessments, we show that the Marseille isolate is a previously unrecognized organism within the Actinobaculum genus, and we propose placement of the organism in the taxon "Actinobaculum massiliae."

Guinebretiere, M.-H., V. Broussolle, et al. (2002). "Enterotoxigenic Profiles of Food-Poisoning and Food-Borne Bacillus cereus Strains." J. Clin. Microbiol. **40**(8): 3053-3056.

http://jcm.asm.org/cgi/content/abstract/40/8/3053

The enterotoxigenic profiles of 51 B. cereus food-related strains were compared to those of 37 B. cereus food-poisoning strains. cytK and association of hbl-nhe-cytK enterotoxin genes were more frequent among diarrheal strains (73 and 63%) than among food-borne strains (37 and 33%). Unlike diarrheal strains, food-borne strains showed frequent nhe and hbl gene polymorphisms and were often low toxin producers.

Habib-Bein, N. F., W. H. Beckwith, III, et al. (2003). "Comparison of SmartCycler Real-Time Reverse Transcription-PCR Assay in a Public Health Laboratory with Direct Immunofluorescence and Cell Culture Assays in a Medical Center for Detection of Influenza A Virus." J. Clin. Microbiol. **41**(8):

3597-3601.

http://jcm.asm.org/cgi/content/abstract/41/8/3597

A single-tube real-time (fluorogenic) reverse transcription (RT)-PCR with the SmartCycler instrument (SmartCycler RT-PCR) for influenza A virus detection was evaluated with 238 respiratory specimens. Direct immunofluorescence antibody staining (DFA) and primary rhesus monkey kidney cell culture were performed on-site at Yale-New Haven Hospital. Specimens were transported to the Connecticut Department of Public Health Laboratory for real-time RT-PCR. Cell culture detected influenza A virus in all 150 influenza A virus-positive specimens, DFA detected the virus in 148 influenza A virus-positive specimens, and SmartCycler RT-PCR detected the virus 143 influenza A virus-positive specimens. The sensitivity and specificity of RT-PCR were 95.3 and 100%, respectively. The high sensitivity and specificity and the rapid turnaround time made the SmartCycler RT-PCR valuable for the rapid diagnosis of influenza A, especially in a public health laboratory. The closed real-time RT-PCR system avoided cross-contamination possible with RT-PCR and the excessive manipulations required for conventional RT-PCR analysis and saved time and labor as well. In a medical center, rapid diagnosis by DFA was labor intensive but was 98.7% sensitive and 100% specific compared to the results of culture and provided results within 2 h throughout operating hours, helping with bed allocation on admission and patient management.

Hall, L., K. A. Doerr, et al. (2003). "Evaluation of the MicroSeq System for Identification of Mycobacteria by 16S Ribosomal DNA Sequencing and Its Integration into a Routine Clinical Mycobacteriology Laboratory." J. Clin. Microbiol. **41**(4): 1447-1453.

http://jcm.asm.org/cgi/content/abstract/41/4/1447

An evaluation of the MicroSeq 500 microbial identification system by nucleic acid sequencing and the Mayo Clinic experience with its integration into a routine clinical laboratory setting are described. Evaluation of the MicroSeq 500 microbial identification system was accomplished with 59 American Type Culture Collection (ATCC) strains and 328 clinical isolates of mycobacteria identified by conventional and 16S ribosomal DNA sequencing by using the MicroSeq 500 microbial identification system. Nucleic acid sequencing identified 58 of 59 (98.3%) ATCC strains to the species level or to the correct group or complex level. The identification results for 219 of 243 clinical isolates (90.1%) with a distance score of <1% were concordant with the identifications made by phenotypic methods. The remaining 85 isolates had distance scores of >1%; 35 (41.1%) were identified to the appropriate species level or group or complex level; 13 (15.3%) were identified to the species level. All 85 isolates were determined to be mycobacterial species, either novel species or species that exhibited significant genotypic divergence from an organism in the database with the closest match. Integration of nucleic acid sequencing into the routine mycobacteriology laboratory and use of the MicroSeq 500 microbial identification system and Mayo Clinic databases containing additional genotypes of common species and added species significantly reduced the number of organisms that could not be identified by phenotypic methods. The turnaround time was shortened to 24 h, and results were reported much earlier. A limited number of species could not be differentiated from one another by 16S ribosomal DNA sequencing; however, the method provides for the identification of unusual species and more accurate identifications and offers the promise of being the most accurate method available.

Harper, C. G., Y. Feng, et al. (2002). "Helicobacter cetorum sp. nov., a Urease-Positive Helicobacter Species Isolated from Dolphins and Whales." J. Clin. Microbiol. **40**(12): 4536-4543.

http://jcm.asm.org/cgi/content/abstract/40/12/4536

A novel helicobacter with the proposed name Helicobacter cetorum, sp. nov. (type strain MIT 99-5656; GenBank accession number AF 292378), was cultured from the main stomach of two wild, stranded Atlantic white-sided dolphins (Lagenorhynchus acutus) and from the feces of three captive cetaceans (a Pacific white-sided dolphin [Lagenorhynchus obliquidens]; an Atlantic bottlenose dolphin [Tursiops truncatus]; and a beluga whale [Delphinapterus leucas]). The infected captive cetaceans were either subclinical, or clinical signs included intermittent regurgitation, inappetance, weight loss, and lethargy. Ulcers were observed in the esophagus and forestomach during endoscopic examination in two of the three captive animals. In the third animal, esophageal linear erosions were visualized endoscopically, and histopathological evaluation of the main stomach revealed multifocal lymphoplasmacytic gastritis with silver-stained spiral-shaped bacteria. Helicobacter cetorum is a fusiform gram-negative bacterium with a single bipolar flagellum. The isolates grow under microaerobic conditions at 37 and 42{degrees}C but not at 25{degrees}C. H. cetorum is urease, catalase, and oxidase positive, and it is sensitive to cephalothin. The isolates from the wild, stranded dolphins were sensitive to nalidixic acid, whereas the isolates from the collection animals were resistant. By 16S rRNA sequencing it was determined that H. cetorum represented a distinct taxon that clusters most closely with H. pylori. Further studies are necessary to determine the role of H. cetorum in the development of gastric ulcers and gastritis of cetaceans. This is the first description and formal naming of a novel Helicobacter species from a marine mammal.

Hartman, A. B., I. I. Essiet, et al. (2003). "Epidemiology of Tetracycline Resistance Determinants in Shigella spp. and Enteroinvasive Escherichia coli: Characterization and Dissemination of tet(A)-1." J. Clin. Microbiol. **41**(3): 1023-1032.

http://jcm.asm.org/cgi/content/abstract/41/3/1023

To make a comprehensive study of tetracycline resistance determinant distribution in the genus Shigella, a collection of 577 clinical isolates of Shigella spp. and enteroinvasive Escherichia coli (EIEC) from a variety of geographical locations was screened to identify tetracycline-resistant strains. The 459 tetracycline-resistant isolates identified were then screened by PCR analysis to determine the distribution in these strains of tetracycline efflux resistance determinants belonging to classes A to E, G, and H that have been identified in gram-negative bacteria. Only classes A to D were represented in these strains. Although Tet B was the predominant determinant in all geographical locations, there were geographical and species differences in the distribution of resistance determinants. An allele of tet(A), designated tet(A)-1, was identified and sequenced, and the 8.6-kb plasmid containing determinant Tet A-1, designated pSSTA-1, was found to have homologies to portions of a Salmonella enterica cryptic plasmid and the broad-host-range resistance plasmid RSF1010. This allele and pSSTA-1 were used as epidemiological markers to monitor clonal and horizontal transmission of determinant Tet A-1. An analysis of serotype, distribution of tetracycline resistance determinants, and resistance profiles indicated that both clonal spread and horizontal transfer had contributed to the spread of specific tetracycline resistance determinants in these populations and demonstrated the use of these parameters as an epidemiological tool to follow the transmission of determinants and strains.

Hawkey, P. M., E. G. Smith, et al. (2003). "Mycobacterial Interspersed Repetitive Unit Typing of Mycobacterium tuberculosis Compared to IS6110-Based Restriction Fragment Length Polymorphism Analysis for Investigation of Apparently Clustered Cases of Tuberculosis." J. Clin. Microbiol. **41**(8): 3514-3520.

http://jcm.asm.org/cgi/content/abstract/41/8/3514

An evaluation of the utility of IS6110-based restriction fragment length polymorphism (RFLP) typing compared to a combination of variable number tandem repeat (VNTR) typing and mycobacterial interspersed repetitive unit (MIRU) typing was undertaken. A total of 53 patient isolates of Mycobacterium tuberculosis from four presumed episodes of cross-infection were examined. Genomic DNA was extracted from the isolates by a cetyl trimethylammonium bromide method. The number of copies of tandem repeats of the five loci ETRA to ETRE and 12 MIRU loci was determined by PCR amplification and agarose gel electrophoresis of the amplicons. VNTR typing identified the major clusters of strains in the three investigations in which they occurred (each representing a different evolutionary clade: 32333, 42235, and 32433). The majority of unrelated isolates (by epidemiology and RFLP typing) were also identified by VNTR typing. The concordance between the RFLP and MIRU typing was complete, with the exception of two isolates with RFLP patterns that differed by one band each from the rest of the major epidemiologically linked groups of isolates in investigation A. All of these isolates had identical MIRU and VNTR types. A further pair of isolates differed in the number of tandem repeat copies at two MIRU alleles but had identical RFLP patterns. The speed of the combined VNTR and MIRU typing approach enabled results for some of the investigations to be supplied in "real time," influencing choices in contact tracing. The ease of comparison of results of MIRU and VNTR typing, which are recorded as single multidigit numbers, was also found to greatly facilitate investigation management and the communication of results to health care professionals.

Hazbon, M. H. and D. Alland (2004). "Hairpin Primers for Simplified Single-Nucleotide Polymorphism Analysis of Mycobacterium tuberculosis and Other Organisms." J. Clin. Microbiol. **42**(3): 1236-1242.

http://jcm.asm.org/cgi/content/abstract/42/3/1236

We describe a novel, simple, rapid, and highly sensitive method to detect single-nucleotide polymorphisms (SNPs) in Mycobacterium tuberculosis and other organisms. Amplification refractory mutation (ARMS) SNP assays were modified by converting the SNP-detecting linear primers in the ARMS assay to hairpin-shaped primers (HPs) through the addition of a 5' tail complementary to the 3' end of the linear primer. The improved ability of these primers to detect SNPs in M. tuberculosis was compared in a real-time PCR with SYBR-I green dye. Linear primers resulted in incorrect or indeterminate allele designation for 6 of the 13 SNP alleles tested in seven different SNP assays, while HPs determined the correct SNP in all cases. We compared the cycle threshold differences ({Delta}Ct) between the reactions containing primer-template matches and the reactions containing primer-template mismatches (where a larger {Delta}Ct indicates a more robust assay). The use of HPs dramatically improved the mean {Delta}Ct values for the SNP assays (7.6 for linear primers and 11.2 for HPs). We designed 98 different HP assays for SNPs previously associated with resistance to the antibiotic isoniazid to test the large-scale utility of the HP approach. Assay design was successful in 72.4%, 83.7%, 88.8%, and 92.9% of the assays after one to four rounds of assay design, respectively. HP SNP assays are simple, sensitive, robust, and inexpensive. These advantages favor the application of this technique for SNP assays of M. tuberculosis and other organisms.

Healy, M., K. Reece, et al. (2004). "Identification to the Species Level and Differentiation between Strains of Aspergillus Clinical Isolates by Automated Repetitive-Sequence-Based PCR." J. Clin. Microbiol. **42**(9): 4016-4024.

http://jcm.asm.org/cgi/content/abstract/42/9/4016

A commercially available repetitive-sequence-based PCR (rep-PCR) DNA fingerprinting assay adapted to an automated format, the DiversiLab system, enables rapid microbial identification

and strain typing. We explored the performance of the DiversiLab system as a molecular typing tool for 69 Aspergillus isolates (38 A. fumigatus, 15 A. flavus, and 16 A. terreus isolates) had been previously characterized by morphological analysis. Initially, 27 Aspergillus isolates (10 A. fumigatus, 9 A. flavus, and 8 A. terreus isolates) were used as controls to create a rep-PCR-based DNA fingerprint library with the DiversiLab software. Then, 42 blinded Aspergillus isolates were typed using the system. The rep-PCR-based profile revealed 98% concordance with morphology-based identification. rep-PCR-based DNA fingerprints were reproducible and were consistent for DNA from both hyphae and conidia. DiversiLab dendrogram reports correctly identified all A. fumigatus (n = 28), A. terreus (n = 8), and A. flavus (n = 6) isolates in the 42 blinded Aspergillus isolates. rep-PCR-based identification of all isolates was 100% in agreement with the contiguous internal transcribed spacer (ITS) region (ITS1-5.8S-ITS2) sequence-based identification of the respective isolates. Additionally, the DiversiLab system could demonstrate strain-level differentiation of A. flavus and A. terreus. Automated rep-PCR may be a time-efficient, effective, easy-to-use, novel genotyping tool for identifying and determining the strain relatedness of fungi. This system may be useful for epidemiological studies, molecular typing, and surveillance of Aspergillus species.

Heikkila, T., I. Seppala, et al. (2002). "Species-Specific Serodiagnosis of Lyme Arthritis and Neuroborreliosis Due to Borrelia burgdorferi Sensu Stricto, B. afzelii, and B. garinii by Using Decorin Binding Protein A." J. Clin. Microbiol. **40**(2): 453-460.

http://jcm.asm.org/cgi/content/abstract/40/2/453

The antigenic potential of decorin binding protein A (DbpA) was evaluated in serodiagnosis of human Lyme borreliosis (LB). The dbpA was cloned and sequenced from the three pathogenic Borrelia species common in Europe. Sequence analysis revealed high interspecies heterogeneity. The identity of the predicted amino acid sequences was 43 to 62% among Borrelia burgdorferi sensu stricto, B. afzelii, and B. garinii. The respective recombinant DbpAs (rDbpAs) were produced and tested as antigens by Western blotting and enzyme-linked immunosorbent assay (ELISA). One hundred percent of patients with neuroborreliosis (NB) and 93% of patients with Lyme arthritis (LA) reacted positively. Sera from the majority of patients reacted with one rDbpA only and had no or low cross-reactivity to other two variant proteins. In patients with culture-positive erythema migrans (EM), the sensitivity of rDbpA immunoglobulin G (IgG) or IgM ELISA was low. The DbpA seems to be a sensitive and specific antigen for the serodiagnosis of LA or NB, but not of EM, provided that variants from all three pathogenic borrelial species are included in the combined set of antigens.

Heininger, A., M. Binder, et al. (2003). "DNase Pretreatment of Master Mix Reagents Improves the Validity of Universal 16S rRNA Gene PCR Results." J. Clin. Microbiol. **41**(4): 1763-1765.

http://jcm.asm.org/cgi/content/abstract/41/4/1763

DNase I pretreatment of 16S rRNA gene PCR reagents was tested. The DNase I requirement for the elimination of false-positive results varied between 0.1 and 70 IU per master mix depending on the applied Taq polymerase. PCR sensitivity was mostly maintained when 0.1 IU of DNase I was used.

Heo, E.-j., J.-h. Park, et al. (2002). "Serologic and Molecular Detection of Ehrlichia chaffeensis and Anaplasma phagocytophila (Human Granulocytic Ehrlichiosis Agent) in Korean Patients." J. Clin.

Microbiol. **40**(8): 3082-3085.

http://jcm.asm.org/cgi/content/abstract/40/8/3082

Sera from 491 Korean patients with acute febrile diseases were tested for Ehrlichia chaffeensis and Anaplasma phagocytophila antibodies by indirect immunofluorescence assay (IFA), Western blotting, and TaqMan real-time PCR. Overall, 0.4% of sera reacted with E. chaffeensis, and 1.8% reacted with A. phagocytophila in IFAs. This is the first report of detection of antibodies to A. phagocytophila and E. chaffeensis in Korea and suggests the presence of A. phagocytophila and E. chaffeensis or antigenically similar species.

Herpers, B. L., B. M. de Jongh, et al. (2003). "Real-Time PCR Assay Targets the 23S-5S Spacer for Direct Detection and Differentiation of Legionella spp. and Legionella pneumophila." J. Clin. Microbiol. **41**(10): 4815-4816.

http://jcm.asm.org/cgi/content/abstract/41/10/4815

A real-time PCR for the ABI Prism 7000 system targeting the 23S-5S spacer of Legionella spp. was developed. Simultaneous detection and differentiation of Legionella spp. and Legionella pneumophila within 90 min and without post-PCR melting-curve analysis was achieved using two TaqMan probes. In sputum samples from 23 controls and 17 patients with legionellosis, defined by positive culture, urinary antigen testing, or seroconversion, 94% sensitivity and 100% specificity were observed.

Hindiyeh, M., V. Levy, et al. (2005). "Evaluation of a Multiplex Real-Time Reverse Transcriptase PCR Assay for Detection and Differentiation of Influenza Viruses A and B during the 2001-2002 Influenza Season in Israel." J. Clin. Microbiol. **43**(2): 589-595.

http://jcm.asm.org/cgi/content/abstract/43/2/589

The ability to rapidly diagnose influenza virus infections is of the utmost importance in the evaluation of patients with upper respiratory tract infections. It is also important for the influenza surveillance activities performed by national influenza centers. In the present study we modified a multiplex real-time reverse transcriptase PCR (RT-PCR) assay (which uses TaqMan chemistry) and evaluated it for its ability to detect and concomitantly differentiate influenza viruses A and B in 370 patient samples collected during the 2001-2002 influenza season in Israel. The performance of the TaqMan assay was compared to those of a multiplex one-step RT-PCR with gel detection, a shell vial immunofluorescence assay, and virus isolation in tissue culture. The TaqMan assay had an excellent sensitivity for the detection of influenza viruses compared to that of tissue culture. The overall sensitivity and specificity of the TaqMan assay compared to the results of culture were 98.4 and 85.5%, respectively. The sensitivity and specificity of the TaqMan assay for the detection of influenza virus A alone were 100 and 91.1%, respectively. On the other hand, the sensitivity and specificity for the detection of influenza virus B alone were 95.7 and 98.7%, respectively. The rapid turnaround time for the performance of the TaqMan assay (4.5 h) and the relatively low direct cost encourage the routine use of this assay in place of tissue culture. We conclude that the multiplex TaqMan assay is highly suitable for the rapid diagnosis of influenza virus infections both in well-established molecular biology laboratories and in reference clinical laboratories.

Hu, A., M. Colella, et al. (2003). "Simultaneous Detection, Subgrouping, and Quantitation of Respiratory Syncytial Virus A and B by Real-Time PCR." J. Clin. Microbiol. **41**(1): 149-154.

http://jcm.asm.org/cgi/content/abstract/41/1/149

Timely diagnosis of respiratory syncytial virus (RSV) infection is critical for appropriate treatment of lower respiratory infection in young children. To facilitate diagnosis, we developed a rapid, specific, and sensitive TaqMan PCR method for detection of RSV A and RSV B. Two sets of primer-probe pairs were selected from the nucleotide sequences encoding the nucleocapsid protein--one targeting RSV A and the other targeting RSV B. The specificity of the TaqMan reverse transcription-PCR assay was evaluated by testing each primer-probe pair against various viruses derived from laboratory virus stocks, as well as clinical respiratory specimens. Fluorescent signals were observed only in the presence of RSV A and/or RSV B. The sensitivity of our quantitative PCR assay was determined on the basis of PFU and virus particle counts. The resulting assay sensitivity was found to be 0.023 PFU, or two copies of viral RNA, for RSV A and 0.018 PFU, or nine copies of viral RNA, for RSV B. This quantitative TaqMan PCR assay was utilized to diagnose 175 nasopharyngeal aspirates obtained from children in Hong Kong with respiratory symptoms during the winter of 2000 and 2001. Among these specimens, TaqMan PCR detected 36 RSV-positive samples, 10 of which were identified as RSV A and 26 of which were identified as RSV B, whereas culture confirmation identified 21 RSV-positive specimens and immunofluorescence identified 32 RSV-positive specimens, all of which were among those identified by PCR. The results confirmed the accuracy of our TaqMan PCR assay and demonstrated its improved sensitivity versus classical methods.

Huang, F. F., G. Haqshenas, et al. (2002). "Heterogeneity and Seroprevalence of a Newly Identified Avian Hepatitis E Virus from Chickens in the United States." J. Clin. Microbiol. **40**(11): 4197-4202.

http://jcm.asm.org/cgi/content/abstract/40/11/4197

We recently identified and characterized a novel virus, designated avian hepatitis E virus (avian HEV), from chickens with hepatitis-splenomegaly syndrome (HS syndrome) in the United States. Avian HEV is genetically related to but distinct from human and swine HEVs. To determine the extent of genetic variation and the seroprevalence of avian HEV infection in chicken flocks, we genetically identified and characterized 11 additional avian HEV isolates from chickens with HS syndrome and assessed the prevalence of avian HEV antibodies from a total of 1,276 chickens of different ages and breeds from 76 different flocks in five states (California, Colorado, Connecticut, Virginia, and Wisconsin). An enzyme-linked immunosorbent assay using a truncated recombinant avian HEV ORF2 antigen was developed and used to determine avian HEV seroprevalence. About 71% of chicken flocks and 30% of chickens tested in the study were positive for antibodies to avian HEV. About 17% of chickens younger than 18 weeks were seropositive, whereas about 36% of adult chickens were seropositive. By using a reverse transcription-PCR (RT-PCR) assay, we tested 21 bile samples from chickens with HS syndrome in California, Connecticut, New York, and Wisconsin for the presence of avian HEV RNA. Of the 21 bile samples, 12 were positive for 30- to 35-nm HEV-like virus particles by electron microscopy (EM). A total of 11 of the 12 EM-positive bile samples and 6 of the 9 EM-negative bile samples were positive for avian HEV RNA by RT-PCR. The sequences of a 372-bp region within the helicase gene of 11 avian HEV isolates were determined. Sequence analyses revealed that the 11 field isolates of avian HEV had 78 to 100% nucleotide sequence identities to each other, 79 to 88% identities to the prototype avian HEV, 76 to 80% identities to chicken big liver and spleen disease virus, and 56 to 61% identities to other known strains of human and swine HEV. The data from this study indicated that, like swine and human HEVs, avian HEV isolates are genetically heterogenic and that avian HEV infection is enzoonotic in chicken flocks in the United States.

Huber, B. S., D. V. Allred, et al. (2002). "Random Amplified Polymorphic DNA and Amplified Fragment Length Polymorphism Analyses of Pasteurella multocida Isolates from Fatal Fowl Cholera Infections." J. Clin. Microbiol. **40**(6): 2163-2168.

http://jcm.asm.org/cgi/content/abstract/40/6/2163

Fowl cholera, a disease caused by Pasteurella multocida, continues to be a major problem for the poultry industry. The sources of pathogenic organisms responsible for most sporadic epidemics remain unconfirmed, although attenuated vaccines that retain a low level of virulence have occasionally been implicated in outbreaks of the disease. One of the vaccines most commonly used to prevent fowl cholera is the M-9 strain. In the present study, 61 clinical isolates from turkeys that died of fowl cholera from 1997 to 1999 on 36 Utah farms were analyzed and compared to the M-9 vaccine strain. Genetic analyses of the isolates were done by random amplified polymorphic DNA (RAPD) analysis and amplified fragment length polymorphism (AFLP) fingerprinting. The results of these genetic analyses were correlated with the vaccination status of the flock, isolate serotype, and geographic location. Although both genetic techniques effectively identified similar subtle genomic differences, RAPD analysis provided only 77% of the detail provided by AFLP analysis. While a relationship between genetic profile and serotype was evident, no significant relationship indicating geographic influence was found (P = 0.351). Interestingly, organisms isolated from vaccinated flocks were significantly closer genetically to the M-9 vaccine strain than isolates from unvaccinated birds were (P = 0.020). Statistical analyses revealed that this relationship could not have been determined by serotyping alone (P = 0.320), demonstrating the value of AFLP and RAPD analyses in the characterization of disease-causing strains.

Huijsdens, X. W., R. K. Linskens, et al. (2002). "Quantification of Bacteria Adherent to Gastrointestinal Mucosa by Real-Time PCR." J. Clin. Microbiol. **40**(12): 4423-4427.

http://jcm.asm.org/cgi/content/abstract/40/12/4423

The use of real-time quantitative PCR (5' nuclease PCR assay) as a tool to study the gastrointestinal microflora that adheres to the colonic mucosa was evaluated. We developed primers and probes based on the 16S ribosomal DNA gene sequences for the detection of Escherichia coli and Bacteroides vulgatus. DNA was isolated from pure cultures and from gut biopsy specimens and quantified by the 5' nuclease PCR assay. The assay showed a very high sensitivity: as little as 1 CFU of E. coli and 9 CFU of B. vulgatus could be detected. The specificities of the primer-probe combinations were evaluated with samples that were spiked with the species most closely related to E. coli and B. vulgatus and with eight other gut microflora species. Mucosal samples spiked with known amounts of E. coli or B. vulgatus DNA showed no PCR inhibition. We conclude that the 5' nuclease PCR assay may be a useful alternative to conventional culture techniques to study the actual in vivo composition of a complex microbial community like the gut microflora.

Hurtle, W., E. Bode, et al. (2004). "Detection of the Bacillus anthracis gyrA Gene by Using a Minor Groove Binder Probe." J. Clin. Microbiol. **42**(1): 179-185.

http://jcm.asm.org/cgi/content/abstract/42/1/179

Identification of chromosomal markers for rapid detection of Bacillus anthracis is difficult because significant chromosomal homology exists among B. anthracis, Bacillus cereus, and Bacillus thuringiensis. We evaluated the bacterial gyrA gene as a potential chromosomal marker for B.

anthracis. A real-time PCR assay was developed for the detection of B. anthracis. After analysis of the unique nucleotide sequence of the B. anthracis gyrA gene, a fluorescent 3' minor groove binding probe was tested with 171 organisms from 29 genera of bacteria, including 102 Bacillus strains. The assay was found to be specific for all 43 strains of B. anthracis tested. In addition, a test panel of 105 samples was analyzed to evaluate the potential diagnostic capability of the assay. The assay showed 100% specificity, demonstrating the usefulness of the gyrA gene as a specific chromosomal marker for B. anthracis.

Hurtle, W., L. Lindler, et al. (2003). "Detection and Identification of Ciprofloxacin-Resistant Yersinia pestis by Denaturing High-Performance Liquid Chromatography." J. Clin. Microbiol. **41**(7): 3273-3283.

http://jcm.asm.org/cgi/content/abstract/41/7/3273

Denaturing high-performance liquid chromatography (DHPLC) has been used extensively to detect genetic variation. We used this method to detect and identify Yersinia pestis KIM5 ciprofloxacin-resistant isolates by analyzing the quinolone resistance-determining region (QRDR) of the gyrase A gene. Sequencing of the Y. pestis KIM5 strain gyrA QRDR from 55 ciprofloxacin-resistant isolates revealed five mutation types. We analyzed the gyrA QRDR by DHPLC to assess its ability to detect point mutations and to determine whether DHPLC peak profile analysis could be used as a molecular fingerprint. In addition to the five mutation types found in our ciprofloxacin-resistant isolates, several mutations in the QRDR were generated by site-directed mutagenesis and analyzed to further evaluate this method for the ability to detect QRDR mutations. Furthermore, a blind panel of 42 samples was analyzed by screening for two mutant types to evaluate the potential diagnostic value of this method. Our results showed that DHPLC is an efficient method for detecting mutations in genes that confer antibiotic resistance.

Huy, T. T.-T., H. Ushijima, et al. (2003). "High Prevalence of Hepatitis B Virus Pre-S Mutant in Countries Where It Is Endemic and Its Relationship with Genotype and Chronicity." J. Clin. Microbiol. **41**(12): 5449-5455.

http://jcm.asm.org/cgi/content/abstract/41/12/5449

It has been reported that hepatitis B virus (HBV) mutants carrying mutations in the pre-S region can be found in infected patients. In this study, we investigated the prevalence of the HBV variant with the pre-S mutant in different geographic regions, including countries with low and high levels of endemic HBV infection, and analyzed the correlation with clinical findings. We examined 387 HBV DNA-positive serum samples from individuals among 12 countries, consisting of Vietnam, Myanmar, Thailand, China, Korea, Nepal, Japan, Russia, Spain, United States, Bolivia, and Ghana. HBV pre-S mutants were detected in 71 (18.3%) of 387 serum samples tested. This mutant was the most prevalent in Vietnam (36%), followed by Nepal (27.3%), Myanmar (23.3%), China (22.4%), Korea (14.3%), Thailand (10.5%), Japan (7.7%), and Ghana (4.3%). In contrast, no case with this mutation was found in Russia, Spain, United States, and Bolivia. Among the HBV deletion mutations, 15.5% (11 of 71) occurred in the pre-S1 and 46.5% (33 of 71) in the pre-S2 regions. Eight (11.3%) cases had a mutation in both the pre-S1 and pre-S2 regions. In addition, a point mutation at the pre-S2 starting codon was observed in 19 (26.7%) cases. The detection rate of the HBV mutant in patients with hepatocellular carcinoma was significantly higher than in other patients ($P < 0.05$). Furthermore, these mutants were found more frequently in genotype B (25%) and genotype C (24.5%) than in the other genotypes ($P < 0.05$). Our results indicated that there was a high prevalence of HBV pre-S mutation in regions of endemic HBV infection in Asia. Furthermore, the pre-S mutation appeared to be correlated with hepatocellular carcinoma and HBV genotypes.

Imada, Y., Y. Mori, et al. (2003). "Enzyme-Linked Immunosorbent Assay Employing a Recombinant Antigen for Detection of Protective Antibody against Swine Erysipelas." J. Clin. Microbiol. **41**(11): 5015-5021.

http://jcm.asm.org/cgi/content/abstract/41/11/5015

The specificities and sensitivities of five recombinant proteins of the surface protective antigen (SpaA) of Erysipelothrix rhusiopathiae were examined by indirect enzyme-linked immunosorbent assay (ELISA) with the aim of developing a reliable serological test for the detection of protective antibody against E. rhusiopathiae. Fully mature protein and the N-terminal 416 amino acids (SpaA416) showed sufficient antigenicities, and further examination was done with SpaA416 because of its higher yield. The antibody titers of pigs experimentally immunized with commercial live vaccine and two types of inactivated vaccines clearly increased after immunization, and all pigs were completely protected against challenge with virulent strains. On the other hand, the antibody titers of nonimmunized control pigs remained very low until they were challenged, and all showed severe symptoms or subsequently died. Interference with the production of antibody against live vaccine by maternal antibody or porcine respiratory and reproductive syndrome virus infection 1 week after vaccination was also clearly detected. Because the ELISA titer correlated well with the protection results, the specificity and sensitivity of the ELISA were further evaluated with sera collected from pigs reared on 1 farm on which animals had acute septicemia, 2 farms on which the animals were infected or free from infection, and 10 farms on which the animals were vaccinated with live vaccine, among others. The ELISA titers clearly revealed the conditions of the herds. These results indicate that the SpaA416 ELISA is an effective method not only for evaluating pigs for the presence of protective antibody levels resulting from vaccination or maternal antibody but also for detecting antibody produced by natural infection. This test has important potential for the effective control of swine erysipelas.

Imada, Y., A. Takase, et al. (2004). "Serotyping of 800 Strains of Erysipelothrix Isolated from Pigs Affected with Erysipelas and Discrimination of Attenuated Live Vaccine Strain by Genotyping." J. Clin. Microbiol. **42**(5): 2121-2126.

http://jcm.asm.org/cgi/content/abstract/42/5/2121

Eight hundred Erysipelothrix strains isolated between 1992 and 2002 from swine with erysipelas in Japan were serotyped. Thirty-seven, 47, 73, and 643 strains were isolated from animals with acute septicemia, urticaria, chronic endocarditis, and chronic arthritis, respectively, of which 381, 146, 254, and 19 isolates belonged to serotypes 1a, 1b, and 2b and other serotypes, respectively. All serotype 1a isolates were further examined for acriflavine resistance and their genotypes to discriminate them from the attenuated live vaccine strain, defined as serotype 1a, which is resistant to 0.02% acriflavine and which shows low levels of pathogenicity in mice. Of the serotype 1a isolates, 64.6% were acriflavine resistant, with 98.4% of these acriflavine-resistant strains having been isolated from animals with chronic arthritis. By randomly amplified polymorphic DNA (RAPD) analysis, almost all the acriflavine-resistant serotype 1a strains showed the 253-bp band characteristic of vaccine strains and were easily discriminated from all 113 strains of acriflavine-sensitive serotype 1a strains from animals with acute and subacute swine erysipelas. The incidence of acriflavine-resistant strains of the distinctive RAPD type 1-2 was markedly higher than that of the other RAPD types and serotypes. RAPD type 1-2 strains also included a specific group identifiable by restriction fragment length polymorphism DNA analysis. Furthermore, the pathogenicities of 29 isolates of RAPD type 1-2 for mice were lower than those of the 21 isolates of other RAPD types. Our results indicate that RAPD type 1-2 strains are live vaccine strains and that 37% of the cases of chronic swine erysipelas detected in the past 11 years in Japan have occurred as a side effect of live vaccine use.

Ito, Y., J.-C. Grivel, et al. (2003). "Real-Time PCR Assay of Individual Human Immunodeficiency Virus Type 1 Variants in Coinfected Human Lymphoid Tissues." J. Clin. Microbiol. **41**(5): 2126-2131.

http://jcm.asm.org/cgi/content/abstract/41/5/2126

In the course of infection, human immunodeficiency virus type 1 (HIV-1) mutates, diverging into a "swarm" of viral quasispecies, and the predominance of CCR5- or CXCR4-utilizing quasispecies is strongly associated with the pattern of disease progression. Quantification of CCR5- and CXCR4-utilizing viruses in viral swarms is important in the investigation of the mechanisms of this phenomenon. Here, we report on a new real-time PCR-based methology for the evaluation of replication of individual CCR5- and CXCR4-utilizing variants. The assay is highly reproducible, with a coefficient of variation of <3%, and it accurately estimates the numbers of virus-specific RNA copies even when their difference in the mixture is 2 orders of magnitude. We demonstrate that replications of CCR5- and CXCR4-utilizing variants can be evaluated and distinguished in experimentally coinfected human lymphoid tissue. The assay we developed may facilitate study of the mechanisms of the R5-to-X4 switch in viral swarms in human tissues infected with HIV-1.

Ivshina, A. V., G. M. Vodeiko, et al. (2004). "Mapping of Genomic Segments of Influenza B Virus Strains by an Oligonucleotide Microarray Method." J. Clin. Microbiol. **42**(12): 5793-5801.

http://jcm.asm.org/cgi/content/abstract/42/12/5793

Similar to other segmented RNA viruses, influenza viruses can exchange genome segments and form a wide variety of reassortant strains upon coreplication within a host cell. Therefore, the mapping of genome segments of influenza viruses is essential for understanding their phenotypes. In this work, we have developed an oligonucleotide microarray hybridization method for simultaneous genotyping of all genomic segments of two highly homologous strains of influenza B virus. A few strain-specific oligonucleotide probes matching each of the eight segments of the viral genomes of the B/Beijing/184/93 and B/Shangdong/7/97 strains were hybridized with PCR-amplified fluorescently labeled single-stranded DNA. Even though there were a few mismatches among the genomes of the studied virus strains, microarray hybridization showed highly significant and reproducible discrimination ability and allowed us to determine the origins of individual genomic segments in a series of reassortant strains prepared as vaccine candidates. Additionally, we were able to detect the presence of at least 5% of mixed genotypes in virus stocks even when conventional sequencing methods failed, for example, for the NS segment. Thus, the proposed microarray method can be used for (i) rapid and reliable genome mapping of highly homologous influenza B viruses and (ii) extensive monitoring of influenza B virus reassortants and the mixed genotypes. The array can be expanded by adding new oligoprobes and using more quantitative assays to determine the origin of individual genomic segments in series of reassortant strains prepared as vaccine candidates or in mixed virus populations.

Jensen, J. S., M. B. Borre, et al. (2003). "Detection of Mycoplasma genitalium by PCR Amplification of the 16S rRNA Gene." J. Clin. Microbiol. **41**(1): 261-266.

http://jcm.asm.org/cgi/content/abstract/41/1/261

In order to develop a species-specific PCR for the detection of Mycoplasma genitalium, the sequence of 1,490 bases of the 16S rRNA gene was determined for M. genitalium G37 (type

strain) and four Danish isolates of M. genitalium. The sequences of the four Danish strains, mutually different with respect to their MgPa gene, were 100% homologous, although they carried a single common base substitution compared to the type strain. Among members of the Mycoplasma pneumoniae phylogenetic cluster, M. genitalium showed the most-prominent homology to the 16S rRNA sequence of M. pneumoniae (98% homology). From regions showing the least homology to the M. pneumoniae 16S rRNA gene sequence, primers were chosen to amplify DNA from M. genitalium only. Two sets of primers were selected for their ability to detect <10 to 50 M. genitalium genome copies without cross-reactions with M. pneumoniae. The performance of these primers was compared to the performance of two pairs of primers amplifying parts of the MgPa adhesin gene; 1,030 randomly selected specimens submitted for Chlamydia trachomatis culture were screened with one of the 16S rRNA gene primer sets. A total of 41 specimens were found to be positive for this gene; 40 of these could be confirmed by one of the MgPa primer sets, whereas the other MgPa primer set detected only 21 positive specimens out of 40. These results indicate that estimates of the prevalence of M. genitalium in various populations using MgPa PCR primers could be incorrectly low if the PCR primers are located in variable regions of the MgPa gene.

Johansen, I. S., B. Lundgren, et al. (2003). "Direct Detection of Multidrug-Resistant Mycobacterium tuberculosis in Clinical Specimens in Low- and High-Incidence Countries by Line Probe Assay." J. Clin. Microbiol. **41**(9): 4454-4456.

http://jcm.asm.org/cgi/content/abstract/41/9/4454

The INNO-LiPA Rif.TB assay is designed for the detection of rpoB gene mutations causing rifampin resistance in isolates. We applied the method directly to 60 Lithuanian and Danish clinical specimens to detect rifampin resistance rapidly. Results were obtained in 78.3% of clinical specimens, and all were concordant with those obtained by BACTEC 460. The assay could have major impact on the management of multidrug-resistant tuberculosis.

Johansen, T. B., B. Djonne, et al. (2005). "Distribution of IS1311 and IS1245 in Mycobacterium avium Subspecies Revisited." J. Clin. Microbiol. **43**(5): 2500-2502.

http://jcm.asm.org/cgi/content/abstract/43/5/2500

We demonstrated that IS1245 is not present in Mycobacterium avium subsp. paratuberculosis by restriction fragment length polymorphism and that the designated three-banded bird pattern of IS1245 in M. avium subsp. avium consists of one copy of IS1245 and two copies of IS1311. Cross hybridization between the two elements can be avoided by using more specific probes.

Johnson, G., M. Ayers, et al. (2003). "Detection and Identification of Bartonella Species Pathogenic for Humans by PCR Amplification Targeting the Riboflavin Synthase Gene (ribC)." J. Clin. Microbiol. **41**(3): 1069-1072.

http://jcm.asm.org/cgi/content/abstract/41/3/1069

Several Bartonella species have now been implicated as human pathogens. The recovery of these fastidious organisms in the clinical microbiology laboratory remains difficult, and current methods are still relatively insensitive. Thus, the bartonellae are good candidates for detection by PCR. We have developed a PCR assay which uses a single primer pair targeting the riboflavin

synthase gene (ribC) and detected six Bartonella species that have been implicated in human disease, B. henselae, B. quintana, B. bacilliformis, B. clarridgeiae, B. elizabethae, and B. vinsonii subsp. berkhoffii. Species identification is achieved simply by restriction enzyme digestion of the amplicon. This PCR assay appears to be specific for the Bartonella genus because it failed to amplify DNA from several other bacterial species.

Johnson, J. R. and T. T. O'Bryan (2004). "Detection of the Escherichia coli Group 2 Polysaccharide Capsule Synthesis Gene kpsM by a Rapid and Specific PCR-Based Assay." J. Clin. Microbiol. **42**(4): 1773-1776.

http://jcm.asm.org/cgi/content/abstract/42/4/1773

A rapid and simple PCR-based assay for detection of the group 2 capsule synthesis gene kpsM of Escherichia coli was designed and validated. When combined with the published group 2 primers (kpsIIf, 5'-GCGCATTTGCTGATACTGTTG-3'; kpsIIr, 5'-CATCCAGACGATAAGCATGAGCA-3'), the new primers (the kpsIIf primer and a new reverse primer K2r, 5'-AGGTAGTTCAGACTCACACCT-3') allowed specific identification by exclusion of the heretofore elusive K2 kpsM variant. The primers yielded the predicted amplicon when multiplexed with other primers and used under varied assay conditions, including a range of concentrations of individual reaction mixture ingredients and of annealing temperatures (from 54 to 64{degrees}C).

Johnson, S. M., K. A. Simmons, et al. (2004). "Amplification of Coccidioidal DNA in Clinical Specimens by PCR." J. Clin. Microbiol. **42**(5): 1982-1985.

http://jcm.asm.org/cgi/content/abstract/42/5/1982

Coccidioides DNA was amplified from serum by a PCR using coccidioid-specific primers. A 239-bp product was visualized when 10 fg of exogenous coccidioidal DNA was subjected to amplification. This product was demonstrated in some human and mouse sera prior to the detection of coccidioidal antibodies.

Jordens, J. Z., J. N. Williams, et al. (2002). "Detection of Meningococcal Carriage by Culture and PCR of Throat Swabs and Mouth Gargles." J. Clin. Microbiol. **40**(1): 75-79.

http://jcm.asm.org/cgi/content/abstract/40/1/75

The standard method for detecting meningococcal carriage is culture of throat swabs on selective media, but the levels of carriage determined depend heavily on the skills of the individuals taking the swab and interpreting the cultures. This study aimed to determine the most sensitive detection method for meningococcal carriage. Throat swabs and saline mouth gargles, obtained from 89 university students, were processed in parallel by conventional culture and TaqMan ctrA PCR. Carriage of meningococci, as detected by the combined methods, was 20%. The sensitivities of throat swab culture, throat swab PCR, gargle culture, and gargle PCR were 72, 56, 56, and 50%, respectively, and the probabilities that these techniques would correctly identify the absence of carriage (negative predictive value [NPV]) were 93.4, 89.9, 89.9, and 88.8%. Culturing both throat swabs and gargles increased the NPV to 98.6%. The further addition of throat swab PCR increased this to 100%. Testing gargles by both culture and PCR was as sensitive as testing throat swabs by both methods, suggesting that gargles may be a suitable alternative for large-

scale screening studies when throat swabs are difficult to obtain, although they required more lengthy laboratory processing. PCR was a useful adjunct to culture for detecting nasopharyngeal carriage, but it failed to detect some nongroupable strains. For maximum sensitivity, a combination of techniques was required. This study indicates the confidence with which health care professionals involved in meningococcal screening can regard laboratory results.

Karmali, M. A., M. Mascarenhas, et al. (2003). "Association of Genomic O Island 122 of Escherichia coli EDL 933 with Verocytotoxin-Producing Escherichia coli Seropathotypes That Are Linked to Epidemic and/or Serious Disease." J. Clin. Microbiol. **41**(11): 4930-4940.

http://jcm.asm.org/cgi/content/abstract/41/11/4930

The distribution of EDL 933 O island 122 (OI-122) was investigated in 70 strains of Verocytotoxin-producing Escherichia coli (VTEC) of multiple serotypes that were classified into five "seropathotypes" (A through E) based on the reported occurrence of serotypes in human disease, in outbreaks, and/or in the hemolytic-uremic syndrome (HUS). Seropathotype A comprised 10 serotype O157:H7 and 3 serotype O157:NM strains. Seropathotype B (associated with outbreaks and HUS but less commonly than serotype O157:H7) comprised three strains each of serotypes O26:H11, O103:H2, O111:NM, O121:H19, and O145:NM. Seropathotype C comprised four strains each of serotypes O91:H21 and O113:H21 and eight strains of other serotypes that have been associated with sporadic HUS but not typically with outbreaks. Seropathotype D comprised 14 strains of serotypes that have been associated with diarrhea but not with outbreaks or HUS, and seropathotype E comprised animal VTEC strains of serotypes not implicated in human disease. All strains were tested for four EDL 933 OI-122 virulence genes (Z4321, Z4326, Z4332, and Z4333) by PCR. Negative PCRs were confirmed by Southern hybridization. Overall, 28 (40%) strains contained OI-122 (positive for all four virulence genes), 27 (38.6%) contained an "incomplete" OI-122 (positive for one to three genes), and 15 (21.4%) strains did not contain OI-122. The seropathotype distribution of complete OI-122 was as follows: 100% for seropathotype A, 60% for B, 36% for C, 15% for D, and 0% for E. The differences in the frequency of OI-122 between seropathotypes A, B, and C (associated with HUS) and seropathotypes D and E (not associated with HUS) and between seropathotypes A and B (associated with epidemic disease) and seropathotypes C, D, and E (not associated with epidemic disease) were highly significant ($P < 0.0001$).

Kazor, C. E., P. M. Mitchell, et al. (2003). "Diversity of Bacterial Populations on the Tongue Dorsa of Patients with Halitosis and Healthy Patients." J. Clin. Microbiol. **41**(2): 558-563.

http://jcm.asm.org/cgi/content/abstract/41/2/558

The primary purpose of the present study was to compare the microbial profiles of the tongue dorsa of healthy subjects and subjects with halitosis by using culture-independent molecular methods. Our overall goal was to determine the bacterial diversity on the surface of the tongue dorsum as part of our ongoing efforts to identify all cultivable and not-yet-cultivated species of the oral cavity. Tongue dorsum scrapings were analyzed from healthy subjects with no complaints of halitosis and subjects with halitosis, defined as an organoleptic score of 2 or more and volatile sulfur compound levels greater than 200 ppb. 16S rRNA genes from DNA isolated from tongue dorsum scrapings were amplified by PCR with universally conserved bacterial primers and cloned into Escherichia coli. Typically, 50 to 100 clones were analyzed from each subject. Fifty-one strains isolated from the tongue dorsa of healthy subjects were also analyzed. Partial sequences of approximately 500 bases of cloned inserts from the 16S rRNA genes of isolates were compared with sequences of known species or phylotypes to determine species identity or closest relatives. Nearly complete sequences of about 1,500 bases were obtained for potentially

novel species or phylotypes. In an analysis of approximately 750 clones, 92 different bacterial species were identified. About half of the clones were identified as phylotypes, of which 29 were novel to the tongue microbiota. Fifty-one of the 92 species or phylotypes were detected in more than one subject. Those species most associated with healthy subjects were Streptococcus salivarius, Rothia mucilaginosa, and an uncharacterized species of Eubacterium (strain FTB41). Streptococcus salivarius was the predominant species in healthy subjects, as it represented 12 to 40% of the total clones analyzed from each healthy subject. Overall, the predominant microbiota on the tongue dorsa of healthy subjects was different from that on the tongue dorsa of subjects with halitosis. Those species most associated with halitosis were Atopobium parvulum, a phylotype (clone BS095) of Dialister, Eubacterium sulci, a phylotype (clone DR034) of the uncultivated phylum TM7, Solobacterium moorei, and a phylotype (clone BW009) of Streptococcus. On the basis of our ongoing efforts to obtain full 16S rRNA sequences for all cultivable and not-yet-cultivated species that colonize the oral cavity, there are now over 600 species.

Keller, A. P., M. L. Beggs, et al. (2002). "Evidence of the Presence of IS1245 and IS1311 or Closely Related Insertion Elements in Nontuberculous Mycobacteria outside of the Mycobacterium avium Complex." J. Clin. Microbiol. **40**(5): 1869-1872.

http://jcm.asm.org/cgi/content/abstract/40/5/1869

A PCR assay based on the simultaneous detection of IS1245 and IS1311 was developed and used to determine the host range of these insertion elements. Specific PCR products were observed in Mycobacterium malmoense, Mycobacterium scrofulaceum, and Mycobacterium nonchromogenicum, indicating that IS1245 and IS1311 are not limited to the Mycobacterium avium complex.

Key, K. F., D. K. Guenette, et al. (2003). "Development of a Heteroduplex Mobility Assay To Identify Field Isolates of Porcine Reproductive and Respiratory Syndrome Virus with Nucleotide Sequences Closely Related to Those of Modified Live-Attenuated Vaccines." J. Clin. Microbiol. **41**(6): 2433-2439.

http://jcm.asm.org/cgi/content/abstract/41/6/2433

Porcine reproductive and respiratory syndrome has been devastating the swine industry since the late 1980s. The disease has been controlled, to some extent, through the use of modified live-attenuated (MLV) vaccines once available. However, such a practice periodically resulted in isolation or detection of vaccine-like viruses from pigs as determined by a partial genomic sequencing. In this study, we developed a heteroduplex mobility assay (HMA) for quickly identifying porcine reproductive and respiratory syndrome virus (PRRSV) isolates with significant nucleotide sequence identities ([>=]98%) with the modified live-attenuated vaccines. The major envelope gene (ORF5) of 51 PRRSV field isolates recovered before and after the introduction of the vaccines was amplified, denatured, and reannealed with the HMA reference vaccine strains Ingelvac PRRS MLV and Ingelvac PRRS ATP, respectively. Nine of the 51 field isolates and the VR2332 parent virus of Ingelvac PRRS MLV, which were all highly related to Ingelvac PRRS MLV with [<=]2% nucleotide sequence divergence as determined by sequence analysis, were all identified by the HMA to form homoduplexes with the reference Ingelvac PRRS MLV. No homoduplex-forming field isolate was identified when Ingelvac PRRS ATP was used as the HMA reference except for its parent virus JA142. Other field isolates with more than 2% nucleotide sequence divergence with the respective reference vaccine strain resulted in the formation of heteroduplexes with reduced mobility in polyacrylamide gel electrophoresis. The HMA results also correlated well with the results of phylogenetic analyses. The data indicated that the HMA

developed in the study may be a rapid and efficient method for large-scale screening of potential vaccine-like PRRSV field isolates for further genetic characterization.

Khanna, M., J. Fan, et al. (2005). "The Pneumoplex Assays, a Multiplex PCR-Enzyme Hybridization Assay That Allows Simultaneous Detection of Five Organisms, Mycoplasma pneumoniae, Chlamydia (Chlamydophila) pneumoniae, Legionella pneumophila, Legionella micdadei, and Bordetella pertussis, and Its Real-Time Counterpart." J. Clin. Microbiol. **43**(2): 565-571.

http://jcm.asm.org/cgi/content/abstract/43/2/565

Respiratory disease caused by atypical bacteria remains an important cause of morbidity and mortality for adults and children, despite the widespread use of effective antimicrobials agents. Culture remains the "gold standard" for the detection of these agents. However, culture is labor-intensive, takes several days to weeks for growth, and can be very insensitive for the detection of some of these organisms. Newer singleplex PCR diagnostic tests are sensitive and specific, but multiple assays would be needed to detect all of the common pathogens. Therefore, we developed the Pneumoplex assays, a multiplex PCR-enzyme hybridization assay (the standard assay) and a multiplex real-time assay to detect the most common atypical pathogens in a single test. Primer and probe sequences were designed from conserved regions of specific genes for each of these organisms. The limits of detection were as follows: for Bordetella pertussis, 2 CFU/ml; for Legionella pneumophila (serotypes 1 to 15) and Legionella micdadei, 9 and 80 CFU/ml, respectively; for Mycoplasma pneumoniae, 5 CFU/ml; and for Chlamydia (Chlamydophila) pneumoniae, 0.01 50% tissue culture infective doses. Recombinant DNA controls for each of these organisms were constructed, and the number of copies for each DNA control was calculated. The Pneumoplex could detect each DNA control down to 10 copies/ml. The analytical specificity demonstrated no cross-reactivity between 23 common respiratory pathogens. One hundred twenty-five clinical bronchoalveolar lavage fluid samples tested by the standard assay demonstrated that the Pneumoplex yielded a sensitivity and a specificity of 100 and 98.5%, respectively. This test has the potential to assist clinicians in establishing a specific etiologic diagnosis before initiating therapy, to decrease hospital costs, and to prevent inappropriate antimicrobial therapy.

Khare, S., T. A. Ficht, et al. (2004). "Rapid and Sensitive Detection of Mycobacterium avium subsp. paratuberculosis in Bovine Milk and Feces by a Combination of Immunomagnetic Bead Separation-Conventional PCR and Real-Time PCR." J. Clin. Microbiol. **42**(3): 1075-1081.

http://jcm.asm.org/cgi/content/abstract/42/3/1075

Immunomagnetic bead separation coupled with bead beating and real-time PCR was found to be a very effective procedure for the isolation, separation, and detection of Mycobacterium avium subsp. paratuberculosis from milk and/or fecal samples from cattle and American bison. Samples were spiked with M. avium subsp. paratuberculosis organisms, which bound to immunomagnetic beads and were subsequently lysed by bead beating; then protein and cellular contaminants were removed by phenol-chloroform-isopropanol extraction prior to DNA precipitation. DNA purified by this sequence of procedures was then analyzed by conventional and real-time IS900-based PCR in order to detect M. avium subsp. paratuberculosis in feces and milk. By use of this simple and rapid technique, 10 or fewer M. avium subsp. paratuberculosis organisms were consistently detected in milk (2-ml) and fecal (200-mg) samples, making this sensitive procedure very useful and cost-effective for the diagnosis of clinical and subclinical Johne's disease (paratuberculosis) compared to bacteriological culture, which is constrained by time, labor, and expense under diagnostic laboratory conditions.

Kivi, M., Y. Tindberg, et al. (2003). "Concordance of Helicobacter pylori Strains within Families." J. Clin. Microbiol. **41**(12): 5604-5608.

http://jcm.asm.org/cgi/content/abstract/41/12/5604

Helicobacter pylori infection is typically acquired in early childhood, and a predominantly intrafamilial transmission has been postulated. To what extent family members share the same strains is poorly documented. Our aim was to explore patterns of shared strains within families by using molecular typing. Family members of H. pylori-infected 10- to 12-year-old index children identified in a school survey were invited to undergo gastroscopy. Bacterial isolates were typed with random amplified polymorphic DNA and PCR-restriction fragment length polymorphism of the genes ureA-B, glmM, or flaA. The presence or absence of the cag pathogenicity island, a bacterial virulence factor, was determined by PCR. GelCompar II software, supplemented with visual inspection, was used in the cluster analysis. In 39 families, 104 individuals contributed 208 bacterial isolates from the antrum and corpus. A large proportion, 29 of 36 (81%) of the offspring in a sibship, harbored the same strain as at least one sibling. Mother-offspring strain concordance was detected in 10 of 18 (56%) of the families. Of 17 investigated father-offspring relations in eight families, none were strain concordant. Spouses were infected with the same strains in 5 of 23 (22%) of the couples. Different strains in the antrum and corpus were found in 8 of 104 (8%) of the subjects. Our family-based fingerprinting study demonstrates a high proportion of shared strains among siblings. Transmission between spouses seems to be appreciable. The data support mother-child and sib-sib transmission as the primary transmission pathways of H. pylori.

Klieforth, R., G. Maalouf, et al. (2002). "Malignant Catarrhal Fever-Like Disease in Barbary Red Deer (Cervus elaphus barbarus) Naturally Infected with a Virus Resembling Alcelaphine Herpesvirus 2." J. Clin. Microbiol. **40**(9): 3381-3390.

http://jcm.asm.org/cgi/content/abstract/40/9/3381

Eight Barbary red deer (Cervus elaphus barbarus) developed clinical signs suggestive of malignant catarrhal fever (MCF) over a 28-day period. These animals were housed outdoors with four other species of ruminants. Affected red deer had lethargy, ocular signs, and nasal discharge and were euthanatized within 48 h. Lesions included ulcers of the muzzle, lips, and oral cavity associated with infiltrates of neutrophils and lymphocytes. Serologically, six of seven red deer tested during the outbreak were positive by competitive enzyme-linked immunosorbent assay for antibodies to a shared MCF virus antigen. PCR using oligonucleotide primers designed for a conserved protein of alcelaphine herpesviruses 1 (AlHV-1) and 2 (AlHV-2) and for conserved regions of a herpesvirus DNA polymerase gene was positive for tissues from all eight clinically affected animals and negative for eight out of eight red deer without clinical signs of MCF. DNA sequencing of PCR amplicons from the diseased red deer indicated that they were infected with a novel herpesvirus closely related to AlHV-2; immunohistochemistry using polyclonal anti-AlHV-2 serum and in situ hybridization demonstrated the presence of virus within salivary glands adjacent to oral lesions of affected animals. A survey of other ruminants near the outbreak subsequently showed that normal Jackson's hartebeest (Alcelaphus buselaphus jacksoni) that were cohoused with the diseased red deer were infected with the same virus and were shedding the virus in nasal excretions. These findings suggest that a herpesvirus closely related to AlHV-2 caused the MCF-like disease epizootic in Barbary red deer and that the virus may have originated from Jackson's hartebeest.

Klotz, M., S. Opper, et al. (2003). "Detection of Staphylococcus aureus Enterotoxins A to D by Real-Time Fluorescence PCR Assay." J. Clin. Microbiol. **41**(10): 4683-4687.

http://jcm.asm.org/cgi/content/abstract/41/10/4683

Staphylococcus aureus is one of the most significant pathogens causing nosocomial and community-acquired infections. Among the secreted staphylococcal virulence factors, there is a growing list of enterotoxins which can induce gastroenteric syndrome and toxic shock syndrome. Here, we developed a real-time fluorescence PCR assay (TaqMan PCR) for the detection of genes encoding staphylococcal enterotoxins A, B, C1, and D (SEA, SEB, SEC1, and SED) of S. aureus as well as the mecA gene encoding methicillin resistance and the femB gene as a specific genomic marker for S. aureus. SEA to SED were selected because they are the four classically described enterotoxins of S. aureus and because they were detected by latex agglutination. In order to evaluate the reliability of TaqMan PCR, we investigated 93 isolates of S. aureus derived from patients at our hospital over 5 months and compared the results with data obtained by a commerciall available reversed passive latex agglutination assay (SET-RPLA) for these isolates. Thirteen enterotoxin genes were detected by TaqMan PCR; however, no proteins expressed by these genes were detected by SET-RPLA. As a result, more isolates of S. aureus (n = 44) were found positive by TaqMan PCR for one or more enterotoxin genes than by SET-RPLA for the respective proteins expressed by these genes (n = 40). We conclude that TaqMan PCR is more sensitive because it offers the possibility for determining enterotoxins on a genotypic basis. Additionally, the assay allows the parallel detection of genes for SEA to SED and methicillin resistance in S. aureus. Furthermore, real-time PCR is well suited for screening large numbers of samples at the same time, allowing rapid, reliable, efficient, and cost-saving routine laboratory diagnosis.

Knutsson, R., C. Lofstrom, et al. (2002). "Modeling of 5' Nuclease Real-Time Responses for Optimization of a High-Throughput Enrichment PCR Procedure for Salmonella enterica." J. Clin. Microbiol. **40**(1): 52-60.

http://jcm.asm.org/cgi/content/abstract/40/1/52

The performance of a 5' nuclease real-time PCR assay was studied to optimize an automated method of detection of preenriched Salmonella enterica cells in buffered peptone water (BPW). The concentrations and interactions of the PCR reagents were evaluated on the basis of two detection responses, the threshold cycle (CT) and the fluorescence intensity by a normalized reporter value ({Delta}Rn). The CT response was identified as the most suitable for detection modeling to describe the PCR performances of different samples. DNA extracted from S. enterica serovar Enteritidis was studied in double-distilled H2O (ddH2O) and in two different enrichment media (brain heart infusion and BPW) with two PCR mixtures based on AmpliTaq Gold or rTth. A descriptive model was proposed and fitted to the available experimental data. Equivalent PCR performances for the two PCR mixtures were obtained when DNA was diluted in ddH2O. However, the level of detection of DNA was affected when BPW was present during amplification. Use of the rTth mixture generated a 1-log-unit wider linear range of amplification, and the DNA detection levels were 2 x 10-13 g/microwell for the rTth mixture and 2 x 10-12 g/microwell for the AmpliTaq Gold mixture. To verify the improved amplification capacity of the rTth mixture, BPW was inoculated with 1 CFU of S. enterica serovar Enteritidis per ml and the mixture was incubated at 30{degrees}C. Samples for PCR were withdrawn every 4 h during a 36-h enrichment. Use of the rTth mixture resulted in an earlier PCR detection during enrichment than use of the AmpliTaq Gold mixture. For accurate detection (CT [<=] 30) of S. enterica serovar Enteritidis inoculated in BPW, the rTth mixture required 8.4 h of enrichment, while the AmpliTaq Gold mixture needed 11.6 h. In conclusion, the principle applied can improve the methodology of 5' nuclease real-time PCR for numerical optimization of sample pretreatment strategies to provide automated diagnostic PCR procedures.

Konnick, E. Q., M. Erali, et al. (2005). "Evaluation of the COBAS Amplicor HBV Monitor Assay and Comparison with the Ultrasensitive HBV Hybrid Capture 2 Assay for Quantification of Hepatitis B Virus DNA." J. Clin. Microbiol. **43**(2): 596-603.

http://jcm.asm.org/cgi/content/abstract/43/2/596

Performance characteristics of the COBAS Amplicor HBV Monitor test (Roche Diagnostics), which measures hepatitis B virus (HBV) DNA quantitatively, were evaluated and compared with the Ultrasensitive HBV Hybrid Capture 2 (HC2; Digene Corporation) assay. Linearity and within-run precision were assessed for both methods by using eight HBV DNA-positive samples serially diluted to obtain a range of <100 to 500,000 HBV DNA copies/ml and run in triplicate. Agreement between the methods was studied with 100 clinical samples. HC2 assay performance near the limit of detection was investigated through repeat testing of 149 samples with HC2 and testing of 37 samples with HC2 results of <4,700 HBV DNA copies/ml by Amplicor assay and a qualitative PCR assay. The linearity experiment for Amplicor had regression of observed values compared to expected values (y = 1.073x - 0.247; R2 = 0.993, n = 32; for HC2, y = 0.855x + 0.759, R2 = 0.729, n = 18). Within-run standard deviation of log HBV DNA copies/ml ranged from 0.003 to 0.348 (Amplicor) and 0.027 to 0.253 (HC2). Agreement assessed by Deming regression was poor [Amplicor = 1.197(HC2) - 0.961; R2 = 0.799, standard error of the estimate (SEE) = 0.710, n = 94]. Near the lower limit of detection, 32 of 149 repeat HC2 results were <4,700 HBV DNA copies/ml. Of the 37 samples with HC2 results of <4,700 HBV DNA copies/ml, HBV DNA was not detected in 15 samples, while HBV DNA was detected by at least one PCR method in 12 samples. Amplicor is linear from 200 to 200,000 HBV DNA copies/ml with undiluted samples, and this range can be expanded through dilution. Inconsistent HC2 results near the limit of detection justify use of a grey zone.

Konomi, N., E. Lebwohl, et al. (2002). "Detection of Mycobacterial DNA in Andean Mummies." J. Clin. Microbiol. **40**(12): 4738-4740.

http://jcm.asm.org/cgi/content/abstract/40/12/4738

The identification of genetic material from pathogenic organisms in ancient tissues provides a powerful tool for the study of certain infectious diseases in historic populations. We have obtained tissue samples from the genital areas of 12 mummies in the American Museum of Natural History collection in New York, N.Y. The mummies were excavated in the Andes Mountain region of South America, and radiocarbon dating estimates that the mummies date from A.D. 140 to 1200. DNAs were successfully extracted from all tissues and were suitable for PCR analysis. PCRs were carried out to detect Mycobacterium tuberculosis complex and mycobacteria other than M. tuberculosis (MOTB). M. tuberculosis complex was detected in 2 out of 12 samples, and MOTB were detected in 7 samples. This study confirmed the adequate preservation of genetic material in mummified tissues and the existence of mycobacteria, including M. tuberculosis, in historic populations in South America.

Korman, T. M., A. Boers, et al. (2004). "Fatal Case of Toxic Shock-Like Syndrome Due to Group C Streptococcus Associated with Superantigen Exotoxin." J. Clin. Microbiol. **42**(6): 2866-2869.

http://jcm.asm.org/cgi/content/abstract/42/6/2866

Group C streptococci have been reported to cause invasive disease similar to that classically

associated with group A streptococcus (GAS). We describe a fatal case of toxic shock-like syndrome due to Streptococcus equi subsp. zooepidemicus. The causative organism did not possess any known GAS superantigen exotoxin genes but did show evidence of superantigen production.

Kornegay, J. R., M. Roger, et al. (2003). "International Proficiency Study of a Consensus L1 PCR Assay for the Detection and Typing of Human Papillomavirus DNA: Evaluation of Accuracy and Intralaboratory and Interlaboratory Agreement." J. Clin. Microbiol. **41**(3): 1080-1086.

http://jcm.asm.org/cgi/content/abstract/41/3/1080

The PGMY L1 consensus primer pair combined with the line blot assay allows the detection of 27 genital human papillomavirus (HPV) genotypes. We conducted an intralaboratory and interlaboratory agreement study to assess the accuracy and reproducibility of PCR for HPV DNA detection and typing using the PGMY primers and typing amplicons with the line blot (PGMY-LB) assay. A test panel of 109 samples consisting of 29 HPV-negative (10 buffer controls and 19 genital samples) and 80 HPV-positive samples (60 genital samples and 20 controls with small or large amounts of HPV DNA plasmids) were tested blindly in triplicate by three laboratories. Intralaboratory agreement ranged from 86 to 98% for HPV DNA detection. PGMY-LB assay results for samples with a low copy number of HPV DNA were less reproducible. The rate of intralaboratory agreement excluding negative results for HPV typing ranged from 78 to 96%. Interlaboratory reliability for HPV DNA positivity and HPV typing was very good, with levels of agreement of >95% and kappa values of >0.87. Again, low-copy-number samples were more prone to generating discrepant results. The accuracy varied from 91 to 100% for HPV DNA positivity and from 90 to 100% for HPV typing. HPV testing can thus be accomplished reliably with PCR by using a standardized written protocol and quality-controlled reagents. The use of validated HPV DNA detection and typing assays demonstrating excellent interlaboratory agreement will allow investigators to better compare results between epidemiological studies.

Kosoy, M., M. Murray, et al. (2003). "Bartonella Strains from Ground Squirrels Are Identical to Bartonella washoensis Isolated from a Human Patient." J. Clin. Microbiol. **41**(2): 645-650.

http://jcm.asm.org/cgi/content/abstract/41/2/645

The most likely animal source of a human case of cardiac disease in Washoe County, Nev., was identified by comparison of DNA sequences of three genes (citrate synthase gltA, 60-kDa heat shock protein gene groEL, and 16S rRNA gene) of Bartonella washoensis cultured from the human patient in question and of Bartonella isolates obtained from the following Nevada rodents: Peromyscus maniculatus (17 isolates), Tamias minimus (11 isolates), Spermophilus lateralis (3 isolates), and Spermophilus beecheyi (7 isolates). Sequence analyses of gltA amplicons obtained from Bartonella from the rodents demonstrated considerable heterogeneity and resulted in the identification of 16 genetic variants that were clustered within three groups in phylogenetic analysis. Each of the three groups was associated with a rodent genus, Peromyscus, Tamias, or Spermophilus. The gltA, 16S rRNA gene, and groEL sequences of a Bartonella isolate obtained from a California ground squirrel (S. beecheyi) were completely identical to homologous sequences of B. washoensis, strongly suggesting that these animals were the source of infection in the human case.

Kotlowski, R., I. C. Shamputa, et al. (2004). "PCR-Based Genotyping of Mycobacterium tuberculosis with

New GC-Rich Repeated Sequences and IS6110 Inverted Repeats Used as Primers." J. Clin. Microbiol. **42**(1): 372-377.

http://jcm.asm.org/cgi/content/abstract/42/1/372

In the present study we attempted to develop a PCR-based epidemiological tool for the differentiation of Mycobacterium tuberculosis isolates. Use of the designed primers Mtb1 (5'-CCG-GCG-GGG-CCG-GCG-G) and Mtb2 (5'-CGG-CGG-CAA-CGG-CGG-C) targeting frequently repeated 16-bp sequences in combination with primers sited at the inverted repeats flanking IS6110 allowed differentiation of M. tuberculosis isolates.

Krafft, A. E., K. L. Russell, et al. (2005). "Evaluation of PCR Testing of Ethanol-Fixed Nasal Swab Specimens as an Augmented Surveillance Strategy for Influenza Virus and Adenovirus Identification." J. Clin. Microbiol. **43**(4): 1768-1775.

http://jcm.asm.org/cgi/content/abstract/43/4/1768

Viral culture isolation has been widely accepted as the "gold standard" for laboratory confirmation of viral infection; however, it requires ultralow temperature specimen storage. Storage of specimens in ethanol at room temperature could expand our ability to conduct active surveillance and retrospective screenings of viruses with rapid and inexpensive real-time PCR tests, including isolates from remote regions where freezing specimens for culture is not feasible. Molecular methods allow for rapid identification of viral pathogens without the need to maintain viability. We hypothesized that ethanol, while inactivating viruses, can preserve DNA and RNA for PCR-based methods. To evaluate the use of ethanol-stored specimens for augmenting surveillance for detection of influenza viruses A and B and adenoviruses (AdV), paired nasal swab specimens were collected from 384 recruits with febrile respiratory illness at Fort Jackson, S.C., in a 2-year study. One swab was stored at ambient temperature in 100% ethanol for up to 6 months, and the other swab was stored at -70{degrees}C in viral medium. For viral detection, frozen specimens were cultured for a variety of respiratory viruses, and ethanol-fixed specimens were tested with TaqMan (TM) probe and LightCycler SYBR green (SG) melting curve assays with at least two different PCR targets for each virus. The sensitivities of the TM and SG assays on specimens stored in ethanol for 1 month were 75% and 58% for influenza A, 89% and 67% for influenza B, and 93 to 98% and 57% for AdV, respectively. Lower specificities of the real-time assays corresponded to the increased detection of PCR-positive but culture-negative specimens. Influenza virus RNA was detected as well or better after 6 months of storage in ethanol.

Kuboki, N., N. Inoue, et al. (2003). "Loop-Mediated Isothermal Amplification for Detection of African Trypanosomes." J. Clin. Microbiol. **41**(12): 5517-5524.

http://jcm.asm.org/cgi/content/abstract/41/12/5517

While PCR is a method of choice for the detection of African trypanosomes in both humans and animals, the expense of this method negates its use as a diagnostic method for the detection of endemic trypanosomiasis in African countries. The loop-mediated isothermal amplification (LAMP) reaction is a method that amplifies DNA with high specificity, efficiency, and rapidity under isothermal conditions with only simple incubators. An added advantage of LAMP over PCR-based methods is that DNA amplification can be monitored spectrophotometrically and/or with the naked eye without the use of dyes. Here we report our conditions for a highly sensitive, specific, and easy diagnostic assay based on LAMP technology for the detection of parasites in the Trypanosoma brucei group (including T. brucei brucei, T. brucei gambiense, T. brucei

rhodesiense, and T. evansi) and T. congolense. We show that the sensitivity of the LAMP-based method for detection of trypanosomes in vitro is up to 100 times higher than that of PCR-based methods. In vivo studies in mice infected with human-infective T. brucei gambiense further highlight the potential clinical importance of LAMP as a diagnostic tool for the identification of African trypanosomiasis.

Kuhne, S. A., W. S. Hawes, et al. (2004). "Isolation of Recombinant Antibodies against EspA and Intimin of Escherichia coli O157:H7." J. Clin. Microbiol. **42**(7): 2966-2976.

http://jcm.asm.org/cgi/content/abstract/42/7/2966

Intimin, Tir, and EspA proteins are expressed by attaching-effacing Escherichia coli, which include enteropathogenic and enterohemorrhagic E. coli pathotypes. EspA proteins are part of the type three secretion system needle complex that delivers Tir to the host epithelial cell, while surface arrayed intimin docks the bacterium to the translocated Tir. This intimate attachment leads to attaching and effacing lesions. Recombinant forms of these effector proteins from enterohemorrhagic E. coli O157:H7 were produced by using E. coli expression vectors. Binding of intimin and Tir fragments in enzyme-linked immunosorbent assay (ELISAs) demonstrated the interaction of intimin fragments containing the C-terminal 282 or 188 amino acids to a Tir fragment containing amino acid residues 258 to 361. Recombinant intimin and EspA proteins were used to elicit immune responses in rabbits and immune phage-display antibody libraries were produced. Screening of these immune libraries by conventional phage-antibody panning and colony filter screening produced a panel of antibodies with specificity for EspA or intimin. Antibodies recognizing different C-terminal epitopes on intimin bound specifically to the gamma intimin of O157:H7 and not to other classes of intimin. Antibodies recognizing EspA from E. coli O157 also recognized the protein from the eae-deficient O157 mutant DM3 and from E. coli O111. Anti-intimin antibodies were also produced as fusion proteins coupled to the reporter molecule alkaline phosphatase, allowing the one-step detection of {gamma} intimin. The isolated recombinant monoclonal antibodies were functional in a range of assay formats, including ELISA, Western blotting, and dot blots, thus demonstrating their diagnostic potential.

Kwara, A., R. Schiro, et al. (2003). "Evaluation of the Epidemiologic Utility of Secondary Typing Methods for Differentiation of Mycobacterium tuberculosis Isolates." J. Clin. Microbiol. **41**(6): 2683-2685.

http://jcm.asm.org/cgi/content/abstract/41/6/2683

Spoligotyping and mycobacterial interspersed repetitive unit-variable-number tandem repeat analysis (MIRU-VNTR) were evaluated for the ability to differentiate 64 Mycobacterium tuberculosis isolates from 10 IS6110-defined clusters. MIRU-VNTR performed slightly better than spoligotyping in reducing the number of clustered isolates and the sizes of the clusters. All epidemiologically related isolates remained clustered by MIRU-VNTR but not by spoligotyping.

La Scola, B., Z. Liang, et al. (2002). "Genotypic Characteristics of Two Serotypes of Bartonella henselae." J. Clin. Microbiol. **40**(6): 2002-2008.

http://jcm.asm.org/cgi/content/abstract/40/6/2002

The study of 16S rRNA gene sequences of all isolates of Bartonella henselae obtained in our laboratory and others from human patients or cats has revealed two genotypes according to the

sequence of the 16S rRNA gene. Two isolates of these genotypes have previously been related to two different serotypes, and lack of cross-protection of the two serotypes has been demonstrated in cats. We investigated the grouping of eight strains of B. henselae on the basis of 16S ribosomal DNA, 35-kDa protein, Pap 31 protein, and internal transcribed spacer (ITS) gene sequencing; sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) profiles; and monoclonal antibody reactivity studies. Houston-1, 90-615, and SA2 strains showed the same patterns in SDS-PAGE, but they differed from the patterns of B. henselae isolates URBHLLY8, URBHLIE9, Cat6, Fizz, and CAL-1. Nine monoclonal antibodies derived from BALB/c mice immunized with B. henselae Houston-1 strain reacted only with strains Houston-1, 90-615, and SA2, and not with any other Bartonella strains. The two serogroups corresponded with two genotypes based on differences in the sequences of the genes encoding 16S rRNA, 35-kDa protein, and Pap 31 protein. Sequences of ITS genes were highly divergent among strains, as each had a unique sequence and the subdivision was not supported by DNA-DNA relatedness study. Study of 22 additional strains of B. henselae isolated from French bacteremic cats demonstrated that they all belong to one or the other of the proposed serotype or genotype.

Lai, K. K.-Y., L. Cook, et al. (2003). "Evaluation of Real-Time PCR versus PCR with Liquid-Phase Hybridization for Detection of Enterovirus RNA in Cerebrospinal Fluid." J. Clin. Microbiol. **41**(7): 3133-3141.

http://jcm.asm.org/cgi/content/abstract/41/7/3133

A LightCycler and two TaqMan real-time PCR assays were evaluated against an older PCR with liquid-phase hybridization method for the detection of enterovirus RNA in 74 patient samples. The two-step LightCycler and the two-step TaqMan formats correlated well with each other (r2 = 0.90) and were equally sensitive compared to the liquid-phase hybridization method, whereas the one-step recombinant Tth DNA polymerase format was rather insensitive, detecting enterovirus RNA in only about one-half of those patient samples previously positive by liquid-phase hybridization. The two-step TaqMan method was optimized utilizing 10 {micro}l of cDNA and demonstrated the highest degree of analytical sensitivity among the methods evaluated in our study, being able to reproducibly quantify down to 510 copies of enteroviral RNA/ml of cerebrospinal fluid. This new assay can be performed in 4 h, is much less labor intensive, and showed less cross-reactivity with rhinovirus than the liquid-phase hybridization assay. Thus, the two-step TaqMan assay should prove useful in the diagnosis of enteroviral meningitis versus bacterial meningitis, thereby resulting in timely and appropriate clinical management that can amount to significant cost savings to the patient and health care system.

Lan, R. and P. R. Reeves (2002). "Pandemic Spread of Cholera: Genetic Diversity and Relationships within the Seventh Pandemic Clone of Vibrio cholerae Determined by Amplified Fragment Length Polymorphism." J. Clin. Microbiol. **40**(1): 172-181.

http://jcm.asm.org/cgi/content/abstract/40/1/172

The seventh cholera pandemic started in 1961 and continues today. A collection of 45 seventh pandemic isolates of V. cholerae sampled over a 33-year period were analyzed by amplified fragment length polymorphism (AFLP) fingerprinting. All but four pairs and one set of three isolates were distinguished. AFLP revealed far more variation than ribotyping, which was until now the most useful method of revealing variation within the pandemic clone. Unfortunately, the ribotype variation observed is mainly due to recombination between the multiple copies of the rrn genes (R. Lan and P. R. Reeves, Microbiology 144:1213-1221, 1998), which makes changes susceptible to repeat occurrences and reversion. This AFLP study shows that particularly for the common ribotypes G and H, such events have indeed occurred. AFLP grouped most of the 45

isolates into two clusters. Cluster I consists mainly of strains from the 1960s and 1970s, while cluster II contains mainly strains from the 1980s and 1990s, revealing a temporal pattern of change in the clone. This is best seen in the relationships of the strains from Africa, which correlate with the epidemiology of epidemics on that continent. The data confirm independent introductions to Africa during the 1970s outbreak and reveal several other African introductions. In the 1991 cholera upsurge, isolates from the Southern and Eastern African epidemic focus are markedly different from those from the West African epidemic focus. An isolate from 1987 in Algeria was identical to the West epidemic isolates, suggesting that the strain was present in Africa at least 3 years before causing large outbreaks. These observations have major implications for our understanding of cholera epidemiology.

Lapa, S., M. Mikheev, et al. (2002). "Species-Level Identification of Orthopoxviruses with an Oligonucleotide Microchip." J. Clin. Microbiol. **40**(3): 753-757.

http://jcm.asm.org/cgi/content/abstract/40/3/753

A method for species-specific detection of orthopoxviruses pathogenic for humans and animals is described. The method is based on hybridization of a fluorescently labeled amplified DNA specimen with the oligonucleotide DNA probes immobilized on a microchip (MAGIChip). The probes identify species-specific sites within the crmB gene encoding the viral analogue of tumor necrosis factor receptor, one of the most important determinants of pathogenicity in this genus of viruses. The diagnostic procedure takes 6 h and does not require any sophisticated equipment (a portable fluorescence reader can be used).

Laperche, S., F. Lunel, et al. (2005). "Comparison of Hepatitis C Virus NS5b and 5' Noncoding Gene Sequencing Methods in a Multicenter Study." J. Clin. Microbiol. **43**(2): 733-739.

http://jcm.asm.org/cgi/content/abstract/43/2/733

A national evaluation study was performed in 11 specialized laboratories with the objective of assessing their capacities to genotype hepatitis C virus (HCV) and define the applicability of a given genotyping method. The panel consisted of 14 samples positive for HCV RNA of different genotypes (including 3 samples with two different artificially mixed genotypes) and 1 HCV-negative sample. Seventeen sets of data were gathered from the 11 participating laboratories. The sensitivities ranged from 64.3 to 100% and from 42.7 to 85.7% for the methods that used sequencing of the NS5b region and the 5' noncoding (5' NC) region, respectively. When the data for the artificially mixed samples were excluded, NS5b genotyping gave correct results for 80% of the samples, 1.7% of the samples were misclassified, and 18.3% of the samples had false-negative results. By 5' NC-region genotyping methods, 58.3% of the results were correct, 29.7% were incomplete, 8.3% were misclassifications, 1.2% were false positive, and 2.4% were false negative. Only two procedures based on NS5b sequencing correctly identified one of the three samples with mixtures of genotypes; the other methods identified the genotype corresponding to the strain with the highest viral load in the sample. Our results suggest that HCV 5' NC-region genotyping methods give sufficient information for clinical purposes, in which the determination of the subtype is not essential, and that NS5b genotyping methods are more reliable for subtype determination, which is required in epidemiological studies.

Lasker, B. A. (2002). "Evaluation of Performance of Four Genotypic Methods for Studying the Genetic Epidemiology of Aspergillus fumigatus Isolates." J. Clin. Microbiol. **40**(8): 2886-2892.

http://jcm.asm.org/cgi/content/abstract/40/8/2886

In the present investigation, 49 Aspergillus fumigatus isolates obtained from four nosocomial outbreaks were typed by Afut1 restriction fragment length polymorphism (RFLP) analysis and three PCR-based molecular typing methods: random amplified polymorphic DNA (RAPD) analysis, sequence-specific DNA primer (SSDP) analysis, and polymorphic microsatellite markers (PMM) analysis. The typing methods were evaluated with respect to discriminatory power (D), reproducibility, typeability, ease of use, and ease of interpretation to determine their performance and utility for outbreak and surveillance investigations. Afut1 RFLP analysis detected 40 types. Thirty types were observed by RAPD analysis. PMM analysis detected 39 allelic types, but SSDP analysis detected only 14 types. All four methods demonstrated 100% typeability. PMM and RFLP analyses had comparable high degrees of discriminatory power (D = 0.989 and 0.988, respectively). The discriminatory power of RAPD analysis was slightly lower (D = 0.971), whereas SSDP analysis had the lowest discriminatory power (D = 0.889). Overall, SSDP analysis was the easiest method to interpret and perform. The profiles obtained by PMM analysis were easier to interpret than those obtained by RFLP or RAPD analysis. Bands that differed in staining intensity or that were of low intensity were observed by RAPD analysis, making interpretation more difficult. The reproducibilities with repeated runs of the same DNA preparation or with different DNA preparations of the same strain were high for all the methods. A high degree of genetic variation was observed in the test population, but isolates were not always similarly divided by each method. Interpretation of band profiles requires understanding of the molecular mechanisms responsible for genetic alternations. PMM analysis and Afut1 RFLP analysis, or their combination, appear to provide the best overall discriminatory power, reproducibility, ease of interpretation, and ease of use. This investigation will aid in planning epidemiologic and surveillance studies of A. fumigatus.

Lavigne, J.-P., N. Bouziges, et al. (2004). "Molecular Epidemiology of Enterobacteriaceae Isolates Producing Extended-Spectrum {beta}-Lactamases in a French Hospital." J. Clin. Microbiol. **42**(8): 3805-3808.

http://jcm.asm.org/cgi/content/abstract/42/8/3805

In 2002, 80 isolates of Enterobacteriaceae producing extended-spectrum {beta}-lactamases (ESBLs) were collected from infected patients in our hospital. Enterobacter aerogenes was the most common bacterium isolated from all specimens (36.5%). The ESBLs were predominantly (90%) TEM derivatives (TEM-24, TEM-3). Pulsed-field gel electrophoresis highlighted that E. aerogenes, Klebsiella pneumoniae, and Citrobacter koseri had a clonal propagation.

Lawrence, E. R., D. B. Griffiths, et al. (2003). "Evaluation of Semiautomated Multiplex PCR Assay for Determination of Streptococcus pneumoniae Serotypes and Serogroups." J. Clin. Microbiol. **41**(2): 601-607.

http://jcm.asm.org/cgi/content/abstract/41/2/601

A semiautomated method for the determination of five serotypes and three serogroups in Streptococcus pneumoniae was developed. Primers specific for serotypes 1, 3, 14, 19F, and 23F and serogroups 6, 19, and 23 were combined in three multiplex PCRs. Products were separated by capillary electrophoresis with a 7-min run time, and a serotype or serogroup was assigned on the basis of fragment size. The method was used to test 93 clinical isolates, and all isolates of the serotypes concerned were correctly detected. The strategy would allow the detection of multiple serotypes in a single sample. Detection of additional serotypes could be included as capsule

locus sequences become available.

Leary, T. P., J. C. Erker, et al. (2002). "Detection of Mammalian Reovirus RNA by Using Reverse Transcription-PCR: Sequence Diversity within the {lambda}3-Encoding L1 Gene." J. Clin. Microbiol. **40**(4): 1368-1375.

http://jcm.asm.org/cgi/content/abstract/40/4/1368

Reoviruses infect virtually all mammalian species, and infection of humans is associated with mild gastrointestinal or upper respiratory illnesses. To improve reovirus detection strategies, we developed a reverse transcription-PCR technique to amplify a fragment of the reovirus L1 gene segment. This assay was capable of detecting 44 of 44 reovirus field isolate strains and was sufficiently sensitive to detect nearly a single viral particle (1.16 {+/-} 0.13) per PCR of prototype strain type 3 Dearing. Pairwise comparisons of the 44 partial L1 gene sequences revealed that nucleotide variability ranged from 0 to 24.7%, with most of the nucleotide polymorphism occurring at synonymous positions. Phylogenetic trees generated from amplified L1 gene sequences suggest that multiple alleles of the L1 gene cocirculate in nature and that genetic diversity of the L1 gene is largely independent of the host species, geographic locale, or date of isolation. Phylogenetic trees constructed from the L1 gene sequences are distinct from those constructed from the four reovirus S-class gene segments, which supports the hypothesis that reovirus gene segments reassort in nature. This study establishes a new sensitive and specific technique for the identification of mammalian reoviruses and enhances our understanding of reovirus evolution.

Legoff, J., E. Guerot, et al. (2005). "High Prevalence of Respiratory Viral Infections in Patients Hospitalized in an Intensive Care Unit for Acute Respiratory Infections as Detected by Nucleic Acid-Based Assays." J. Clin. Microbiol. **43**(1): 455-457.

http://jcm.asm.org/cgi/content/abstract/43/1/455

Forty-seven bronchoalveolar lavages (BAL) were obtained from 41 patients with acute pneumonia attending an intensive care unit. By molecular diagnosis, 30% of total BAL and 63% of bacteria-negative BAL were positive for respiratory viruses. Molecular detection allows for high-rate detection of respiratory viral infections in adult patients suffering from severe pneumonia.

Li, H., N. S. Taus, et al. (2004). "Shedding of Ovine Herpesvirus 2 in Sheep Nasal Secretions: the Predominant Mode for Transmission." J. Clin. Microbiol. **42**(12): 5558-5564.

http://jcm.asm.org/cgi/content/abstract/42/12/5558

Ovine herpesvirus 2 (OvHV-2), the major causative agent of malignant catarrhal fever in ruminant species worldwide, has never been propagated in vitro. Using real-time PCR, a striking, short-lived, peak of viral DNA, ranging from 105 to over 108 copies/2 {micro}g of DNA, was detected in nasal secretions from over 60.7% of adolescent sheep (n = 56) at some point during the period from 6 to 9 months of age. In contrast, only about 18% of adult sheep (n = 33) experienced a shedding episode during the study period. The general pattern of the appearance of viral DNA in nasal secretions was a dramatic rise and subsequent fall within 24 to 36 h, implying a single cycle of viral replication. These episodes occurred sporadically and infrequently, but over the 3-month period most of the 56 lambs (33, or 60.7%) experienced at least one episode. No corresponding fluctuations in DNA levels were found in either peripheral blood leukocytes or plasma. In a DNase

protection assay, complete, enveloped OvHV-2 virions were demonstrated in the nasal secretions of all sheep examined during the time when they were experiencing an intense shedding episode. OvHV-2 infectivity in nasal secretions was also demonstrated by aerosolization of the secretions into OvHV-2-negative sheep. The data herein show that nasal shedding is the major mode of OvHV-2 transmission among domestic sheep and that adolescents represent the highest risk group for transmission.

Li, J., D. S. Gerhard, et al. (2003). "Denaturing High-Performance Liquid Chromatography for Detecting and Typing Genital Human Papillomavirus." J. Clin. Microbiol. **41**(12): 5563-5571.

http://jcm.asm.org/cgi/content/abstract/41/12/5563

Human papillomaviruses (HPVs) are important in the development of human cancers, including cervical and oral tumors. However, most existing methods for HPV typing cannot routinely distinguish among the more than 100 distinct types of HPV or the natural HPV intratypic variants that have also been documented. To address this problem, we developed a novel method, general primer-denaturing high-performance liquid chromatography (GP-dHPLC), for the detection and typing of genital HPV using an automated 96-well plate format. GP-dHPLC uses general primer PCR (GP-PCR) to amplify the viral DNA and then analyzes the GP-PCR products by denaturing high-performance liquid chromatography (dHPLC). A number of different primer pairs with homology to most known genital HPV types were tested, and the L1C1-L1C2M pair specific for the L1 region of the viral genome was chosen. A set of HPV standard control patterns, consisting of those for HPV types 16, 18, 31, 33, 39, 45, 51, 52, 56, 58, 59, 6, and 11, was established for genital HPV typing. One hundred eighty-six frozen and formalin-fixed cervical cancer tissue samples were analyzed for the presence of HPV and the HPV type by this method, and 95.8% of them were found to contain HPV DNA. GP-dHPLC accurately discriminated among HPV variants that differed by as little as one nucleotide. Several new variants of HPV types 16, 18, 39, 45, 52, and 59 were identified. Moreover, multiple HPV infections were detected in 26.6% of the samples. Our results indicate that HPV typing by GP-dHPLC permits discrimination of common genital HPV types, detection of multiple HPV infections, and identification of HPV variants in clinical samples.

Liddell, A. M., S. L. Stockham, et al. (2003). "Predominance of Ehrlichia ewingii in Missouri Dogs." J. Clin. Microbiol. **41**(10): 4617-4622.

http://jcm.asm.org/cgi/content/abstract/41/10/4617

To investigate the species distribution of Ehrlichia present in Missouri dogs, we tested 78 dogs suspected of having acute ehrlichiosis and 10 healthy dogs. Blood from each dog was screened with a broad-range 16S rRNA gene PCR assay that detects known pathogenic species of Ehrlichia and Anaplasma. The species was determined by using species-specific PCR assays and nucleotide sequencing. Ehrlichia antibody testing was performed by using an indirect immunofluorescence assay with Ehrlichia chaffeensis as the antigenic substrate. The broad-range assay detected Ehrlichia or Anaplasma DNA in 20 (26%) of the symptomatic dogs and 2 (20%) of the asymptomatic dogs. E. ewingii accounted for 20 (91%), and E. chaffeensis accounted for 1 (5%) of the positives. Anaplasma phagocytophilum DNA was detected in one dog, and the sequences of regions of the 16S rRNA gene and the groESL operon amplified from the blood of this dog matched the published sequences of this organism. Antibodies reactive with E. chaffeensis were detected in 14 (67%) of the 21 PCR-positive dogs and in 12 (19%) of the 64 PCR-negative dogs. Combining the results of PCR and serology indicated that 33 (39%) of 85 evaluable dogs had evidence of past or current Ehrlichia infection. We conclude that E. ewingii is the predominant etiologic agent of canine ehrlichiosis in the areas of Missouri included in this

survey. E. canis, a widely recognized agent of canine ehrlichiosis, was not detected in any animal. The finding of E. ewingii in asymptomatic dogs suggests that dogs could be a reservoir for this Ehrlichia species.

Lievano, F. A., M. A. Reynolds, et al. (2002). "Issues Associated with and Recommendations for Using PCR To Detect Outbreaks of Pertussis." J. Clin. Microbiol. **40**(8): 2801-2805.

http://jcm.asm.org/cgi/content/abstract/40/8/2801

Two outbreaks of respiratory tract illness associated with prolonged cough occurring in 1998 and 1999 in New York State were investigated. A PCR test for Bordetella pertussis was primarily used by a private laboratory to confirm 680 pertussis cases. Several clinical specimens had positive culture results for B. pertussis during both outbreaks, which confirmed that B. pertussis was circulating during the outbreaks. However, testing by the New York State Department of Health reference laboratory suggested that some of the PCR results may have been falsely positive. In addition, features of the outbreak that suggested that B. pertussis may not have been the primary agent of infection included a low attack rate among incompletely vaccinated children and a significant amount of illness among patients testing PCR negative for B. pertussis. These investigations highlight the importance of appropriate clinical laboratory quality assurance programs, of the limitations of the PCR test, and of interpreting laboratory results in context of clinical disease.

Lindecrona, R. H., T. K. Jensen, et al. (2002). "Application of a 5' Nuclease Assay for Detection of Lawsonia intracellularis in Fecal Samples from Pigs." J. Clin. Microbiol. **40**(3): 984-987.

http://jcm.asm.org/cgi/content/abstract/40/3/984

A 5' nuclease assay was developed to detect Lawsonia intracellularis in porcine fecal samples. The specific probe and primers were chosen by using the 16S ribosomal DNA gene as a target. The 5' nuclease assay was used with a total of 204 clinical samples, and the results were compared to those of immunohistochemistry (IM) on ileal sections of the same animals. There was 91% agreement between the results of IM and the 5' nuclease assay. In the 5' nuclease assay, 111 (54%) of the pigs tested positive for L. intracellularis infection, with a mean cycle threshold (Ct) value of 27.2, whereas 98 (48%) of the pigs tested positive by IM. On average, the Ct and {Delta}Rn values for the positive samples were 27.2 (standard deviation [SD], 3.7) and 1.6 (SD, 0.7), respectively. A Ct value of 27.2 corresponds to a fecal excretion of approximately 107 L. intracellularis cells per g of feces. Furthermore, a total of 40 fecal samples derived from a herd known to be free from infection with L. intracellularis all tested negative, with a Ct value of 40. By using a Ct value of 36 as the cutoff limit, the detection limit of the assay was 1 L. intracellularis cell per PCR tube. In conclusion, the 5' nuclease assay that has been developed represents an applicable fast method for detection of L. intracellularis in fecal samples, with a sensitivity and specificity comparable to those of IM.

Lindell, A. T., L. Grillner, et al. (2005). "Molecular Epidemiology of Norovirus Infections in Stockholm, Sweden, during the Years 2000 to 2003: Association of the GGIIb Genetic Cluster with Infection in Children." J. Clin. Microbiol. **43**(3): 1086-1092.

http://jcm.asm.org/cgi/content/abstract/43/3/1086

The incidence of norovirus-associated gastroenteritis and the molecular epidemiology of norovirus strains were studied during three seasons (2000-2001, 2001-2002, and 2002-2003) among patients of all ages, mainly from the Stockholm region in Sweden. A total of 3,252 fecal samples were analyzed by reverse transcription-PCR. The incidences of norovirus infection among adults were 23, 26, and 30% during the three seasons studied and 18, 11, and 15% among children 0 to 15 years of age. During the first season, all norovirus strains detected by PCR were typed either by reverse line blot hybridization or nucleotide sequence analysis. During the two successive seasons, a total of 60 norovirus-positive strains from the beginning, peak, and end of the seasons were selected for nucleotide sequence analysis. We identified two dominant norovirus variants over the seasons: a new norovirus variant, recently described as the GGIIb genetic cluster, dominated among children during the first season, and during the following two seasons, a GGII-4 variant dominated. Our data suggest that norovirus infections are common, not only among adults, but also among children, and that some strains may predominantly affect children.

Lindstedt, B.-A., E. Heir, et al. (2003). "DNA Fingerprinting of Salmonella enterica subsp. enterica Serovar Typhimurium with Emphasis on Phage Type DT104 Based on Variable Number of Tandem Repeat Loci." J. Clin. Microbiol. **41**(4): 1469-1479.

http://jcm.asm.org/cgi/content/abstract/41/4/1469

Seventy-eight human and environmental strains of Salmonella enterica subsp. enterica serovar Typhimurium, as well as 18 isolates of other Salmonella serovars and 6 isolates of Escherichia coli, were subjected to a novel variable number of tandem repeats (VNTR)-based fingerprinting method that showed high discrimination and reproducibility for typing serovar Typhimurium isolates. The method is based on capillary separation of PCR products from fluorescence-labeled VNTR in the serovar Typhimurium genome. The serovar Typhimurium isolates displayed 54 VNTR patterns, and the VNTR assay correctly identified strains from a well-characterized outbreak. Among 37 serovar Typhimurium phage type DT104 isolates, 28 distinct VNTR patterns were found. This VNTR-based method is fast and suitable for complete automation. Our VNTR-based method was capable of high discrimination within the homogeneous serovar Typhimurium DT104 phage type and can be used to trace outbreaks and to monitor DT104 as well as other phage types. The VNTR assay was compared to XbaI pulsed-field gel electrophoresis, amplified fragment length polymorphism analysis, integron-cassette profiles and gene PCR of intI1, qacE{Delta}1, sulI1, and floR. The VNTR assay showed greatly improved resolution compared to all other tested methods in this study.

Lindstrom, A., J. Odeberg, et al. (2004). "Pyrosequencing for Detection of Lamivudine-Resistant Hepatitis B Virus." J. Clin. Microbiol. **42**(10): 4788-4795.

http://jcm.asm.org/cgi/content/abstract/42/10/4788

Chronic hepatitis B virus (HBV) infection can cause severe liver disease, including cirrhosis and hepatocellular carcinoma. Lamivudine is a relatively recent alternative to alpha interferon for the treatment of HBV infection, but unfortunately, resistance to lamivudine commonly develops during monotherapy. Lamivudine-resistant HBV mutants display specific mutations in the YMDD (tyrosine, methionine, aspartate, aspartate) motif of the viral polymerase (reverse transcriptase [rt]), which is the catalytic site of the enzyme, i.e., methionine 204 to isoleucine (rtM204I) or valine (rtM204V). The latter mutation is often accompanied by a compensatory leucine-to-methionine change at codon 180 (rtL180M). In the present study, a novel sequencing method, pyrosequencing, was applied to the detection of lamivudine resistance mutations and was compared with direct Sanger sequencing. The new pyrosequencing method had advantages in

terms of throughput. Experiments with mixtures of wild-type and resistant viruses indicated that pyrosequencing can detect minor sequence variants in heterogeneous virus populations. The new pyrosequencing method was evaluated with a small number of patient samples, and the results showed that the method could be a useful tool for the detection of lamivudine resistance in the clinical setting.

Lo Cascio, G., M. Ligozzi, et al. (2004). "Utility of Molecular Identification in Opportunistic Mycotic Infections: a Case of Cutaneous Alternaria infectoria Infection in a Cardiac Transplant Recipient." J. Clin. Microbiol. **42**(11): 5334-5336.

http://jcm.asm.org/cgi/content/abstract/42/11/5334

We report on a case of cutaneous infection caused by Alternaria infectoria in a cardiac transplant recipient. A rapid molecular diagnosis was obtained by sequence analysis of the internal transcribed spacer domain of the 5.8S ribosomal DNA region amplified from colonies developed on Sabouraud medium. Treatment consisted of a combination of systemic antifungal therapy, first with amphotericin B and then with itraconazole.

Lovmar, L., C. Fock, et al. (2003). "Microarrays for Genotyping Human Group A Rotavirus by Multiplex Capture and Type-Specific Primer Extension." J. Clin. Microbiol. **41**(11): 5153-5158.

http://jcm.asm.org/cgi/content/abstract/41/11/5153

Human group A rotavirus (HRV) is the major cause of severe gastroenteritis in infants worldwide. HRV shares the feature of a high degree of genetic diversity with many other RNA viruses, and therefore, genotyping of this organism is more complicated than genotyping of more stable DNA viruses. We describe a novel microarray-based method that allows high-throughput genotyping of RNA viruses with a high degree of polymorphism by multiplex capture and type-specific extension on microarrays. Denatured reverse transcription (RT)-PCR products derived from two outer capsid genes of clinical isolates of HRV were hybridized to immobilized capture oligonucleotides representing the most commonly occurring P and G genotypes on a microarray. Specific primer extension of the type-specific capture oligonucleotides was applied to incorporate the fluorescent nucleotide analogue cyanine 5-labeled dUTP as a detectable label. Laser scanning and fluorescence detection of the microarrays was followed by visual or computer-assisted interpretation of the fluorescence patterns generated on the microarrays. Initially, the method detected HRV in all 40 samples and correctly determined both the G and the P genotypes of 35 of the 40 strains analyzed. After modification by inclusion of additional capture oligonucleotides specific for the initially unassigned genotypes, all genotypes could be correctly defined. The results of genotyping with the microarray fully agreed with the results obtained by nucleotide sequence analysis and sequence-specific multiplex RT-PCR. Owing to its robustness, simplicity, and general utility, the microarray-based method may gain wide applicability for the genotyping of microorganisms, including highly variable RNA and DNA viruses.

Lovseth, A., S. Loncarevic, et al. (2004). "Modified Multiplex PCR Method for Detection of Pyrogenic Exotoxin Genes in Staphylococcal Isolates." J. Clin. Microbiol. **42**(8): 3869-3872.

http://jcm.asm.org/cgi/content/abstract/42/8/3869

A modified multiplex PCR method for detection of nine Staphylococcus aureus enterotoxin genes

(sea, seb, sec, sed, see, seg, seh, sei, and sej) and one form of immunoreactive toxic shock syndrome toxin based on a previously published method (S. R. Monday and G. A. Bohach, J. Clin. Microbiol. 37:3411-3414, 1999) has been developed. The modified PCR protocol seems robust and gives reliable results.

Luo, W., H. Yang, et al. (2005). "Detection of Human Immunodeficiency Virus Type 1 DNA in Dried Blood Spots by a Duplex Real-Time PCR Assay." J. Clin. Microbiol. **43**(4): 1851-1857.

http://jcm.asm.org/cgi/content/abstract/43/4/1851

A dried blood spot (DBS) is a well-accepted means for the collection, transport, and storage of blood samples for various epidemiologic, serologic, and molecular assays for human immunodeficiency virus (HIV) studies. It is particularly important for mother-to-infant-transmission studies of affected individuals living in remote areas. We have developed a real-time PCR method to detect HIV type 1 (HIV-1) DNA in dried blood spots. A cellular gene, RNase P, was coamplified with the HIV-1 DNA in the same tube to monitor the DNA extraction efficiency and the overall assay performance. Our assay is a one-tube, single-step closed-system assay and uses a dUTP/uracil DNA glycosidase anti-PCR contamination control. The HIV-1 primers and probe were derived from a conserved region of the long terminal repeat. The detection of RNase P is attenuated by lowering the forward and reverse primer concentrations so that its amplification will not overwhelm the HIV-1 amplification and yet will provide a semiquantitative measurement of the quality of the isolated DBS DNA. We examined 103 HIV-1-seropositive and 56 seronegative U.S. adults and found that our assay has a sensitivity of 98.1% (95% confidence interval [CI], 95.5% to 100%) and specificity of 100% (95% CI, 99% to 100%). The positive and negative predictive values are 100% and 96.6%, respectively. This duplex PCR assay may be useful in identifying HIV-1-infected persons, particularly infants born to seropositive mothers in remote areas of the world.

Ma, L. and D. H. Martin (2004). "Single-Nucleotide Polymorphisms in the rRNA Operon and Variable Numbers of Tandem Repeats in the Lipoprotein Gene among Mycoplasma genitalium Strains from Clinical Specimens." J. Clin. Microbiol. **42**(10): 4876-4878.

http://jcm.asm.org/cgi/content/abstract/42/10/4876

We characterized the single-nucleotide polymorphisms in the rRNA operon and variable numbers of tandem repeats in the lipoprotein gene MG309 among Mycoplasma genitalium strains from clinical specimens by PCR and sequencing. Analysis of 31 M. genitalium-infected patient specimens and 7 American Type Culture Collection strains identified six types of rRNA sequences and 11 different numbers of MG309 repeats. Examination of sequential specimens from 10 patients showed that these genotypes were stable for at least 5 weeks. These data suggest the potential usefulness of the rRNA genotypes and the MG309 repeats for genotyping of M. genitalium.

Maes, N., J. Magdalena, et al. (2002). "Evaluation of a Triplex PCR Assay To Discriminate Staphylococcus aureus from Coagulase-Negative Staphylococci and Determine Methicillin Resistance from Blood Cultures." J. Clin. Microbiol. **40**(4): 1514-1517.

http://jcm.asm.org/cgi/content/abstract/40/4/1514

A triplex PCR targeting the 16S rRNA, mecA, and nuc genes was developed for identification of staphylococci and detection of methicillin resistance. After validation of the assay with a collection of strains of staphylococci and enterococci (n = 169), the assay was evaluated with cultures of blood with gram-positive cocci from 40 patients. Accurate results were obtained for 59 (98%) of 61 cultures within 6 h of growth detection.

Maggi, R. G. and E. B. Breitschwerdt (2005). "Potential Limitations of the 16S-23S rRNA Intergenic Region for Molecular Detection of Bartonella Species." J. Clin. Microbiol. **43**(3): 1171-1176.

http://jcm.asm.org/cgi/content/abstract/43/3/1171

PCR targeting the 16S-23S rRNA gene intergenic transcribed spacer (ITS) region has been proposed as a rapid and reliable method for the detection of Bartonella species DNA in clinical samples. Because of variation in ITS sequences among Bartonella species, a single PCR amplification can be used to detect different species within this genus. Therefore, by targeting the ITS region, multiple PCRs or additional sample-processing steps beyond the primary amplification can be avoided when attempting to achieve molecular diagnostic detection of Bartonella species. Although PCR amplification targeting this region is considered highly sensitive, amplification specificity obviously depends on primer design. We report evidence of nonspecific PCR amplification of Mesorhizobium species with previously published primers that were designed to amplify the Bartonella consensus ITS region. Use of these or other, less species-specific, primers could lead to a false-positive diagnostic test result when evaluating clinical samples. We also report the presence of Mesorhizobium species DNA as a contaminant in molecular-grade water, a series of homologous sequences in the ITS region that are common to Bartonella and Mesorhizobium species, the amplification of Mesorhizobium DNA with unpublished primers designed in our laboratory targeting the ITS region, and the subsequent design of unambiguous ITS primers that avoid nonspecific amplification of Mesorhizobium species. Our results define some potential limitations associated with the molecular detection of Bartonella species in patient samples and indicate that primer specificity is of critical importance if the ITS region is used as a diagnostic target for detection of Bartonella species.

Maraha, B., H. Berg, et al. (2004). "Is the Perceived Association between Chlamydia pneumoniae and Vascular Diseases Biased by Methodology?" J. Clin. Microbiol. **42**(9): 3937-3941.

http://jcm.asm.org/cgi/content/abstract/42/9/3937

Inter- and intralaboratory inconsistencies in detection rates of Chlamydia pneumoniae in vascular specimens have been demonstrated. In this study, 66 vascular tissue specimens from 66 patients with vascular disease were tested by three PCR assays: a 16S PCR-based reverse line blot (RLB) assay, a single-step PCR, and a nested PCR. Also, we explored the impacts of different DNA polymerase enzymes on the results based on gel electrophoresis and hybridization. The PCR results by gel electrophoresis in the single-step PCR depended on which DNA polymerase was used. All samples were negative with AmpliTaq Gold DNA polymerase, and 54.5% (36 of 66) were positive with the conventional Taq DNA polymerase. All samples were negative after hybridization with a C. pneumoniae-specific probe. In the nested PCR, all specimens were negative by gel electrophoresis and after hybridization. The RLB assay failed to detect C. pneumoniae in any specimen; however, 20 specimens were Chlamydia sp. positive. The sequence analysis of six of these samples demonstrated Chlamydia-like organisms. RLB detected Chlamydia sp. DNA in water and in the elution buffer after passage of the Qiagen columns (11 of 40). This study identified factors that may influence the detection of C. pneumoniae DNA in vascular tissues and consequently bias the perception of a link between C. pneumoniae and vascular diseases. The following are strongly recommended: to use DNA

polymerases that have to be activated, to decontaminate with dUTP-uracil-DNA glycosylase, to hybridize with specific probes, to include sufficient controls, and to use molecular grade water.

Marianelli, C., F. Ciuchini, et al. (2004). "Genetic Bases of the Rifampin Resistance Phenotype in Brucella spp." J. Clin. Microbiol. **42**(12): 5439-5443.

http://jcm.asm.org/cgi/content/abstract/42/12/5439

Rifampin is one of the most potent and broad-spectrum antibiotics against bacterial pathogens. Its bactericidal activity is due to its ability to bind to the {beta} subunit of the DNA-dependent RNA polymerase encoded by the rpoB gene. Mutations of the rpoB gene have been characterized in rifampin-resistant (Rifr) strains of Escherichia coli and Mycobacterium tuberculosis. The genetic bases of Rifr in Brucella spp. are still unknown. In the present study, the nucleotide sequences of the rpoB gene of the Rifr vaccine strain Brucella abortus RB51 and of 20 Rifr clones derived in our laboratory from two Brucella melitensis isolates were determined. These sequences were then compared to those of the respective rifampin-susceptible (Rifs) parental strains and to the published B. melitensis strain 16M. All Rifr strains carried one or more missense mutations mapping in two regions of the rpoB gene. These two "hot" regions were investigated in eight additional Rifr Brucella laboratory mutants and in 20 reference Rifs Brucella strains. rpoB mutations were found in all Rifr mutants. In contrast, no missense mutations were found in any analyzed Rifs strains. Our results represent the first from a study of the molecular characterization of rpoB mutations in resistant Brucella strains and provide an additional proof of the association of specific rpoB mutations with the development of the Rifr phenotype in prokaryotes. In addition, because of the relationship between Rifr and the attenuation of virulence in Brucella spp., studies of virulence in these mutants may provide useful information about the genetic basis of pathogenesis in Brucella.

Martella, V., M. Ciarlet, et al. (2003). "Molecular Analysis of the VP7, VP4, VP6, NSP4, and NSP5/6 Genes of a Buffalo Rotavirus Strain: Identification of the Rare P[3] Rhesus Rotavirus-Like VP4 Gene Allele." J. Clin. Microbiol. **41**(12): 5665-5675.

http://jcm.asm.org/cgi/content/abstract/41/12/5665

We report the detection and molecular characterization of a rotavirus strain, 10733, isolated from the feces of a buffalo calf affected with diarrhea in Italy. Strain 10733 was classified as a P[3] rotavirus, as the VP8* trypsin cleavage product of the VP4 protein revealed a high amino acid identity (96.2%) with that of rhesus rotavirus strain RRV (P5B[3]), used as the recipient virus in the human-simian reassortant vaccine. Analysis of the VP7 gene product revealed that strain 10733 possessed G6 serotype specificity, a type common in ruminants, with an amino acid identity to G6 rotavirus strains ranging from 88 to 98%, to Venezuelan bovine strain BRV033, and Hungarian human strain Hun4. Phylogenetic analysis based on the VP7 gene of G6 rotaviruses identified at least four lineages and an apparent linkage between each lineage and the VP4 specificity, suggesting the occurrence of repeated interspecies transmissions and genetic reassortment events between ruminant and human rotaviruses. Moreover, strain 10733 displayed a bovine-like NSP4 and NSP5/6 and a subgroup I VP6 specificity, as well as a long electropherotype pattern. The detection of the rare P[3] genotype in ruminants provides additional evidence for the wide genetic and antigenic diversity of group A rotaviruses.

Marty, F. M., D. H. Barouch, et al. (2003). "Disseminated Trichosporonosis Caused by Trichosporon

loubieri." J. Clin. Microbiol. **41**(11): 5317-5320.

http://jcm.asm.org/cgi/content/abstract/41/11/5317

Trichosporonosis is an emerging invasive fungal infection in immunosuppressed patients; a case of disseminated infection caused by Trichosporon loubieri presented confirms its role as a human pathogen.

Massung, R. F. and K. G. Slater (2003). "Comparison of PCR Assays for Detection of the Agent of Human Granulocytic Ehrlichiosis, Anaplasma phagocytophilum." J. Clin. Microbiol. **41**(2): 717-722.

http://jcm.asm.org/cgi/content/abstract/41/2/717

Human granulocytic ehrlichiosis is an emerging infectious disease in the United States and Europe, and PCR methods have been shown to be effective for the diagnosis of acute infections. Numerous PCR assays and primer sets have been reported in the literature. The analytical sensitivities (limits of detection) of 13 published PCR primer sets were compared using DNA extracted from serial dilutions of Anaplasma phagocytophilum-infected HL-60 cells. The specificity of the assays that were able to detect [<=]2.5 infected cells was tested by the use of template DNA extracted from Ehrlichia chaffeensis, Rickettsia rickettsii, and Bartonella henselae. The assays with the lowest limits of detection were shown to be a nested assay that amplifies the 16S rRNA gene (primer pairs ge3a-ge10 [primary] and ge9-ge3 [nested]; detects 0.25 infected cell), a direct assay that amplifies the major surface protein gene msp2 (primer pair msp2-3f-msp2-3r; detects 0.25 infected cell), and a direct assay that amplifies the 16S rRNA gene (primer pair ehr521-ehr790; detects 0.25 infected cell). The specificity and limit of detection of the MSP2 and 16S rRNA direct assays were further tested by use of A. phagocytophilum template DNA from both North America and Europe and from human, tick, white-footed mouse, equine, deer, bovine, and wood rat samples and of template DNA from closely related species (Anaplasma marginale, the white-tailed deer agent, and additional E. chaffeensis-positive samples). Three manufacturers' PCR kits were tested and showed distinct variations in the limit of detection, specificity, and nonspecific background amplification. The importance of these results for the molecular diagnosis of human granulocytic ehrlichiosis is discussed.

Menotti, J., G. Vilela, et al. (2003). "Comparison of PCR-Enzyme-Linked Immunosorbent Assay and Real-Time PCR Assay for Diagnosis of an Unusual Case of Cerebral Toxoplasmosis in a Stem Cell Transplant Recipient." J. Clin. Microbiol. **41**(11): 5313-5316.

http://jcm.asm.org/cgi/content/abstract/41/11/5313

A PCR-enzyme-linked immunosorbent assay and a real-time PCR assay were compared for diagnosis and follow-up of cerebral toxoplasmosis in a stem cell transplant recipient. The sensitivity of detection was similar for both assays but was higher when the assays were performed on buffy coat rather than on whole blood or serum.

Mittelholzer, C., L. Englund, et al. (2003). "Detection and Sequence Analysis of Danish and Swedish Strains of Mink Astrovirus." J. Clin. Microbiol. **41**(11): 5192-5194.

http://jcm.asm.org/cgi/content/abstract/41/11/5192

The sequences of mink astroviruses collected from 11 farms in Denmark and Sweden were analyzed and found to be homologous with one another but different from those of other astroviruses. A species-specific reverse transcriptase-PCR for mink astrovirus was established and shown to be suitable for the analysis of clinical samples.

Moberg, M., I. Gustavsson, et al. (2003). "Real-Time PCR-Based System for Simultaneous Quantification of Human Papillomavirus Types Associated with High Risk of Cervical Cancer." J. Clin. Microbiol. **41**(7): 3221-3228.

http://jcm.asm.org/cgi/content/abstract/41/7/3221

We have previously shown that women with a high titer of human papillomavirus type 16 (HPV16) in cervical epithelial cells have an increased risk of developing cervical carcinoma in situ. In order to study the relationship between viral DNA amount and risk of cervical carcinoma for the HPV types most commonly found in cervical tumors, we developed a real-time PCR assay for the detection and quantification of HPV16, -18, -31, -33, -35, -39, -45, -52, -58, and -67. These HPV types are analyzed in two reaction tubes, allowing for independent quantification of three viral types, or groups of viral types, in each reaction. A separate reaction is used for estimating the number of a nuclear single-copy gene and is used to calculate the HPV copy number per genomic DNA equivalent in the sample. The system has a dynamic range from 102 to 107 HPV copies per assay and is applicable to both fresh clinical samples and DNA extracted from archival samples. Reconstitution experiments, made to mimic infections with several HPV types, shows that individual HPV types can be detected in a mixture as long as they represent 1 to 10% of the main type. The system was evaluated with respect to technical specificity and sensitivity, reproducibility, reagent stability, and sample preparation protocol and then used to analyze clinical samples. This homogeneous assay provides a fast and sensitive way for estimating the viral load of a series of the most frequent oncogenic HPV types in biopsies, as well as cervical smear samples.

Moissenet, D., H. Vu-Thien, et al. (2003). "DNA Fingerprinting of Ralstonia paucula by Infrequent-Restriction-Site PCR and Randomly Amplified Polymorphic DNA Analysis." J. Clin. Microbiol. **41**(12): 5747-5749.

http://jcm.asm.org/cgi/content/abstract/41/12/5747

Ralstonia paucula (formerly CDC group IV c-2) is an environmental organism that can cause serious human infections, occasionally clusters of nosocomial infections. In the present work, 26 strains of R. paucula (4 from the American Centers for Disease Control and Prevention collection, 10 from the Belgian Laboratorium voor Microbiologie [LMG] collection, and 12 French clinical isolates) were analyzed with infrequent-restriction-site PCR and randomly amplified polymorphic DNA analysis. Both techniques accurately distinguished between collection strains. Two close patterns obtained for all the French isolates suggested a clonal strain. Two LMG collection strains originating from human sources in the United States also showed patterns close to those of French isolates.

Molano, M., C. J. L. M. Meijer, et al. (2004). "Combination of PCR Targeting the VD2 of omp1 and Reverse Line Blot Analysis for Typing of Urogenital Chlamydia trachomatis Serovars in Cervical Scrape Specimens." J. Clin. Microbiol. **42**(7): 2935-2939.

http://jcm.asm.org/cgi/content/abstract/42/7/2935

In this study we developed and evaluated a new PCR-based typing assay, directed to the VD2 region of the omp1 gene, for the detection and typing of urogenital Chlamydia trachomatis infections. A nested VD2 PCR-reverse line blot (RLB) assay was developed for the typing of nine different urogenital serovars of C. trachomatis. The assay developed was tested with reference strains of C. trachomatis serovars and cervical scrapes of 86 Colombian women previously found to be positive for C. trachomatis by using plasmid PCR. Two sets of primers directed to the VD2 region of the omp1 gene of C. trachomatis were designed, and fragments of 220 and 166 bp were generated in the primary and nested PCRs, respectively. In addition, an RLB assay was developed to identify nine different urogenital serovars of C. trachomatis (Ba, D, E, F, G, H, I, J, and K) and group controls, including group B (Ba, D, and E), group C (I, J, K, and H), and an intermediate group (F and G). Using this assay, we were able to type 81 of the 86 samples (94.2%). Of these samples, 91.3% were single C. trachomatis infections, and 8.7% were multiple infections. The most common serovars identified were serovars D (22.2%), F (18.5%), G (13.6%), and E (12.3%). Of the women with multiple C. trachomatis infections, >50% contained both serovars D and E. The nested VD2 PCR-RLB developed is a simple, fast, and specific method for the identification of individual urogenital C. trachomatis serovars previously detected by using plasmid PCR. Moreover, it is an appropriate method for studying multiple C. trachomatis infections and for use in large epidemiological studies.

Mothershed, E. A., P. K. Cassiday, et al. (2002). "Development of a Real-Time Fluorescence PCR Assay for Rapid Detection of the Diphtheria Toxin Gene." J. Clin. Microbiol. **40**(12): 4713-4719.

http://jcm.asm.org/cgi/content/abstract/40/12/4713

We developed and evaluated a real-time fluorescence PCR assay for detecting the A and B subunits of diphtheria toxin (tox) gene. When 23 toxigenic Corynebacterium diphtheriae strains, 9 nontoxigenic C. diphtheriae strains, and 44 strains representing the diversity of pathogens and normal respiratory flora were tested, this real-time PCR assay exhibited 100% sensitivity and specificity. It allowed for the detection of both subunits of the tox gene at 750 times greater sensitivity (2 CFU) than the standard PCR (1,500 CFU). When used directly on specimens collected from patients with clinical diphtheria, one or both subunits of the tox gene were detected in 34 of 36 specimens by using the real-time PCR assay; only 9 specimens were found to be positive by standard PCR. Reamplification by standard PCR and DNA sequencing of the amplification product confirmed all real-time PCR tox-positive reactions. This real-time PCR format is a more sensitive and rapid alternative to standard PCR for detection of the tox gene in clinical material.

Muyldermans, G., D. Pierard, et al. (2004). "Simple Algorithm for Identification of Bordetella pertussis Pertactin Gene Variants." J. Clin. Microbiol. **42**(4): 1614-1619.

http://jcm.asm.org/cgi/content/abstract/42/4/1614

Studies performed in several countries have demonstrated the recent emergence and subsequent dominance of circulating Bordetella pertussis strains harboring pertactin and pertussis toxin variants not included in pertussis vaccines. Determination of the pertactin gene variants is commonly performed using a time-consuming and expensive sequence analysis. We developed a simple and reliable pertactin typing algorithm suitable for large-scale screening. The assay correctly identified all pertactin alleles in representative strains. The typing of 231 clinical strains of B. pertussis routinely isolated in Belgium showed that this algorithm was adequate to

identify less-frequent prn types like prn9 and prn11.

Nadkarni, M. A., C. E. Caldon, et al. (2004). "Carious Dentine Provides a Habitat for a Complex Array of Novel Prevotella-Like Bacteria." J. Clin. Microbiol. **42**(11): 5238-5244.

http://jcm.asm.org/cgi/content/abstract/42/11/5238

Previous analysis of the microbiology of advanced caries by culture and real-time PCR emphasized the high incidence and abundance of gram-negative anaerobic species, particularly Prevotella-like bacteria. The diversity of Prevotella-like bacteria was further explored by analyzing pooled bacterial DNA from lesions of carious dentine. This was achieved by amplification of a region of the 16S ribosomal DNA with a Prevotella genus-specific forward primer and a universal bacterial reverse primer, followed by cloning and sequencing. Cultured Prevotella species commonly associated with oral tissues constituted only 12% of the Prevotella clones isolated from advanced carious lesions. The remaining 88% consisted of a diverse range of phylotypes. These included five clusters of previously recognized but uncultured oral Prevotella spp. and a major cluster containing Prevotella-like bacteria most closely related to uncharacterized rumen bacteria. Cluster-specific primers were designed, and the numbers of bacteria within clusters were quantified by real-time PCR, confirming the abundance of these organisms. The data indicated that advanced dental caries provides a unique environment for a complex array of novel and uncultured Prevotella and Prevotella-like bacteria which, in some cases, may dominate the diverse polymicrobial community associated with the disease.

Nagai, T., H. Sobajima, et al. (2003). "Neonatal Sudden Death Due to Legionella Pneumonia Associated with Water Birth in a Domestic Spa Bath." J. Clin. Microbiol. **41**(5): 2227-2229.

http://jcm.asm.org/cgi/content/abstract/41/5/2227

We report the first case of neonatal Legionnaires' disease associated with water birth in a spa bath at home. Legionella pneumophila serogroup 6 was detected from postmortem lung tissue.

Nakano, K., R. Nomura, et al. (2004). "Demonstration of Streptococcus mutans with a Cell Wall Polysaccharide Specific to a New Serotype, k, in the Human Oral Cavity." J. Clin. Microbiol. **42**(1): 198-202.

http://jcm.asm.org/cgi/content/abstract/42/1/198

Streptococcus mutans organisms are occasionally isolated from the blood of patients with infective endocarditis, though the mechanisms of invasion and survival remain to be elucidated. Two of four blood isolates from patients with bacteremia or infective endocarditis (strains TW295 and TW871) were serologically untypeable by immunodiffusion testing, which was due to a lack of the glucose side chain of the serotype-specific polysaccharide antigen of S. mutans. Immunodiffusion analyses using antisera against these strains demonstrated that 2 of 100 isolates from 100 subjects showed a positive reaction, while further analysis of 2,500 isolates from 50 subjects revealed that all 50 isolates from a single subject were not reactive with anti-c, -e, and -f antisera, though they were reactive with anti-TW295 and -TW871 antisera. The oral isolates showed biological properties similar to those of the reference S. mutans strain MT8148, including high levels of sucrose-dependent adhesion and cellular hydrophobicity, along with expression of glucosyltransferases and a protein antigen, PA. We designated these organisms

serotype k. A glucose side chain-defective mutant strain was then constructed by insertional inactivation of the gluA gene of strain MT8148, which showed biological properties similar to those of serotype k of S. mutans. Serotype k oral isolates were less susceptible to phagocytosis, as were the gluA-inactivated mutant of strain MT8148 and blood isolates. These results indicate that S. mutans serotype k strains are present in the oral cavity in humans and may be able to survive longer in blood owing to their low susceptibility to phagocytosis.

Naser, S., F. L. Thompson, et al. (2005). "Phylogeny and Identification of Enterococci by atpA Gene Sequence Analysis." J. Clin. Microbiol. **43**(5): 2224-2230.

http://jcm.asm.org/cgi/content/abstract/43/5/2224

The relatedness among 91 Enterococcus strains representing all validly described species was investigated by comparing a 1,102-bp fragment of atpA, the gene encoding the alpha subunit of ATP synthase. The relationships observed were in agreement with the phylogeny inferred from 16S rRNA gene sequence analysis. However, atpA gene sequences were much more discriminatory than 16S rRNA for species differentiation. All species were differentiated on the basis of atpA sequences with, at a maximum, 92% similarity. Six members of the Enterococcus faecium species group (E. faecium, E. hirae, E. durans, E. villorum, E. mundtii, and E. ratti) showed >99% 16S rRNA gene sequence similarity, but the highest value of atpA gene sequence similarity was only 89.9%. The intraspecies atpA sequence similarities for all species except E. faecium strains varied from 98.6 to 100%; the E. faecium strains had a lower atpA sequence similarity of 96.3%. Our data clearly show that atpA provides an alternative tool for the phylogenetic study and identification of enterococci.

Nguyen, T. V., P. Le Van, et al. (2005). "Detection and Characterization of Diarrheagenic Escherichia coli from Young Children in Hanoi, Vietnam." J. Clin. Microbiol. **43**(2): 755-760.

http://jcm.asm.org/cgi/content/abstract/43/2/755

Diarrhea continues to be one of the most common causes of morbidity and mortality among infants and children in developing countries. Escherichia coli is an emerging agent among pathogens that cause diarrhea. The development of a highly applicable technique for the detection of different categories of diarrheagenic E. coli is important. We have used multiplex PCR by combining eight primer pairs specific for enteroaggregative E. coli (EAEC), enteroinvasive E. coli (EIEC), enterohemorrhagic E. coli, enteropathogenic E. coli (EPEC), and enterotoxigenic E. coli (ETEC). This facilitates the identification of five different categories of diarrheagenic E. coli from stool samples in a single reaction simultaneously. The prevalences of diarrheagenic E. coli were 22.5 and 12% in the diarrhea group and the control group, respectively. Among 587 fecal samples from Vietnamese children under 5 years of age with diarrhea, this technique identified 132 diarrheagenic E. coli strains. This included 68 samples (11.6%) with EAEC, 12 samples (2.0%) with EIEC, 39 samples (6.6%) with EPEC, and 13 samples (2.2%) with ETEC. Among the 249 age-matched controls, 30 samples were positive for diarrheagenic E. coli. The distribution was 18 samples (7.2%) with EAEC, 11 samples (4.4%) with EPEC, and 1 sample (0.4%) with ETEC.

Nicolas, M. M., I. H. Stalis, et al. (2005). "Systemic Disease in Vaal Rhebok (Pelea capreolus) Caused by Mycoplasmas in the Mycoides Cluster." J. Clin. Microbiol. **43**(3): 1330-1340.

http://jcm.asm.org/cgi/content/abstract/43/3/1330

In the winter of 2002, an outbreak of mycoplasma infection in Vaal rhebok (Pelea capreolus) originating from South Africa occurred 15 weeks after their arrival in San Diego, Calif. Three rhebok developed inappetence, weight loss, lethargy, signs related to pulmonary or arthral dysfunction, and sepsis. All three rhebok died or were euthanized. Primary postmortem findings were erosive tracheitis, pleuropneumonia, regional cellulitis, and necrotizing lymphadenitis. Mycoplasmas were detected in numerous tissues by electron microscopy, immunohistochemistry, and PCR. The three deceased rhebok were coinfected with ovine herpesvirus-2, and two animals additionally had a novel gammaherpesvirus. However, no lesions indicative of herpesvirus were seen microscopically in any animal. The rheboks' mycoplasmas were characterized at the level of the 16S rRNA gene, the 16S-23S intergenic spacer region, and the fructose biphosphate aldolase gene. Denaturing gradient gel electrophoresis was carried out to address the possibility of infection with multiple strains. Two of the deceased rhebok were infected with a single strain of Mycoplasma capricolum subsp. capricolum, and the third animal had a single, unique strain most closely related to Mycoplasma mycoides subsp. mycoides large-colony. A PCR survey of DNA samples from 46 other ruminant species demonstrated the presence of several species of mycoplasmas in the mycoides cluster, including a strain of M. capricolum subsp. capricolum identical to that found in two of the rhebok. These findings demonstrate the pervasiveness of mycoplasmas in the mycoides cluster in small ruminants and the potential for interspecies transmission and disease when different animal taxa come in contact.

Nielsen, E. M. and M. T. Andersen (2003). "Detection and Characterization of Verocytotoxin-Producing Escherichia coli by Automated 5' Nuclease PCR Assay." J. Clin. Microbiol. **41**(7): 2884-2893.

http://jcm.asm.org/cgi/content/abstract/41/7/2884

In recent years increased attention has been focused on infections caused by isolates of verocytotoxin-producing Escherichia coli (VTEC) serotypes other than O157. These non-O157 VTEC isolates are commonly present in food and food production animals. Easy detection, isolation, and characterization of non-O157 VTEC isolates are essential for improving our knowledge of these organisms. In the present study, we detected VTEC isolates in bovine fecal samples by a duplex 5' nuclease PCR assay (real-time PCR) that targets vtx1 and vtx2. VTEC isolates were obtained by colony replication by use of hydrophobic-grid membrane filters and DNA probe hybridization. Furthermore, we have developed 5' nuclease PCR assays for the detection of virulence factors typically present in VTEC isolates, including subtypes of three genes of the locus of enterocyte effacement (LEE) pathogenicity island. The 22 assays included assays for the detection of verocytotoxin genes (vtx1, vtx2), pO157-associated genes (ehxA, katP, espP, and etpD), a recently identified adhesin (saa), intimin (eae, all variants), seven subtypes of eae, four subtypes of tir, and three subtypes of espD. A number of reference strains (VTEC and enteropathogenic E. coli strains) and VTEC strains isolated from calves were tested to validate the PCR assays. The expected virulence profiles were detected for all reference strains. In addition, new information on the subtypes of LEE genes was obtained. For reference strains as well as bovine isolates, a consistent relationship between subtypes of the LEE genes was found, so that a total of seven different combinations of these were recognized (corresponding to the seven subtypes of eae). Isolates with 15 different serogroup-virulence profiles were isolated from 16 calves. Among these, 53% harbored LEE and 73% harbored factors carried by the large virulence plasmid. One LEE-negative isolate had the gene for the adhesin Saa. The most common virulence profile among the bovine isolates was vtx1, eae-{zeta}, tir-{alpha}, ehxA, and espP. This panel of assays offers an easy method for the extensive characterization of VTEC isolates.

Nijhuis, M., N. van Maarseveen, et al. (2002). "Rapid and Sensitive Routine Detection of All Members of the Genus Enterovirus in Different Clinical Specimens by Real-Time PCR." J. Clin. Microbiol. **40**(10): 3666-3670.

http://jcm.asm.org/cgi/content/abstract/40/10/3666

We developed a rapid and sensitive method for the routine detection of all members of the enterovirus genus in different clinical specimens by using real-time TaqMan quantitative PCR. Multiple primer and probe sets were selected in the highly conserved 5'-untranslated region of the enterovirus genome. Our assay detected all 60 different enterovirus species tested, whereas no reactivity was observed with the viruses from the other genera of the picornaviridae family, e.g., hepatovirus and parechovirus. Weak cross-reactivity was observed with 7 of the 90 different high-titer rhinovirus stocks but not with rhinovirus-positive clinical isolates. Analysis of a well-characterized reference panel containing different enteroviruses at various concentrations demon-strated that the enterovirus real-time TaqMan PCR is as sensitive as most of the currently used molecular detection assays. Evaluation of clinical isolates demonstrated that the assay is more sensitive than the "gold standard" method, i.e., viral culture. Moreover, the PCR assay can be used on different clinical specimens, such as plasma, serum, nose and throat swabs, cerebrospinal fluid, and bronchoalveolar lavage, without apparent inhibition. Our data demonstrate that the real-time TaqMan PCR is a rapid and sensitive assay for the detection of enterovirus infection. The assay has a robust character and is easily standardized, which makes it an excellent alternative for the conventional time-consuming viral culture.

Nilsson, H.-O., I.-S. Ouis, et al. (2004). "High Prevalence of Helicobacter Species Detected in Laboratory Mouse Strains by Multiplex PCR-Denaturing Gradient Gel Electrophoresis and Pyrosequencing." J. Clin. Microbiol. **42**(8): 3781-3788.

http://jcm.asm.org/cgi/content/abstract/42/8/3781

Rodent models have been developed to study the pathogenesis of diseases caused by Helicobacter pylori, as well as by other gastric and intestinal Helicobacter spp., but some murine enteric Helicobacter spp. cause hepatobiliary and intestinal tract diseases in specific inbred strains of laboratory mice. To identify these murine Helicobacter spp., we developed an assay based on PCR-denaturing gradient gel electrophoresis and pyrosequencing. Nine strains of mice, maintained in four conventional laboratory animal houses, were assessed for Helicobacter sp. carriage. Tissue samples from the liver, stomach, and small intestine, as well as feces and blood, were collected; and all specimens (n = 210) were screened by a Helicobacter genus-specific PCR. Positive samples were identified to the species level by multiplex denaturing gradient gel electrophoresis, pyrosequencing, and a H. ganmani-specific PCR assay. Histologic examination of 30 tissue samples from 18 animals was performed. All mice of eight of the nine strains tested were Helicobacter genus positive; H. bilis, H. hepaticus, H. typhlonius, H. ganmani, H. rodentium, and a Helicobacter sp. flexispira-like organism were identified. Helicobacter DNA was common in fecal (86%) and gastric tissue (55%) specimens, whereas samples of liver tissue (21%), small intestine tissue (17%), and blood (14%) were less commonly positive. Several mouse strains were colonized with more than one Helicobacter spp. Most tissue specimens analyzed showed no signs of inflammation; however, in one strain of mice, hepatitis was diagnosed in livers positive for H. hepaticus, and in another strain, gastric colonization by H. typhlonius was associated with gastritis. The diagnostic setup developed was efficient at identifying most murine Helicobacter spp.

Nurpeisov, V., S. J. Hurwitz, et al. (2003). "Fluorescent Dye Terminator Sequencing Methods for Quantitative Determination of Replication Fitness of Human Immunodeficiency Virus Type 1

Containing the Codon 74 and 184 Mutations in Reverse Transcriptase." J. Clin. Microbiol. **41**(7): 3306-3311.

http://jcm.asm.org/cgi/content/abstract/41/7/3306

The fluorescent dye-labeled dideoxynucleotide automated DNA sequencing system has been routinely used for monitoring the development of resistance mutations in human immunodeficiency virus type 1 reverse transcriptase (RT) and protease genes during therapy. This system has provided information regarding the presence of mixtures of nucleotides in the clinical samples but has not previously been validated for the quantitative determination between peak heights and relative DNA concentration. We evaluated this system by using various ratios of wild-type and mutated DNA fragments and by performing sequencing reactions at actual melting temperatures of specific primers. Several different ratios of purified DNA fragments containing mixtures of L74/V74 and M184/V184 were sequenced, and peak heights were measured. Regression analysis between ratios of peak heights and DNA concentration demonstrated a statistically significant linear correlation, suggesting that the quantification of two different species of DNA in a mixture could be achieved with the fluorescent dye-labeled dideoxynucleotide system. These strategies have broader implications for the quantification of replication fitness of viruses, particularly those containing RT mutations at codons 74 and 184.

O'Donnell, K., D. A. Sutton, et al. (2004). "Genetic Diversity of Human Pathogenic Members of the Fusarium oxysporum Complex Inferred from Multilocus DNA Sequence Data and Amplified Fragment Length Polymorphism Analyses: Evidence for the Recent Dispersion of a Geographically Widespread Clonal Lineage and Nosocomial Origin." J. Clin. Microbiol. **42**(11): 5109-5120.

http://jcm.asm.org/cgi/content/abstract/42/11/5109

Fusarium oxysporum is a phylogenetically diverse monophyletic complex of filamentous ascomycetous fungi that are responsible for localized and disseminated life-threatening opportunistic infections in immunocompetent and severely neutropenic patients, respectively. Although members of this complex were isolated from patients during a pseudoepidemic in San Antonio, Tex., and from patients and the water system in a Houston, Tex., hospital during the 1990s, little is known about their genetic relatedness and population structure. This study was conducted to investigate the global genetic diversity and population biology of a comprehensive set of clinically important members of the F. oxysporum complex, focusing on the 33 isolates from patients at the San Antonio hospital and on strains isolated in the United States from the water systems of geographically distant hospitals in Texas, Maryland, and Washington, which were suspected as reservoirs of nosocomial fusariosis. In all, 18 environmental isolates and 88 isolates from patients spanning four continents were genotyped. The major finding of this study, based on concordant results from phylogenetic analyses of multilocus DNA sequence data and amplified fragment length polymorphisms, is that a recently dispersed, geographically widespread clonal lineage is responsible for over 70% of all clinical isolates investigated, including all of those associated with the pseudoepidemic in San Antonio. Moreover, strains of the clonal lineage recovered from patients were conclusively shown to genetically match those isolated from the hospital water systems of three U.S. hospitals, providing support for the hypothesis that hospitals may serve as a reservoir for nosocomial fusarial infections.

Okitsu, N., S. Kaieda, et al. (2005). "Characterization of ermB Gene Transposition by Tn1545 and Tn917 in Macrolide-Resistant Streptococcus pneumoniae Isolates." J. Clin. Microbiol. **43**(1): 168-173.

http://jcm.asm.org/cgi/content/abstract/43/1/168

In Streptococcus pneumoniae, the ermB gene is carried by transposons, such as Tn917 and Tn1545. This study investigated the relationship between macrolide resistance and the presence of the ermB gene on Tn917 or Tn1545 in 84 Japanese pneumococcal isolates. Macrolide-resistant strains were classified into two groups as follows. Group 1 (19 strains) showed a tendency to high resistance to erythromycin (MIC at which 50% of isolates are inhibited, 4 mg/liter; MIC at which 90% of isolates are inhibited [MIC90], 128 mg/liter) but susceptibility to rokitamycin (MIC90, 1 mg/liter), with the ermB gene located on Tn1545. Group 2 (65 strains) showed a tendency to high resistance to both antibiotics (MIC90s for both erythromycin and rokitamycin, >128 mg/liter), with the ermB gene located on Tn917. There were no strains with constitutive macrolide resistance in either group. All of the strains in group 2 had a deletion in the promoter region of ermB and an insertion of the TAAA motif in the leader peptide. The results of pulsed-field gel electrophoresis and serogrouping showed that Tn1545 spread clonally while Tn917 spread both horizontally and clonally. In conclusion, in Japanese macrolide-resistant S. pneumoniae isolates, the ermB gene is carried and spread primarily by Tn917.

On, S. L. W. and P. J. Jordan (2003). "Evaluation of 11 PCR Assays for Species-Level Identification of Campylobacter jejuni and Campylobacter coli." J. Clin. Microbiol. **41**(1): 330-336.

http://jcm.asm.org/cgi/content/abstract/41/1/330

We examined the sensitivity and specificity of 11 PCR assays described for the species identification of Campylobacter jejuni and Campylobacter coli by using 111 type, reference, and field strains of C. jejuni, C. coli, and Campylobacter lari. For six assays, an additional 21 type strains representing related Campylobacter, Arcobacter, and Helicobacter species were also included. PCR tests were initially established in the laboratory by optimizing conditions with respect to five type and reference strains of C. jejuni, C. coli, and C. lari. One PCR test for C. coli failed to give appropriate results during this initial setup phase and was not evaluated further. The remaining 10 assays were used to examine heated lysate and purified DNA templates as appropriate of well-characterized type, reference, and field strains of C. jejuni (n = 62), C. coli (n = 34), and C. lari (n = 15). The tests varied considerably in their sensitivity and specificity for their respective target species. No assay was found to be 100% sensitive and/or specific for all C. jejuni strains tested, but four assays for C. coli gave appropriate responses for all strains examined. Between one and six strains of C. jejuni gave amplicons in four of seven C. jejuni PCR tests only where purified DNA was used as the template; corresponding results were seen with one strain of C. coli in each of three assays for the latter species. Our findings indicate that a polyphasic strategy for PCR-based identification should be used to identify C. jejuni and C. coli strains. The data may assist laboratories in selecting assays suited for their needs and in designing evaluations of future PCR tests aimed to identify these species.

Osiowy, C. (2002). "Sensitive Detection of HBsAg Mutants by a Gap Ligase Chain Reaction Assay." J. Clin. Microbiol. **40**(7): 2566-2571.

http://jcm.asm.org/cgi/content/abstract/40/7/2566

Hepatitis B virus (HBV) surface gene variants have been associated with diagnostic escape and immune escape following vaccination. The most common mutation observed in these variants is a glycine-to-arginine substitution at amino acid 145 (G145R). In order to sensitively detect the presence of this mutant in serum, a new molecular detection system was developed; in this new system, a gap ligase chain reaction (gLCR) assay was coupled with electrochemiluminescence

detection of reaction products. The gLCR assay could detect approximately 10 copies of mutant DNA and could discriminate low levels of mutant DNA in the presence of excess wild-type DNA. Detection of the G145R mutant in clinical specimens was evaluated by testing 56 suspect serum specimens. The G145R mutation was observed in 18 of 28 HBV-DNA-positive samples. The approximate percentage of mutant present in each specimen was calculated by comparison with a standard curve of an increasing ratio of mutant DNA to wild-type DNA. Most samples contained a very low percentage of mutant virus (approximately 5%), with an observed range of approximately 3 to 74%. The G145R mutation was most frequently observed in specimens producing a diagnostic anomaly or from transplant patients but was also observed in specimens from vaccinated individuals and specimens in which HBsAg diagnostic escape was suspected. Therefore, the gLCR assay is a sensitive and specific method for detection of G145R mutants, which could be modified to include the detection of other HBV mutants.

Osiowy, C. and E. Giles (2003). "Evaluation of the INNO-LiPA HBV Genotyping Assay for Determination of Hepatitis B Virus Genotype." J. Clin. Microbiol. **41**(12): 5473-5477.

http://jcm.asm.org/cgi/content/abstract/41/12/5473

Specific genotypes of hepatitis B virus (HBV) are increasingly recognized for their clinical significance and association with particular viral mutations. Although many HBV genotyping methods exist, there has been no standardized or commercially available method for direct molecular typing of the HBV genome. A newly available line probe assay (INNO-LiPA HBV Genotyping assay; Innogenetics N.V., Ghent, Belgium) that allows the identification of HBV genotypes A to G was assessed by comparison with pre-S1/pre-S2 sequence analysis of the isolates in 188 serum specimens. All seven genotypes were detected by the line probe assay (LiPA), and complete concordance between LiPA and sequence analysis was observed for 152 specimens (81%). LiPA was able to detect 19 mixed genotype infections not detected by amplicon sequencing, which for the most part were confirmed by cloning and sequencing of the pre-S1/pre-S2 amplicon. Four specimens had discrepant results between the two methods, and five specimens had indeterminate results by LiPA. The HBV DNA in four specimens was unable to be amplified by the nested INNO-LiPA HBV DR amplification primers; however, the HBV DNA in six specimens unable to be genotyped by sequencing was clearly genotyped by LiPA. The INNO-LiPA HBV Genotyping assay appears to be useful for the rapid genotyping of HBV, particularly for the sensitive detection of mixed genotype infections.

Ott, S. J., M. Musfeldt, et al. (2004). "Quantification of Intestinal Bacterial Populations by Real-Time PCR with a Universal Primer Set and Minor Groove Binder Probes: a Global Approach to the Enteric Flora." J. Clin. Microbiol. **42**(6): 2566-2572.

http://jcm.asm.org/cgi/content/abstract/42/6/2566

The composition of the human intestinal flora is important for the health status of the host. The global composition and the presence of specific pathogens are relevant to the effects of the flora. Therefore, accurate quantification of all major bacterial populations of the enteric flora is needed. A TaqMan real-time PCR-based method for the quantification of 20 dominant bacterial species and groups of the intestinal flora has been established on the basis of 16S ribosomal DNA taxonomy. A PCR with conserved primers was used for all reactions. In each real-time PCR, a universal probe for quantification of total bacteria and a specific probe for the species in question were included. PCR with conserved primers and the universal probe for total bacteria allowed relative and absolute quantification. Minor groove binder probes increased the sensitivity of the assays 10- to 100-fold. The method was evaluated by cross-reaction experiments and quantification of bacteria in complex clinical samples from healthy patients. A sensitivity of 101 to

103 bacterial cells per sample was achieved. No significant cross-reaction was observed. The real-time PCR assays presented may facilitate understanding of the intestinal bacterial flora through a normalized global estimation of the major contributing species.

Palacios, G., O. Jabado, et al. (2005). "Molecular Identification of Mumps Virus Genotypes from Clinical Samples: Standardized Method of Analysis." J. Clin. Microbiol. **43**(4): 1869-1878.

http://jcm.asm.org/cgi/content/abstract/43/4/1869

A sensitive nested reverse transcription-PCR assay, targeting a short fragment of the gene encoding the small hydrophobic protein (SH gene), was developed to allow rapid characterization of mumps virus in clinical samples. The sensitivity and specificity of the assay were established using representative genotypes A, B, C, D, E, and F. Mumps virus RNA was characterized directly from cerebrospinal fluid (CSF) samples and in extracts of mumps virus isolates from patients with various clinical syndromes. Direct sequencing of products and subsequent phylogenetic analysis enabled genetic classification. A simple web-based system of sequence analysis was established. The study also allowed characterization of mumps virus strains from Argentina as part of a new subgenotype. This PCR assay for characterization of mumps infections coupled to a web-based analytical program provides a rapid method for identification of known and novel strains.

Palmer, S., A. P. Wiegand, et al. (2003). "New Real-Time Reverse Transcriptase-Initiated PCR Assay with Single-Copy Sensitivity for Human Immunodeficiency Virus Type 1 RNA in Plasma." J. Clin. Microbiol. **41**(10): 4531-4536.

http://jcm.asm.org/cgi/content/abstract/41/10/4531

More sensitive assays for human immunodeficiency virus type 1 (HIV-1) RNA are needed to detect, quantify, and characterize persistent viremia in patients who are receiving antiretroviral therapy and whose plasma HIV-1 RNA levels are suppressed to less than 50 to 75 copies/ml. We therefore developed an internally controlled real-time reverse transcriptase-initiated PCR assay that quantifies HIV-1 RNA concentrations down to 1 copy per ml of plasma. This assay with single-copy sensitivity (the single-copy assay) generates a reproducible linear regression plot of input copy number versus threshold cycle by using HIV-1 RNA transcripts at copy numbers ranging from 1 to 106 per reaction mixture. The single-copy assay was compared to the ultrasensitive AMPLICOR HIV-1 MONITOR assay and a more sensitive modification of the ultrasensitive assay by repeatedly testing a low-copy-number panel containing 200 to 0.781 copies of HIV-1 RNA per ml of plasma. This comparison showed that the single-copy assay had a greater sensitivity than the other assays and was the only assay that detected HIV-1 RNA at levels as low as 0.781 copies/ml. Testing of plasma samples from 15 patients who were receiving antiretroviral therapy and who had <75 HIV-1 RNA copies/ml revealed persistent viremia in all 15 patients, with HIV-1 RNA levels ranging from 1 to 32 copies/ml (median, 13 copies/ml). The greater sensitivity of the single-copy assay should allow better characterization of persistent viremia in patients who are receiving antiretroviral therapy and whose HIV-1 RNA levels are suppressed to below the detection limits of present assays.

Pang, X., B. Lee, et al. (2004). "Evaluation and Validation of Real-Time Reverse Transcription-PCR Assay Using the LightCycler System for Detection and Quantitation of Norovirus." J. Clin. Microbiol. **42**(10): 4679-4685.

http://jcm.asm.org/cgi/content/abstract/42/10/4679

We developed an assay for the detection and quantitation of norovirus with the LightCycler SYBR Green-based real-time reverse transcription-PCR (real-time LC RT-PCR) and previously published primers in the capsid and the polymerase gene. One hundred thirty-two stool specimens from the Provincial Laboratory for Public Health (Microbiology), Alberta, Canada, and the Centers for Disease Control and Prevention, Atlanta, Ga., were used to validate the new assay. The samples were collected from patients involved in outbreaks of acute gastroenteritis or children who presented with sporadic gastroenteritis. The real-time LC RT-PCR assay detected norovirus strains from three genogroup I (G-I) clusters (G-I/1, G-I/2, and G-I/3) and 10 genogroup II (G-II) clusters (G-II/1, G-II/2, G-II/3, G-II/4, G-II/6, G-II/7, G-II/10, G-II/12, G-II/15, and G-II/16). There was 100% concordance with the results from 58 stool specimens which tested positive by conventional RT-PCR assays. By dilution analysis, the real-time LC RT-PCR was 10,000 times more sensitive than the conventional RT-PCR. The new assay increased the number of samples in which noroviruses were detected by 19%. The real-time LC RT-PCR had a wide dynamic range, detecting from 5 to 5 x 106 copies of RNA per reaction, resulting in a theoretical lower limit of detection of 25,000 copies of RNA per g of stool. No cross-reactions were found with specimens containing sapovirus, rotavirus, astrovirus, and adenovirus. Because of the high sensitivity and specificity of the assay with a relatively rapid and simple procedure, the real-time LC RT-PCR will be useful as a routine assay for the clinical diagnosis of norovirus infection.

Park, S.-W., C.-S. Lee, et al. (2005). "Rapid Identification of the Coxsackievirus A24 Variant by Molecular Serotyping in an Outbreak of Acute Hemorrhagic Conjunctivitis." J. Clin. Microbiol. **43**(3): 1069-1071.

http://jcm.asm.org/cgi/content/abstract/43/3/1069

We evaluated the clinical applicability of a molecular serotyping method for determination of the cause of epidemic acute hemorrhagic conjunctivitis. Seventy conjunctival swab specimens from individuals involved in a nationwide acute hemorrhagic conjunctivitis outbreak were tested. Viral culture and a molecular biology-based assay were compared by directly using clinical specimens. On the one hand, virus culture was done to isolate the enteroviruses, and serotyping was done by a coxsackievirus A24 variant-specific PCR. On the other hand, the original clinical specimens were directly screened for enterovirus by reverse transcription (RT)-PCR with panenterovirus-specific primers. Enterovirus screening-positive specimens were subjected to RT-PCR for detection of the VP1 region of enterovirus, and the amplicons were sequenced. Molecular serotyping was done by calculating the pairwise identity scores for the sequences with the maximum identities to the sequences of known prototype enteroviruses. Thirty-two specimens (45.7%) were culture positive, whereas 37 specimens (52.8%) were screening PCR positive ($P < 0.001$). The VP1 regions were amplified from 21 of the 37 specimens (56.8%), and the products amplified from 9 specimens were appropriately sequenced. These nine sequences were homologous with the sequence of the coxsackievirus A24 variant. Molecular serotyping by direct use of clinical specimens without cell culture could be applied for the rapid identification of the causative agent of epidemic acute hemorrhagic conjunctivitis.

Paster, B. J., W. A. Falkler, Jr., et al. (2002). "Prevalent Bacterial Species and Novel Phylotypes in Advanced Noma Lesions." J. Clin. Microbiol. **40**(6): 2187-2191.

http://jcm.asm.org/cgi/content/abstract/40/6/2187

The purpose of this study was to determine the bacterial diversity in advanced noma lesions

using culture-independent molecular methods. 16S ribosomal DNA bacterial genes from DNA isolated from advanced noma lesions of four Nigerian children were PCR amplified with universally conserved primers and spirochetal selective primers and cloned into Escherichia coli. Partial 16S rRNA sequences of approximately 500 bases from 212 cloned inserts were used initially to determine species identity or closest relatives by comparison with sequences of known species or phylotypes. Nearly complete sequences of approximately 1,500 bases were obtained for most of the potentially novel species. A total of 67 bacterial species or phylotypes were detected, 25 of which have not yet been grown in vitro. Nineteen of the species or phylotypes, including Propionibacterium acnes, Staphylococcus spp., and the opportunistic pathogens Stenotrophomonas maltophilia and Ochrobactrum anthropi were detected in more than one subject. Other known species that were detected included Achromobacter spp., Afipia spp., Brevundimonas diminuta, Capnocytophaga spp., Cardiobacterium sp., Eikenella corrodens, Fusobacterium spp., Gemella haemoylsans, and Neisseria spp. Phylotypes that were unique to noma infections included those in the genera Eubacterium, Flavobacterium, Kocuria, Microbacterium, and Porphyromonas and the related Streptococcus salivarius and genera Sphingomonas and Treponema. Since advanced noma lesions are infections open to the environment, it was not surprising to detect species not commonly associated with the oral cavity, e.g., from soil. Several species previously implicated as putative pathogens of noma, such as spirochetes and Fusobacterium spp., were detected in at least one subject. However, due to the limited number of available noma subjects, it was not possible at this time to associate specific species with the disease.

Paule, S. M., W. E. Trick, et al. (2003). "Comparison of PCR Assay to Culture for Surveillance Detection of Vancomycin-Resistant Enterococci." J. Clin. Microbiol. **41**(10): 4805-4807.

http://jcm.asm.org/cgi/content/abstract/41/10/4805

Direct multiplex PCR assay using vanA and vanB primers, which provides rapid results, was more sensitive than culture on selective media for samples collected by rectal swab (20 of 46 versus 8 of 46; P < 0.001) or perianal swab (17 of 58 versus 12 of 58; P = 0.059) for the detection of gastrointestinal colonization by vancomycin-resistant enterococci.

Perandin, F., N. Manca, et al. (2004). "Development of a Real-Time PCR Assay for Detection of Plasmodium falciparum, Plasmodium vivax, and Plasmodium ovale for Routine Clinical Diagnosis." J. Clin. Microbiol. **42**(3): 1214-1219.

http://jcm.asm.org/cgi/content/abstract/42/3/1214

A TaqMan-based real-time PCR qualitative assay for the detection of three species of malaria parasites--Plasmodium falciparum, P. ovale, and P. vivax--was devised and evaluated using 122 whole-blood samples from patients who had traveled to areas where malaria is endemic and who presented with malaria-like symptoms and fever. The assay was compared to conventional microscopy and to an established nested-PCR assay. The specificity of the new assay was confirmed by sequencing the PCR products from all the positive samples and by the lack of cross-reactivity with Toxoplasma gondii and Leishmania infantum DNA. Real-time PCR assay showed a detection limit (analytical sensitivity) of 0.7, 4, and 1.5 parasites/{micro}l for P. falciparum, P. vivax, and P. ovale, respectively. Real-time PCR, like nested PCR, brought to light errors in the species identification by microscopic examination and revealed the presence of mixed infections (P. falciparum plus P. ovale). Real-time PCR can yield results within 2 h, does not require post-PCR processing, reduces sample handling, and minimizes the risks of contamination. The assay can therefore be easily implemented in routine diagnostic malaria tests. Future studies are warranted to investigate the clinical value of this technique.

Perilli, M., E. Dell'Amico, et al. (2002). "Molecular Characterization of Extended-Spectrum {beta}-Lactamases Produced by Nosocomial Isolates of Enterobacteriaceae from an Italian Nationwide Survey." J. Clin. Microbiol. **40**(2): 611-614.

http://jcm.asm.org/cgi/content/abstract/40/2/611

Extended-spectrum {beta}-lactamases (ESBLs) are widespread in hospital settings worldwide. The present investigation was undertaken to assess the distribution and prevalence of ESBLs belonging to the TEM and SHV families in 448 ESBL-producing clinical isolates of Enterobacteriaceae collected from 10 different Italian hospitals. The natures of TEM and SHV determinants were identified by direct sequencing of PCR-amplified genes. TEM-52 and SHV-12 were the most common variants, and they were found in most hospitals and in several different species. Other less frequent variants included TEM-5, TEM-12, TEM-15, TEM-19, TEM-20, TEM-24, TEM-26, TEM-43, TEM-60, TEM-72, TEM-87, SHV-2a, SHV-5, and SHV-11. Proteus mirabilis was the most common producer of TEM-type ESBLs, while Klebsiella pneumoniae was the most common producer of SHV-type ESBLs. The distribution of TEM- and SHV-type ESBL variants in Enterobacteriaceae from Italian hospitals exhibited notable differences from those from other geographical settings.

Pillay, A., J. Lewis, et al. (2004). "Evaluation of Xenostrip-Tv, a Rapid Diagnostic Test for Trichomonas vaginalis Infection." J. Clin. Microbiol. **42**(8): 3853-3856.

http://jcm.asm.org/cgi/content/abstract/42/8/3853

An immunochromatographic strip test, Xenostrip-Tv, was compared to wet mount and PCR for the diagnosis of Trichomonas vaginalis infection in women. Of 428 specimens tested, 54 (12.6%) were positive by an "expanded gold standard," defined as either a positive wet mount and PCR test with primers TVK3 and TVK7 and/or a positive PCR test confirmed by a second PCR assay with primers TVA5-1 and TVA6; 26 (6%) were positive by wet mount, and 36 (8.4%) were positive by Xenostrip-Tv test. Since the Xenostrip-Tv test is rapid and easy to perform and proved to be more sensitive than wet mount, it should be considered as an alternative to wet mount for point-of-care diagnosis of trichomoniasis, especially in settings where microscopy is impractical.

Plantier, J.-C., L. Vergne, et al. (2002). "Development and Evaluation of a DNA Enzyme Immunoassay Method for env Genotyping of Subtypes A through G of Human Immunodeficiency Virus Type 1 Group M, with Discrimination of the Circulating Recombinant Forms CRF01_AE and CRF02_AG." J. Clin. Microbiol. **40**(3): 1010-1022.

http://jcm.asm.org/cgi/content/abstract/40/3/1010

The tools currently available for genetic subtyping of human immunodeficiency virus type 1 are laborious or can be used only for the analysis of a limited number of samples and/or subtypes. We developed and evaluated a molecular biology-based method using subtype-specific oligonucleotide probes for env genotyping of subtypes A through G, CRF01_AE, and CRF02_AG. DNA enzyme immunoassay (DEIA) genotyping is based on nested PCR amplification of the 5' end of the env gene (proviral DNA), followed by subtype-specific hybridization and immunoenzymatic detection on microplates. DEIA genotyping was validated with a large number of samples (n = 128) collected in Europe (France; n = 47), West-Central Africa (Cameroon; n = 36), and West Africa (Senegal; n = 45). Three different formats, depending on the distribution of

subtypes in the three countries, were developed. The results were compared with those obtained by sequencing of the V3-V5 region and phylogenetic analysis or an env heteroduplex mobility assay. Additional sequencing and phylogenetic analyses of the DEIA region (the first codon of the env coding sequence to the middle of conserved region C1 of gp120) were performed to investigate the reasons for discrepancies. Intense and highly specific reactions between the oligonucleotide probes and the corresponding samples were observed. Overall, correct identification was achieved for 107 of 128 samples (83.6%). One sample was not amplified, 10 (8%) were nontypeable (NT), and 10 (8%) were misidentified. Six of the 10 discordant samples were further investigated by phylogenetic analysis, which indicated that these samples corresponded to recombinants involving the env 5' end and the V3 and V5 regions of the two parental clades. Sequencing of NT samples showed numerous differences between sample and probe sequences, resulting in a lack of hybridization, and revealed the limitations of the selected probes in terms of specificity and sensitivity. We demonstrated the feasibility of DEIA genotyping: six subtypes plus the two most prevalent circulating recombinant forms were discriminated by using the 5' end of the env gene. This method can be adapted to the local situation by including only probes that correspond to the prevalent strains.

Pottumarthy, S., A. P. Limaye, et al. (2003). "Nocardia veterana, a New Emerging Pathogen." J. Clin. Microbiol. **41**(4): 1705-1709.

http://jcm.asm.org/cgi/content/abstract/41/4/1705

Nocardia veterana is a newly described species named after the veteran's hospital where it was first isolated. This initial type strain was not thought to be clinically significant. We describe three cases of pulmonary disease attributable to N. veterana: two cases in patients presenting with multiple pulmonary nodules in a setting of immunocompromise and one case of exacerbation of chronic pulmonary disease. The isolates were susceptible to ampicillin, imipenem, gentamicin, amikacin, and trimethoprim-sulfamethoxazole and had reduced susceptibilities to ceftriaxone, cefotaxime, minocycline, and ciprofloxacin. The MICs of amoxicillin-clavulanate were higher than that of ampicillin alone, and the bacteria produced a {beta}-lactamase detectable only after induction with clavulanic acid. Phenotypically, the isolates could not be characterized beyond the Nocardia genus level. All three isolates were definitively identified as N. veterana by PCR and sequencing of the 16S rRNA gene. On the basis of their susceptibility and restriction enzyme analysis profiles, our findings indicate that they could potentially be misidentified as N. nova. These cases illustrate the pathogenic potential of this newly described species and emphasize the importance of accurate identification of Nocardia isolates to the species level by integrated use of phenotypic and genotypic methods.

Pounder, J. I., S. Williams, et al. (2005). "Repetitive-Sequence-PCR-Based DNA Fingerprinting Using the DiversiLab System for Identification of Commonly Encountered Dermatophytes." J. Clin. Microbiol. **43**(5): 2141-2147.

http://jcm.asm.org/cgi/content/abstract/43/5/2141

The performance of repetitive-sequence-based PCR (rep-PCR) using the DiversiLab system for identification of dermatophytes commonly isolated in a clinical laboratory was assessed by comparing results to those of conventional tests (colony morphology, microscopic examination of slide cultures, and, for suspected Trichophyton species, use of additional media). Sixty-one cultures were tested in phase 1, the feasibility portion of the study; 64 additional cultures were tested in phase 2, the validation portion conducted to assess reproducibility and confirm accuracy. Discrepancies were resolved by repeating rep-PCR and conventional tests and, in phase 2, sequencing the internal transcribed spacers. After initial testing of the cultures in phase

1 (excluding one contaminated culture), agreement between conventional tests and rep-PCR was 90% (54 of 60). Agreement was 98.3% after resolution of discrepancies, and in all but one case the initial rep-PCR result was correct. After initial testing of cultures in phase 2 (excluding one discarded and one contaminated culture), agreement between rep-PCR and conventional testing was 88.7% (55 of 62). After discrepancies were resolved, agreement was 100%. Initial rep-PCR results were correct, except for one Microsporum canis culture containing two colony variants, which could not be initially identified by rep-PCR. The performance of the DiversiLab system for identification of the dermatophytes commonly encountered in a clinical mycology laboratory--Trichophyton mentagrophytes, Trichophyton rubrum, Trichophyton tonsurans, and M. canis--was excellent. Moreover, the DiversiLab system is technically simple and provides results in <24 h once a pure culture is available for testing, which is considerably more rapid than conventional identification tests.

Prager, R., W. Rabsch, et al. (2003). "Molecular Properties of Salmonella enterica Serotype Paratyphi B Distinguish between Its Systemic and Its Enteric Pathovars." J. Clin. Microbiol. **41**(9): 4270-4278.

http://jcm.asm.org/cgi/content/abstract/41/9/4270

Salmonella enterica serotype O1,4,5,12:Hb:1,2, designated according to the current Kauffmann-White scheme as S. enterica serotype Paratyphi B, is a very diverse serotype with respect to its clinical and microbiological properties. PCR and blot techniques, which identify the presence, polymorphism, and expression of various effector protein genes, help to distinguish between strains with systemic and enteric outcomes of disease. All serotype Paratyphi B strains from systemic infections have been found to be somewhat genetically related with respect to the pattern of their virulence genes sopB, sopD, sopE1, avrA, and sptP as well as other molecular properties (multilocus enzyme electrophoresis type, pulsed-field gel electrophoresis [PFGE] type, ribotype, and IS200 type). They have been classified as members of the systemic pathovar (SPV). All these SPV strains possess a new sopE1-carrying bacteriophage (designated {Phi}SopE309) with high SopE1 protein expression but lack the commonly occurring avrA determinant. They exhibit normal SopB protein expression but lack SopD protein production. In contrast, strains from enteric infections classified as belonging to the enteric pathovar possess various combinations of the respective virulence genes, PFGE pattern, and ribotypes. We propose that the PCR technique for testing for the presence of the virulence genes sopE1 and avrA be used as a diagnostic tool for identifying both pathovars of S. enterica serotype Paratyphi B. This will be of great public health importance, since strains of serotype Paratyphi B have recently reemerged worldwide.

Pratelli, A., N. Decaro, et al. (2004). "Two Genotypes of Canine Coronavirus Simultaneously Detected in the Fecal Samples of Dogs with Diarrhea." J. Clin. Microbiol. **42**(4): 1797-1799.

http://jcm.asm.org/cgi/content/abstract/42/4/1797

Sixty-nine fecal samples from diarrheic puppies were examined by reverse transcription-PCR assays for the M and the S genes of canine coronaviruses (CCoVs). The isolates in 10 samples were recognized as CCoV type I, and the isolates in 6 samples were recognized as CCoV type II, while isolates of both genotypes were simultaneously detected in 53 samples.

Priestnall, S. L., B. Wiinberg, et al. (2004). "Evaluation of "Helicobacter heilmannii" Subtypes in the Gastric Mucosas of Cats and Dogs." J. Clin. Microbiol. **42**(5): 2144-2151.

http://jcm.asm.org/cgi/content/abstract/42/5/2144

Infection with candidatus "Helicobacter heilmannii" is associated with gastritis and mucosa-associated lymphoid tissue lymphoma in people. Infection with "H. heilmannii" type 1 predominates (80%) and is thought to be acquired from dogs, cats, or pigs. We further examined the zoonotic potential of dogs and cats by amplifying gastric DNA from cats (n = 45) and dogs (n = 10) with primers against "H. heilmannii" ureB and 16S rRNA genes and sequencing the products. Fluorescence in situ hybridization (FISH) with eubacterial and "H. heilmannii"-specific probes was employed to directly visualize "H. heilmannii" types and their intragastric distribution. ureB sequences of "H. heilmannii" amplicons clustered with human and feline isolates of "H. heilmannii" and were distinct from the "H. heilmannii"-like organisms (HHLO) H. felis, H. salomonis, and H. bizzozeronii. 16S ribosomal DNA sequences in 20 "H. heilmannii"-infected cats and dogs were distinct from "H. heilmannii" type 1 and "H. suis" and clustered with "H. heilmannii" types 2 and 4. FISH confirmed the presence of "H. heilmannii" types 2 and 4 in dogs but failed to definitively characterize the "H. heilmannii" types present in cats. In infected dogs, "H. heilmannii" inhabited the gastric mucus and glands, and in dogs coinfected with other HHLO it shared the same gastric niche. The results indicate that dogs and cats are predominantly colonized by "H. heilmannii" bacteria that are distinct from type 1 and from "H. suis." As "H. heilmannii" type 1 predominates in people, the zoonotic risk posed by dogs and cats is likely small.

Probert, W. S., K. N. Schrader, et al. (2004). "Real-Time Multiplex PCR Assay for Detection of Brucella spp., B. abortus, and B. melitensis." J. Clin. Microbiol. **42**(3): 1290-1293.

http://jcm.asm.org/cgi/content/abstract/42/3/1290

The identification of Brucella can be a time-consuming and labor-intensive process that places personnel at risk for laboratory-acquired infection. Here, we describe a real-time PCR assay for confirmation of presumptive Brucella isolates. The assay was designed in a multiplex format that will allow the rapid identification of Brucella spp., B. abortus, and B. melitensis in a single test.

Ratcliff, R. M., J. C. Doherty, et al. (2002). "Sensitive Detection of RNA Viruses Associated with Gastroenteritis by a Hanging-Drop Single-Tube Nested Reverse Transcription-PCR Method." J. Clin. Microbiol. **40**(11): 4091-4099.

http://jcm.asm.org/cgi/content/abstract/40/11/4091

The detection of the human RNA viruses, calicivirus and astrovirus, requires high sensitivity and broad reactivity. A novel single-tube nested reverse transcription-PCR (RT-PCR) method is described here, in which all of the required reagents are included in the one tube; however, those required for the nested amplification are separated in a "hanging drop" in the cap to be introduced by centrifugation after the RT and first-round cDNA amplification steps. Broad reactivity was obtained by using primer cocktails covering the published sequence variation in the primer targets. The method was evaluated with clinical fecal samples from outbreak and sporadic cases. Norwalk-like virus types 1 and 2 and rotavirus were the causal agents in 10 of 12 outbreaks. A viral agent was detected in 44% of 197 samples from sporadic infections in patients presenting to community health centers and a children's hospital. Interestingly, whereas rotavirus was more common than astrovirus in patients presenting to the hospital (33 and 7.6%, respectively), the reverse was true for patients presenting to community health centers (4.2 and 34%, respectively).

Rose, C., M. Green, et al. (2002). "Detection of Epstein-Barr Virus Genomes in Peripheral Blood B Cells from Solid-Organ Transplant Recipients by Fluorescence In Situ Hybridization." J. Clin. Microbiol. **40**(7): 2533-2544.

http://jcm.asm.org/cgi/content/abstract/40/7/2533

Resolution of Epstein-Barr Virus (EBV) infection in pediatric solid-organ transplant recipients often leads to an asymptomatic carrier state characterized by a persistently elevated circulating EBV load that is 2 to 4 orders of magnitude greater than the load typical of healthy latently infected individuals. Elevated EBV loads in immunosuppressed individuals are associated with an increased risk for development of posttransplant lymphoproliferative disease. We have performed fluorescence in situ hybridization (FISH) studies with peripheral blood B cells from carriers of persistent EBV loads in order to directly quantitate the number of EBV genomes per infected cell. Patients were assigned to two groups on the basis of the level of the persistent load (low-load carriers, 8 to 200 genomes/105 peripheral blood lymphocytes; high-load carriers, >200 genomes/105 peripheral blood lymphocytes). FISH analysis revealed that the low-load carriers predominantly had circulating virus-infected cells harboring one or two genome copies/cell. High-load carriers also had cells harboring one or two genome copies/cell; in addition, however, they carried a distinct population of cells with high numbers of viral genome copies. The increased viral loads correlated with an increase in the frequency of cells containing high numbers of viral genomes. We conclude that low-load carriers possess EBV-infected cells that are in a state similar to normal latency, whereas high-load carriers possess two populations of virus-positive B cells, one of which carries an increased number of viral genomes per cell and is not typical of normal latency.

Sako, Y., M. Nakao, et al. (2002). "Alveolar Echinococcosis: Characterization of Diagnostic Antigen Em18 and Serological Evaluation of Recombinant Em18." J. Clin. Microbiol. **40**(8): 2760-2765.

http://jcm.asm.org/cgi/content/abstract/40/8/2760

The Echinococcus multilocularis protein Em18 is one of the most promising antigens for use in serodiagnosis of alveolar echinococcosis in human patients. Here we identify an antigenic relationship between Em18 and a 65-kDa immunodominant E. multilocularis surface protein previously identified as either EM10 or EmII/3. The NH2-terminal sequence of native Em18 was determined, revealing it to be a fragment of EM10. Experiments were undertaken to investigate the effect of proteinase inhibitors on the degradation of EM10 in crude extracts of E. multilocularis protoscoleces. Em18 was found to be the product of degradation of EM10 by cysteine proteinase. A recombinant Em18 (RecEm18, derived from 349K to 508K of EM10) was successfully expressed by using Escherichia coli expression system and then evaluated for use in serodiagnosis of alveolar echinococcosis. RecEm18 was recognized by 27 (87.1%) and 28 (90.3%) of 31 serum samples from clinically and/or pathologically confirmed alveolar echinococcosis patients by enzyme-linked immunosorbent assay and immunoblotting, respectively. Of 33 serum samples from cystic echinococcosis patients, 1 was recorded as having a weak positive reaction to RecEm18; however, none of the serum samples which were tested from neurocysticercosis patients (n = 10) or healthy people (n = 15) showed positive reactions. RecEm18 has the potential for use in the differential serodiagnosis of alveolar echinococcosis.

Sanchez, J. L. and G. A. Storch (2002). "Multiplex, Quantitative, Real-Time PCR Assay for Cytomegalovirus and Human DNA." J. Clin. Microbiol. **40**(7): 2381-2386.

http://jcm.asm.org/cgi/content/abstract/40/7/2381

We created a multiplex, quantitative, real-time PCR assay that amplifies cytomegalovirus (CMV) and human DNA in the same reaction tube, allowing for a viral load determination that is normalized to measured human DNA. The assay targets a conserved region of the CMV DNA polymerase gene that is not affected by known drug resistance mutations. All 36 strains of CMV detected by culture or qualitative PCR in a population of lung transplant recipients were detected. The assay detected 1 to 10 copies of CMV plasmid DNA. The analytic sensitivity was not affected by the presence of DNA from 106 human cells but was reduced approximately 10-fold by alkaline lysates of leukocyte preparations. CMV quantitation was linear over a range of 101 to 106 copies. The intraassay and interassay coefficients of variation were 29 and 40%. Human DNA was regularly detected in patient plasma samples, and the amount was increased by storage of blood at room temperature before plasma separation and by plasma separation techniques that allowed leukocyte contamination. Applied to whole blood, the assay provides a measurement of CMV DNA in relation to cellular content without a need for cell counting procedures. Applied to plasma, the assay can reveal artifactual increases in plasma CMV levels resulting from leukocyte contamination. Further study of the utility of this assay to monitor patient populations at risk for CMV disease is warranted.

Schauer, E., S. Webber, et al. (2004). "Surface Immunoglobulin-Deficient Epstein-Barr Virus-Infected B Cells in the Peripheral Blood of Pediatric Solid-Organ Transplant Recipients." J. Clin. Microbiol. **42**(12): 5802-5810.

http://jcm.asm.org/cgi/content/abstract/42/12/5802

Epstein-Barr virus (EBV), a ubiquitous human herpesvirus, normally causes an asymptomatic latent infection with very low levels of circulating virus in the peripheral blood of infected individuals. However, EBV does have pathogenic potential and has been linked to several diseases, including posttransplant lymphoproliferative disease (PTLD), which involves very high circulating viral loads. As a consequence of immunosuppression associated with transplantation, children in particular are at risk for PTLD. Even in the absence of symptoms of PTLD, very high viral loads are often observed in these patients. EBV-infected B cells in the circulations of 16 asymptomatic pediatric solid-organ transplant recipients from Children's Hospital of Pittsburgh were simultaneously characterized for their surface immunoglobulin (sIg) isotypes and EBV genome copy numbers. Patients were characterized as having high and low viral loads on the basis of their stable levels of circulating virus. Patients with high viral loads had both high- and low-copy-number cells. Cells with a high numbers of viral episomes (>20/cell) were predominantly Ig null, and cells with low numbers of episomes were predominantly sIgM positive. Patients with low viral loads carried the vast majority of their viral load in low-copy-number cells, which were predominantly IgM positive. The very rare high-copy-number cells detected in carriers with low viral loads were also predominantly Ig-null cells. This suggests that two distinct types of B-lineage cells contribute to the viral load in transplant recipients, with cells bearing high genome copy numbers having an aberrant Ig-null cellular phenotype.

Schlicht, M. J., S. D. Lovrich, et al. (2004). "High Prevalence of Genital Mycoplasmas among Sexually Active Young Adults with Urethritis or Cervicitis Symptoms in La Crosse, Wisconsin." J. Clin. Microbiol. **42**(10): 4636-4640.

http://jcm.asm.org/cgi/content/abstract/42/10/4636

Sexually active young adults in the small college town of La Crosse, Wisconsin, were evaluated for conventional sexually transmitted pathogens and tested for infections with mycoplasmas. The prevalence in 65 symptomatic men or women and 137 healthy volunteers (67 men and 70 women) was compared. Urine specimens from both cohorts were tested by ligase chain reaction

for Chlamydia trachomatis or Neisseria gonorrhoeae. In addition, the urethral or cervical swabs from the symptomatic subjects were tested by PCR for Mycoplasma genitalium and cultured for Mycoplasma hominis and the ureaplasmas. The results confirmed a relatively low prevalence of gonorrhea among symptomatic men (12%) and chlamydia among symptomatic men (15%) and normal women (3%). In contrast, infections with mycoplasmas, especially the ureaplasmas (57%), were common and the organisms were the only potential sexually transmitted pathogen detected in 40 (62%) symptomatic subjects. Because of the high prevalence, we also evaluated urethral swabs from an additional 25 normal female volunteers and recovered ureaplasmas from 4 (16%) subjects. Additionally, the participants rarely used protection during sexual intercourse and some symptomatic subjects apparently acquired their infections despite using condoms regularly. The findings demonstrate a strong association between abnormal urogenital findings and detection of myoplasmas, particularly ureaplasmas, and suggest the infections will remain common.

Schulz, A., K. Mellenthin, et al. (2003). "Detection, Differentiation, and Quantitation of Pathogenic Leishmania Organisms by a Fluorescence Resonance Energy Transfer-Based Real-Time PCR Assay." J. Clin. Microbiol. **41**(4): 1529-1535.

http://jcm.asm.org/cgi/content/abstract/41/4/1529

Real-time technology eliminates many of the pitfalls of diagnostic PCR, but this method has not been applied to differentiation of Leishmania organisms so far. We have developed a real-time PCR that simultaneously detects, quantitates, and categorizes Leishmania organisms into three relevant groups causing distinct clinical pictures. The analytical sensitivity (detection rate of [>=]95% at 94.1 parasites/ml of blood) was within a range that has been determined previously to facilitate the confirmation of visceral leishmaniasis from peripheral blood. Parasites were successfully detected in 12 different clinical samples (blood, bone marrow, skin, and liver). The Leishmania donovani complex, the Leishmania brasiliensis complex, and species other than these could be clearly discriminated by means of distinct melting temperatures obtained with fluorescence resonance energy transfer probes (melting points, 72.7, 67.1, and 65.0{degrees}C, respectively). All three groups could be quantified within equal ranges. As in other real-time PCRs, the variability in the quantification of DNA was small (coefficient of variation [CV], <2%). However, human samples containing low levels of parasites (100 parasites per ml of blood) showed higher variation (CV, 60.89%). Therefore, despite its superior analytical performance, care must be taken when real-time PCR is utilized for therapy monitoring.

Schuurman, T., R. F. de Boer, et al. (2004). "Prospective Study of Use of PCR Amplification and Sequencing of 16S Ribosomal DNA from Cerebrospinal Fluid for Diagnosis of Bacterial Meningitis in a Clinical Setting." J. Clin. Microbiol. **42**(2): 734-740.

http://jcm.asm.org/cgi/content/abstract/42/2/734

We have evaluated the use of a broad-range PCR aimed at the 16S rRNA gene in detecting bacterial meningitis in a clinical setting. To achieve a uniform DNA extraction procedure for both gram-positive and gram-negative organisms, a combination of physical disruption (bead beating) and a silica-guanidiniumthiocyanate procedure was used for nucleic acid preparation. To diminish the risk of contamination as much as possible, we chose to amplify almost the entire 16S rRNA gene. The analytical sensitivity of the assay was approximately 1 x 102 to 2 x 102 CFU/ml of cerebrospinal fluid (CSF) for both gram-negative and gram-positive bacteria. In a prospective study of 227 CSF samples, broad-range PCR proved to be superior to conventional methods in detecting bacterial meningitis when antimicrobial therapy had already started. Overall, our assay showed a sensitivity of 86%, a specificity of 97%, a positive predictive value of 80%, and a

negative predictive value of 98% compared to culture. We are currently adapting the standard procedures in our laboratory for detecting bacterial meningitis; broad-range 16S ribosomal DNA PCR detection is indicated when antimicrobial therapy has already started at time of lumbar puncture or when cultures remain negative, although the suspicion of bacterial meningitis remains.

Shopsin, B., B. Mathema, et al. (2003). "Prevalence of agr Specificity Groups among Staphylococcus aureus Strains Colonizing Children and Their Guardians." J. Clin. Microbiol. **41**(1): 456-459.

http://jcm.asm.org/cgi/content/abstract/41/1/456

PCR-based assays were used to evaluate agr locus nucleotide polymorphism for the identification of agr autoinducer receptor specificity groups within a population of Staphylococcus aureus isolates colonizing children and their guardians. All isolates could be assigned to one of three major agr groups that had similar prevalences, regardless of whether isolates were implicated in transmission of S. aureus within families. Among healthy carriers, agr groups I to III appear to be equally fit, which may reflect selection for the coexistence of S. aureus strains in a population.

Shukla, S. K., K. A. Bernard, et al. (2003). "Corynebacterium nigricans sp. nov.: Proposed Name for a Black-Pigmented Corynebacterium Species Recovered from the Human Female Urogenital Tract." J. Clin. Microbiol. **41**(9): 4353-4358.

http://jcm.asm.org/cgi/content/abstract/41/9/4353

Six independent isolates of an unusual black-pigmented Corynebacterium species (strains CN-1, CN-2, CN-3415, W70124, 91-0032, and 92-0360) were recovered from the human female urogenital tract. Four of the six source patients had complications of pregnancy, including spontaneous abortion, preterm labor, and low amniotic fluid volume at the time of the pathogen isolation. One isolate was recovered from a vaginal ulcer. All six strains yielded black-pigmented colonies on sheep blood agar, chocolate agar, and colistin-nalidixic acid agar after 24 to 48 h of incubation at 35{degrees}C. The dry, adherent colonies pitted the agar surface. The cells were coccobacillary to rod-shaped, catalase positive, nonmotile, and nonlipophilic. Only five of six isolates were available for characterization. Biochemical and chemotaxonomic studies revealed that the strains belong to the genus Corynebacterium but differ from known corynebacterial species. Comparative 16S rRNA gene sequence analysis showed that the strains are closely related and form a new subline within the genus Corynebacterium. We propose the name Corynebacterium nigricans sp. nov. for this group of coryneforms. The type strain of Corynebacterium nigricans is CN-1. It is deposited in the American Type Culture Collection (assigned strain number ATCC 700975) and in the Institute Pasteur collection (assigned strain number CIP 107346).

Shutt, C. K., J. I. Pounder, et al. (2005). "Clinical Evaluation of the DiversiLab Microbial Typing System Using Repetitive-Sequence-Based PCR for Characterization of Staphylococcus aureus Strains." J. Clin. Microbiol. **43**(3): 1187-1192.

http://jcm.asm.org/cgi/content/abstract/43/3/1187

The DiversiLab System, which includes microfluidics-based detection, reagent kits, and software

for data processing and analysis, is an automated method using repetitive sequence-based PCR (rep-PCR) for microbial strain typing. To assess the reliability of the DiversiLab System for strain characterization of Staphylococcus aureus, we tested clinical isolates sent to ARUP Laboratories for typing and compared results to those of pulsed field electrophoresis (PFGE) aided by the cluster analysis provided by BioNumerics software. spa typing was performed when the results of these two methods for an outbreak were not concordant. The study included 89 S. aureus isolates (65 mecA positive, 24 mecA negative) from 19 outbreaks (2 to 11 isolates/outbreak). The DiversiLab and PFGE-BioNumerics results were concordant for 15 of the 19 outbreaks. For the remaining four outbreaks, there was partial concordance between the two methods. spa typing results were the same as or more similar to rep-PCR results for three of those outbreaks and were more similar to PFGE results for one. With regard to performance, the DiversiLab system was considerably less labor intensive than PFGE and provided results in less than 24 h, compared with 2 to 3 days for PFGE. Additionally, the Web-based DiversiLab software provides standardized comparisons among isolates almost instantaneously and generates user-friendly, customized reports.

Simjee, S., D. G. White, et al. (2002). "Characterization of Tn1546 in Vancomycin-Resistant Enterococcus faecium Isolated from Canine Urinary Tract Infections: Evidence of Gene Exchange between Human and Animal Enterococci." J. Clin. Microbiol. **40**(12): 4659-4665.

http://jcm.asm.org/cgi/content/abstract/40/12/4659

Thirty-five enterococcal isolates were recovered from dogs diagnosed with urinary tract infections at the Michigan State University Veterinary Teaching Hospital over a 2-year period (1996 to 1998). Isolated species included Enterococcus faecium (n = 13), Enterococcus faecalis (n = 7), Enterococcus gallinarum (n = 11), and Enterococcus casseliflavus (n = 4). Antimicrobial susceptibility testing revealed several different resistance phenotypes, with the majority of the enterococcal isolates exhibiting resistance to three or more antibiotics. One E. faecium isolate, CVM1869, displayed high-level resistance to vancomycin (MIC > 32 {micro}g/ml) and gentamicin (MIC > 2,048 {micro}g/ml). Molecular analysis of this isolate revealed the presence of Tn1546 (vanA), responsible for high-level vancomycin resistance, and Tn5281 carrying aac6'-aph2", conferring high-level aminoglycoside resistance. Pulsed-field gel electrophoresis analysis revealed that CVM1869 was a canine E. faecium clone that had acquired Tn1546, perhaps from a human vancomycin-resistant E. faecium. Transposons Tn5281 and Tn1546 were located on two different conjugative plasmids. Sequence analysis revealed that in Tn1546, ORF1 had an 889-bp deletion and an IS1216V insertion at the 5' end and an IS1251 insertion between vanS and vanH. To date, this particular form of Tn1546 has only been described in human clinical vancomycin-resistant enterococcus isolates unique to the United States. Additionally, this is the first report of a vancomycin-resistant E. faecium isolated from a companion animal in the United States.

Singh, S., V. T. K. Chow, et al. (2002). "Direct Detection of Enterovirus 71 (EV71) in Clinical Specimens from a Hand, Foot, and Mouth Disease Outbreak in Singapore by Reverse Transcription-PCR with Universal Enterovirus and EV71-Specific Primers." J. Clin. Microbiol. **40**(8): 2823-2827.

http://jcm.asm.org/cgi/content/abstract/40/8/2823

A recent outbreak of hand, foot, and mouth disease in Singapore in 2000 affected several thousand children and resulted in four deaths. The aim of this study was to determine the applicability of reverse transcription-PCR (RT-PCR) with universal pan-enterovirus primers and enterovirus 71 (EV71) type-specific primers for the direct detection of enteroviruses in clinical specimens derived from this outbreak. With the universal primers, EV71 RNA sequences were

successfully detected by RT-PCR and direct sequencing in 71% of positive specimens. Three pairs of EV71 type-specific primers were evaluated for rapid detection of EV71 directly from clinical specimens and cell culture isolates. By using a seminested RT-PCR strategy, specific identification of EV71 sequences directly in clinical specimens was achieved, with a detection rate of 53%. In contrast, cell culture could isolate EV71 in only 20% of positive specimens. EV71 was detected directly from brain, heart, and lung specimens of two deceased siblings. Although more than one type of enterovirus was identified in clinical specimens from this outbreak, 90% of the enteroviruses were confirmed as EV71. The data demonstrate the clinical applicability of pan-enterovirus and seminested RT-PCR for the detection of EV71 RNA directly from clinical specimens in an outbreak situation.

Sinha, S., R. Chakraborty, et al. (2002). "Escalating Association of Vibrio cholerae O139 with Cholera Outbreaks in India." J. Clin. Microbiol. **40**(7): 2635-2637.

http://jcm.asm.org/cgi/content/abstract/40/7/2635

Between December 1999 and December 2000, teams from the National Institute of Cholera and Enteric Diseases, Calcutta, India, examined eight outbreaks of cholera, which occurred in different parts of the country distant from each other. In two of these outbreaks each, only V. cholerae O1 biotype ElTor or V. cholerae O139 could be isolated, while in the remaining four outbreaks, both O1 and O139 were isolated. The interesting feature is the escalating association of V. cholerae O139 with outbreaks of cholera; two of the most recent outbreaks, one in Calcutta and one in Orissa, were caused exclusively by O139. The O139 strains from the six different outbreaks were genotypically closely related. These trends indicate a shift in the outbreak propensity of V. cholerae O139.

Smole, S. C., F. McAleese, et al. (2002). "Clinical and Epidemiological Correlates of Genotypes within the Mycobacterium avium Complex Defined by Restriction and Sequence Analysis of hsp65." J. Clin. Microbiol. **40**(9): 3374-3380.

http://jcm.asm.org/cgi/content/abstract/40/9/3374

Species identification of isolates of the Mycobacterium avium complex (MAC) remains a difficult task. Although M. avium and Mycobacterium intracellulare can be identified with expensive, commercially available probes, many MAC isolates remain unresolved, including those representing Mycobacterium lentiflavum as well as other potentially undefined species. PCR restriction analysis (PRA) of the hsp65 gene has been proposed as a rapid and inexpensive approach. We applied PRA to 278 MAC isolates, including 126 from blood of human immunodeficiency virus (HIV)-infected patients, 59 from sputum of HIV-negative patients with chronic obstructive pulmonary disease, 88 from environmental sources, and 5 pulmonary isolates from a different study. A total of 15 different PRA patterns were observed. For 27 representative isolates, a 441-bp fragment of the hsp65 gene was sequenced; based on 54 polymorphic sites, 18 different alleles were defined, including 12 alleles not previously reported. Species and phylogenetic relationships were more accurately defined by sequencing than by PRA or commercial probe. The distribution of PRA types and, by implication, phylogenetic lineages among blood isolates was significantly different from that for pulmonary and environmental isolates, suggesting that particular lineages have appreciably greater virulence and invasive potential.

Solnick, J. V., K. Chang, et al. (2003). "Natural Acquisition of Helicobacter pylori Infection in Newborn Rhesus Macaques." J. Clin. Microbiol. **41**(12): 5511-5516.

http://jcm.asm.org/cgi/content/abstract/41/12/5511

Helicobacter pylori infection is usually acquired in childhood, but precise estimates of the age of acquisition are difficult to obtain in young children. Since serial endoscopic biopsies are not feasible in human infants, we examined acquisition of H. pylori infection that is known to occur in socially housed nonhuman primates. By 12 weeks of age, 8 of 20 newborns (40%) were culture positive for H. pylori, and prevalence reached 90% by 1 year of age. Newborns from infected dams were more commonly infected than those from uninfected dams, particularly during the peripartum period, suggesting that close contact during this time may facilitate transmission. Transient infection was uncommon and occurred only after the first positive culture. These results suggest that in a high-prevalence environment, persistent H. pylori infection may be acquired at an earlier age than was previously thought. Since clean, potable water was readily available, contamination of water supply is not essential for widespread infection at an early age in areas where hygiene is otherwise poor. Furthermore, breastfeeding seems to offer little protection, since newborn macaques breastfeed during the first year of life and typically are fully weaned only when another newborn arrives the following spring.

Sotlar, K., D. Diemer, et al. (2004). "Detection and Typing of Human Papillomavirus by E6 Nested Multiplex PCR." J. Clin. Microbiol. **42**(7): 3176-3184.

http://jcm.asm.org/cgi/content/abstract/42/7/3176

A nested multiplex PCR (NMPCR) assay that combines degenerate E6/E7 consensus primers and type-specific primers was evaluated for the detection and typing of human papillomavirus (HPV) genotypes 6/11, 16, 18, 31, 33, 35, 39, 42, 43, 44, 45, 51, 52, 56, 58, 59, 66, and 68 using HPV DNA-containing plasmids and cervical scrapes (n = 1,525). The performance of the NMPCR assay relative to that of conventional PCR with MY09-MY11 and GP5+-GP6+ primers, and nested PCR with these two primer sets (MY/GP) was evaluated in 495 cervical scrapes with corresponding histologic and cytologic findings. HPV prevalence rates determined with the NMPCR assay were 34.7% (102 of 294) in the absence of cervical intraepithelial neoplasia (CIN 0), 94.2% (113 of 120) in the presence of mild or moderate dysplasia (CIN I/II), and 97.8% (44 of 45) in the presence of severe dysplasia (CIN III). The combination of all four HPV detection methods applied in the study was taken as "gold standard": in all three morphological subgroups the NMPCR assay had significantly ($P < 0.0001$) higher sensitivities than the MY09-MY11 and GP5+-GP6+ assays and sensitivities comparable or equal to those of the MY/GP assay. All 18 HPV genotypes investigated were detected among the clinical samples. The ratio of high- to low-risk HPV genotypes increased from 4:1 (80 of 103) in CIN 0 to 19:1 (149 of 157) in CIN I to III. Multiple infections were detected in 47.9% (124 of 259) of the patients. In conclusion, the novel NMPCR method is a sensitive and useful tool for HPV DNA detection, especially when exact HPV genotyping and the identification of multiple HPV infections are required.

Steininger, C., M. Kundi, et al. (2002). "Effectiveness of Reverse Transcription-PCR, Virus Isolation, and Enzyme-Linked Immunosorbent Assay for Diagnosis of Influenza A Virus Infection in Different Age Groups." J. Clin. Microbiol. **40**(6): 2051-2056.

http://jcm.asm.org/cgi/content/abstract/40/6/2051

The degrees of effectiveness of reverse transcription (RT)-PCR, virus isolation, and antigen

enzyme-linked immunosorbent assay (ELISA) for the detection of influenza A virus were evaluated with nasopharyngeal swabs from 150 patients (1 week to 86 years old) with influenza A virus infection. RT-PCR had a sensitivity for influenza A virus in stock virus preparations 103 times higher than virus isolation and 106 to 107 times higher than ELISA. The detection rate achieved by RT-PCR in clinical samples was clearly higher (93%) than that by virus isolation (80%) and ELISA (62%). Despite low overall detection rates achieved by antigen ELISA, samples from patients younger than 5 years old yielded higher-than-average rates in this rapid assay (88%). The likelihood of negative results in the ELISA increased significantly with increasing age of the patient ($P < 0.01$). The degrees of effectiveness of RT-PCR and virus isolation were not influenced by the age of the patient. Neither influenza immunizations nor the interval between onset of symptoms and laboratory investigation (mean, 4.7 days; standard deviation, 3.3 days) affected results obtained by the three test systems. Our results demonstrate that the ELISA is reliable for rapid laboratory diagnosis of influenza in infants and young children, but for older patients application of RT-PCR or virus isolation is necessary to avoid false negative results.

Stevens, M. P., S. N. Tabrizi, et al. (2004). "Characterization of Chlamydia trachomatis omp1 Genotypes Detected in Eye Swab Samples from Remote Australian Communities." J. Clin. Microbiol. **42**(6): 2501-2507.

http://jcm.asm.org/cgi/content/abstract/42/6/2501

Chlamydia trachomatis conjunctival samples collected over a 6-month period from individuals with clinical signs of trachoma and located in remote communities in the Australian Northern Territory were differentially characterized according to serovar and variants. The rationale was to gain an understanding of the epidemiology of an apparent increased prevalence of acute trachoma in areas thought to be less conducive to this disease. Characterization was performed through sequencing of a region of the omp1 gene spanning the four variable domains and encoding the major outer membrane protein. Nucleotide and deduced amino acid sequences were genotyped by using a BLAST similarity search and were examined by phylogenetic analyses to illustrate evolutionary relationships between the clinical and GenBank reference strains. The predominant genotype identified corresponded to that of serovar C (87.1%), followed by the genotype corresponding to serovar Ba (12.9%). All nucleotide and amino acid sequences exhibited minor levels of variation with respect to GenBank reference sequences. The omp1 nucleotide sequences of the clinical samples best aligned with those of the conjunctival C. trachomatis reference strains C/TW-3/OT and Ba/Apache-2. All clinical samples (of serovar C) exhibited four or five nucleotide changes compared with C/TW-3/OT, while all serovar Ba samples had one or two nucleotide differences from Ba/Apache-2. Phylogenetic analyses revealed close relationships between these Northern Territory chlamydial samples and the respective reference strains, although the high proportion of sequence variants suggests an evolutionarily distinct C. trachomatis population causing eye infections in Australia. Given that such genotypic information has gone unreported, these findings provide knowledge and a foundation for trachoma-associated C. trachomatis variants circulating in the Northern Territory.

Stevenson, J., W. Hymas, et al. (2005). "Effect of Sequence Polymorphisms on Performance of Two Real-Time PCR Assays for Detection of Herpes Simplex Virus." J. Clin. Microbiol. **43**(5): 2391-2398.

http://jcm.asm.org/cgi/content/abstract/43/5/2391

Herpes simplex virus (HSV) is the most common cause of acquired, sporadic encephalitis in the United States. PCR identification of HSV in spinal fluid has become the diagnostic gold standard due to its sensitivity and potential for speed, replacing other methods such as culture. We

developed a real-time PCR assay to detect HSV, using a new type of hybridization probe, the Eclipse probe. In this study, we ran 323 samples (171 positives and 152 negatives) with the Eclipse real-time PCR assay and compared these results with another PCR assay using gel detection. The real-time assay agreed with our reference method for 319 out of the 323 samples tested (99%). Using two different real-time PCR platforms, we discovered that SNPs within the amplicon's probe binding region that are used to distinguish HSV-1 from HSV-2 can decrease assay sensitivity. This problem is potentially a general one for assays using fluorescent probes to detect target amplification in a real-time format. While real-time PCR can be a powerful tool in the field of infectious disease, careful sequence evaluation and clinical validation are essential in creating an effective assay.

Stich, R. W., Y. Rikihisa, et al. (2002). "Detection of Ehrlichia canis in Canine Carrier Blood and in Individual Experimentally Infected Ticks with a p30-Based PCR Assay." J. Clin. Microbiol. **40**(2): 540-546.

http://jcm.asm.org/cgi/content/abstract/40/2/540

Detection of vector-borne pathogens is necessary for investigation of their association with vertebrate and invertebrate hosts. The ability to detect Ehrlichia spp. within individual experimentally infected ticks would be valuable for studies to evaluate the relative competence of different vector species and transmission scenarios. The purpose of this study was to develop a sensitive PCR assay based on oligonucleotide sequences from the unique Ehrlichia canis gene, p30, to facilitate studies that require monitoring this pathogen in canine and tick hosts during experimental transmission. Homologous sequences for Ehrlichia chaffeensis p28 were compared to sequences of primers derived from a sequence conserved among E. canis isolates. Criteria for primer selection included annealing scores, identity of the primers to homologous E. chaffeensis sequences, and the availability of similarly optimal primers that were nested within the target template sequence. The p30-based assay was at least 100-fold more sensitive than a previously reported nested 16S ribosomal DNA (rDNA)-based assay and did not amplify the 200-bp target amplicon from E. chaffeensis, the human granulocytic ehrlichiosis agent, or Ehrlichia muris DNA. The assay was used to detect E. canis in canine carrier blood and in experimentally infected Rhipicephalus sanguineus ticks. Optimized procedures for preparing tissues from these hosts for PCR assay are described. Our results indicated that this p30-based PCR assay will be useful for experimental investigations, that it has potential as a routine test, and that this approach to PCR assay design may be applicable to other pathogens that occur at low levels in affected hosts.

Streutker, C. J., C. N. Bernstein, et al. (2004). "Detection of Species-Specific Helicobacter Ribosomal DNA in Intestinal Biopsy Samples from a Population-Based Cohort of Patients with Ulcerative Colitis." J. Clin. Microbiol. **42**(2): 660-664.

http://jcm.asm.org/cgi/content/abstract/42/2/660

The inflammatory bowel diseases are considered an abnormal host immune response to an environmental stimulus. Evidence suggests a role for intestinal bacteria in initiating and/or providing an ongoing stimulus for inflammation in inflammatory bowel disease. Helicobacter pylori is the major cause of active chronic gastritis and peptic ulcers in humans and has been linked to gastric carcinoma and lymphoma. Studies in various animal models, particularly mice, have identified enterohepatic Helicobacter species that are capable of causing hepatitis and enterocolitis. We hypothesize that Helicobacter species may have a role in maintaining inflammation in humans with inflammatory bowel disease. In order to investigate this, biopsy specimens were obtained from patients with and without inflammatory bowel disease. DNA was extracted from the tissues and subjected to PCR with primers designed to detect the ribosomal

DNA of members of the Helicobacter species. DNA from six biopsy samples from 60 inflammatory bowel disease patients tested positive. This included 5 of 33 ulcerative colitis patients that were positive compared to 0 of 29 age-matched controls (P < 0.04). Sequencing of the bands produced by PCR amplification revealed [IMG]=" BORDER="0">99% homology with H. pylori. These results indicate that a member of the Helicobacter species may be involved in some cases of ulcerative colitis.

Suchodolski, J. S., C. G. Ruaux, et al. (2004). "Application of Molecular Fingerprinting for Qualitative Assessment of Small-Intestinal Bacterial Diversity in Dogs." J. Clin. Microbiol. **42**(10): 4702-4708.

http://jcm.asm.org/cgi/content/abstract/42/10/4702

The aims of this study were to evaluate the use of molecular fingerprinting for assessment of bacterial diversity in canine duodenal juice and to evaluate the variation in the small intestinal microflora at repeated sampling. Two groups of dogs were used. Duodenal juice was collected from eight dogs euthanized for an unrelated project (group 1). Duodenal juice was also collected endoscopically from six dogs at weekly intervals for a total of 3 weeks (group 2). The variable V6-V8 region of bacterial 16S ribosomal DNA was amplified, and PCR amplicons separated by denaturing gradient gel electrophoresis (DGGE). The reproducibility of DGGE profiles and variations in bacterial diversity between dogs were evaluated by comparing similarity indices (Dice's coefficient; 100% represents complete identity) of DGGE profiles from group 1 dogs. Weekly variations in the flora of the small intestine were evaluated by comparison of DGGE profiles from different time points within the same individuals in group 2. The mean ({+/-} standard deviation) similarity of DGGE profiles of duodenal juice between the dogs in group 1 was 38.3 {+/-} 15.7% (range, 12.5 to 76.65%). There was a significantly higher variation in DGGE profiles between different dogs than between duplicates obtained from the same dog (P < 0.0001). DGGE profiles from samples collected at different time points varied within individuals, possibly due to variation over time or slight variation in sampling location. DGGE profiles indicate that dogs have a highly diverse microflora of the small intestine, with marked differences between individual dogs.

Sugiura, W., K. Shimada, et al. (2003). "Novel Enzyme-Linked Minisequence Assay for Genotypic Analysis of Human Immunodeficiency Virus Type 1 Drug Resistance." J. Clin. Microbiol. **41**(11): 4971-4979.

http://jcm.asm.org/cgi/content/abstract/41/11/4971

We constructed a novel tool for genotypic analysis of human immunodeficiency virus type 1 (HIV-1) drug resistance by using an enzyme-linked minisequence assay (ELMA). ELMA is a combination of hybridization and a 1-base extension reaction, and we designed the assay to detect five mutations conferring nucleoside analogue resistance (M41L, D67N, K70R, T215Y, and M184V) and six mutations conferring protease inhibitor resistance (D30N, M46I, G48V, V82A, I84V, and L90M). At all detection points, ELMA demonstrated high sensitivity and specificity, sufficient for clinical use. Compared to that obtained by direct sequencing, the genotypic information obtained by ELMA is limited to the targeted loci for which it was designed. However, ELMA proves advantageous in several respects. The assay does not require expensive equipment, such as an autosequencer, and can be performed in regular clinical diagnostic laboratories. Therefore ELMA can be a candidate for a drug resistance monitoring assay to be introduced in developing countries. In addition, ELMA demonstrated higher sensitivity in the detection of minor resistant populations. We successfully detected a minor virus population (10%) by the assay. The high sensitivity and specificity of the assay recommend it as a first screening assay for drug resistance surveillance.

Sukhnanand, S., B. Dogan, et al. (2005). "Molecular Subtyping and Characterization of Bovine and Human Streptococcus agalactiae Isolates." J. Clin. Microbiol. **43**(3): 1177-1186.

http://jcm.asm.org/cgi/content/abstract/43/3/1177

Streptococcus agalactiae causes severe invasive disease in humans and mastitis in cattle. Temporally matched bovine milk isolates and clinical human invasive isolates (52 each) collected in New York State over 18 months were characterized by molecular subtyping and phenotypic methods to probe the interspecies transmission potential of this species. EcoRI ribotyping differentiated 17 ribotypes, and DNA sequencing of the housekeeping gene sodA and the putative virulence gene hylB differentiated 7 and 17 allelic types, respectively. Human and bovine isolates were not randomly distributed between ribotypes or hylB and sodA clusters. The combined analysis of all subtyping data allowed the differentiation of 39 clonal groups; 26 groups contained only bovine isolates, and 2 groups contained both human and bovine isolates. The EcoRI ribotype diversity among bovine isolates (Simpson's numerical index of discrimination [mean {+/-} standard deviation], 0.90 {+/-} 0.05) being significantly higher than that among human isolates (0.42 {+/-} 0.15) further supports that these isolates represent distinct populations. Eight human isolates, but no bovine isolates, showed an IS1548 transposon insertion in hylB, which encodes a hyaluronidase. Based on data for 43 representative isolates, human isolates, on average, showed lower hyaluronidase activities than bovine isolates. Isolates with the IS1548 insertion in hylB showed no hyaluronidase activity. Human and bovine isolates did not differ in their abilities to invade HeLa human epithelial cells. Our data show that (i) EcoRI ribotyping, combined with hylB and sodA sequencing, provides a discriminatory subtype analysis of S. agalactiae; (ii) most human invasive and bovine S. agalactiae isolates represent distinct subtypes, suggesting limited interspecies transmission; and (iii) hyaluronidase activity is not required for all human infections.

Sum, S. S.-M., D. K.-H. Wong, et al. (2004). "Real-Time PCR Assay Using Molecular Beacon for Quantitation of Hepatitis B Virus DNA." J. Clin. Microbiol. **42**(8): 3438-3440.

http://jcm.asm.org/cgi/content/abstract/42/8/3438

Levels of hepatitis B virus (HBV) DNA in the blood serve as an important marker in monitoring the disease progression and treatment efficacy of chronic HBV infection. Several commercial assays are available for accurate measurement of HBV genomic DNA, but many of them are hampered by relatively low sensitivity and limited dynamic range. The aim of this study was to develop a sensitive and accurate assay for measuring HBV genomic DNA using real-time PCR with a molecular beacon (HBV beacon assay). The performance of this assay was validated by testing serial dilutions of the two EUROHEP HBV DNA standards (ad and ay subtypes) of known concentrations. The assay showed low intra-assay (<7%) and interassay (<5%) variations for both subtypes. Its dynamic range was found to be 101 to 107 copies per reaction (1.0 x 102 to 1.0 x 109 copies ml-1). The assay was further evaluated clinically using serum samples from 175 individuals with chronic hepatitis B. The HBV DNA level measured by this assay showed good correlation with that measured by the commercially available COBAS AMPLICOR HBV Monitor test ($r = 0.901$; $P < 0.001$). The higher sensitivity and broader dynamic range of this assay compared to the existing commercial assays will provide an ideal tool for monitoring disease progression and treatment efficacy in HBV-infected patients, in particular for those with low levels of HBV viremia.

Sun, Z. F., C. T. Larsen, et al. (2004). "Generation and Infectivity Titration of an Infectious Stock of Avian Hepatitis E Virus (HEV) in Chickens and Cross-Species Infection of Turkeys with Avian HEV." J. Clin. Microbiol. **42**(6): 2658-2662.

http://jcm.asm.org/cgi/content/abstract/42/6/2658

Avian hepatitis E virus (HEV), a novel virus identified from chickens with hepatitis-splenomegaly syndrome in the United States, is genetically and antigenically related to human HEV. In order to further characterize avian HEV, an infectious viral stock with a known infectious titer must be generated, as HEV cannot be propagated in vitro. Bile and feces collected from specific-pathogen-free (SPF) chickens experimentally infected with avian HEV were used to prepare an avian HEV infectious stock as a 10% suspension of positive fecal and bile samples in phosphate-buffered saline. The infectivity titer of this infectious stock was determined by inoculating 1-week-old SPF chickens intravenously with 200 {micro}l of each of serial 10-fold dilutions (10-2 to 10-6) of the avian HEV stock (two chickens were inoculated with each dilution). All chickens inoculated with the 10-2 to 10-4 dilutions of the infectious stock and one of the two chickens inoculated with the 10-5 dilution, but neither of the chickens inoculated with the 10-6 dilution, became seropositive for anti-avian HEV antibody at 4 weeks postinoculation (wpi). Two serologically negative contact control chickens housed together with chickens inoculated with the 10-2 dilution also seroconverted at 8 wpi. Viremia and shedding of virus in feces were variable in chickens inoculated with the 10-2 to 10-5 dilutions but were not detectable in those inoculated with the 10-6 dilution. The infectivity titer of the infectious avian HEV stock was determined to be 5 x 105 50% chicken infectious doses (CID50) per ml. Eight 1-week-old turkeys were intravenously inoculated with 105 CID50 of avian HEV, and another group of nine turkeys were not inoculated and were used as controls. The inoculated turkeys seroconverted at 4 to 8 wpi. In the inoculated turkeys, viremia was detected at 2 to 6 wpi and shedding of virus in feces was detected at 4 to 7 wpi. A serologically negative contact control turkey housed together with the inoculated ones also became infected through direct contact. This is the first demonstration of cross-species infection by avian HEV.

Thiem, V. D., O. Sethabutr, et al. (2004). "Detection of Shigella by a PCR Assay Targeting the ipaH Gene Suggests Increased Prevalence of Shigellosis in Nha Trang, Vietnam." J. Clin. Microbiol. **42**(5): 2031-2035.

http://jcm.asm.org/cgi/content/abstract/42/5/2031

Shigella spp. are exquisitely fastidious gram-negative organisms which frequently escape detection by traditional culture methods. To get a more complete understanding of the disease burden caused by Shigella in Nha Trang, Vietnam, real-time PCR was used to detect Shigella DNA. Randomly selected rectal swab specimens from 60 Shigella culture-positive patients and 500 Shigella culture-negative patients detected by population-based surveillance of patients seeking care for diarrhea were processed by real-time PCR. The target of the primer pair is the invasion plasmid antigen H gene sequence (ipaH), carried by all four Shigella species and enteroinvasive Escherichia coli. Shigella spp. could be isolated from the rectal swabs of 547 of 19,206 (3%) patients with diarrhea. IpaH was detected in 55 of 60 (93%) Shigella culture-positive specimens, whereas it was detected in 87 of 245 (36%) culture-negative patients free of dysentery ($P < 0.001$). The number of PCR cycles required to detect a PCR product was highest for culture-negative, nonbloody diarrheal specimens (mean number of cycles to detection, 36.6) and was lowest for children with culture-positive, bloody diarrheal specimens (mean number of cycles, 25.3) ($P < 0.001$). The data from real-time PCR amplification indicate that the culture-proven prevalence of Shigella among patients with diarrhea may underestimate the prevalence of Shigella infections. The clinical presentation of shigellosis may be directly related to the bacterial load.

Truman, R., A. B. Fontes, et al. (2004). "Genotypic Variation and Stability of Four Variable-Number Tandem Repeats and Their Suitability for Discriminating Strains of Mycobacterium leprae." J. Clin. Microbiol. **42**(6): 2558-2565.

http://jcm.asm.org/cgi/content/abstract/42/6/2558

It has not been possible to distinguish different strains of Mycobacterium leprae according to their genetic sequence. However, the genome contains several variable-number tandem repeats (VNTR), which have been used effectively in strain typing of other bacteria. To determine their suitability for differentiating M. leprae, we developed PCR systems to amplify 5 different VNTR loci and examined a battery of 12 M. leprae strains derived from patients in different regions of the United States, Brazil, Mexico, and the Philippines, as well as from wild armadillos and a sooty mangabey monkey. We found diversity at four VNTR (D = 0.74), but one system (C16G8) failed to yield reproducible results. Alleles for the GAA VNTR varied in length from 10 to 16 copies, those for AT17 varied in length from 10 to 15 copies, those for GTA varied in length from 9 to 12 copies, and those for TA18 varied in length from 13 to 20 copies. Relatively little variation was seen with interspecies transfer of bacilli or during short-term passage of strains in nude mice or armadillos. The TA18 locus was more polymorphic than other VNTR, and genotypic variation was more common after long-term expansion in armadillos. Most strain genotypes remained fairly stable in passage, but strain Thai-53 showed remarkable variability. Statistical cluster analysis segregated strains and passage samples appropriately but did not reveal any particular genotype associable with different regions or hosts of origin. VNTR polymorphisms can be used effectively to discriminate M. leprae strains. Inclusion of additional loci and other elements will likely lead to a robust typing system that can be used in community-based epidemiological studies and select clinical applications.

Ueti, M. W., G. H. Palmer, et al. (2003). "Expression of Equi Merozoite Antigen 2 during Development of Babesia equi in the Midgut and Salivary Gland of the Vector Tick Boophilus microplus." J. Clin. Microbiol. **41**(12): 5803-5809.

http://jcm.asm.org/cgi/content/abstract/41/12/5803

Equi merozoite antigens 1 and 2 (EMA-1 and EMA-2) are Babesia equi proteins expressed on the parasite surface during infection in horses and are orthologues of proteins in Theileria spp., which are also tick-transmitted protozoal pathogens. We determined in this study whether EMA-1 and EMA-2 were expressed within the vector tick Boophilus microplus. B. equi transitions through multiple, morphologically distinct stages, including sexual stages, and these transitions culminate in the formation of infectious sporozoites in the tick salivary gland. EMA-2-positive B. equi stages in the midgut lumen and midgut epithelial cells of Boophilus microplus nymphs were identified by reactivity with monoclonal antibody 36/253.21. This monoclonal antibody also recognized B. equi in salivary glands of adult Boophilus microplus. In addition, quantification of B. equi in the mammalian host and vector tick indicated that the duration of tick feeding and parasitemia levels affected the percentage of nymphs that contained morphologically distinct B. equi organisms in the midgut. In contrast, there was no conclusive evidence that B. equi EMA-1 was expressed in either the Boophilus microplus midgut or salivary gland when monoclonal antibody 36/18.57 was used. The expression of B. equi EMA-2 in Boophilus microplus provides a marker for detecting the various development stages and facilitates the identification of novel stage-specific Babesia proteins for testing transmission-blocking immunity.

Unemo, M., P. Olcen, et al. (2003). "Comparison of Serologic and Genetic porB-Based Typing of Neisseria gonorrhoeae: Consequences for Future Characterization." J. Clin. Microbiol. **41**(9): 4141-4147.

http://jcm.asm.org/cgi/content/abstract/41/9/4141

Due to temporal changes in the epidemiology of gonorrhea, a precise characterization of Neisseria gonorrhoeae is essential. In the present study genetic heterogeneity in the porB genes of N. gonorrhoeae was examined, and serovar determination was compared to porB gene sequencing. Among 108 N. gonorrhoeae isolates, phylogenetic analysis of the entire porB alleles (924 to 993 bp) identified 87 unique sequences. By analyzing only the four to six most heterogeneous porB gene regions (174 to 363 bp), 86 out of these 87 genetic variants were identified. Consequently, analysis of shorter highly variable regions of the porB gene generates high-level discriminatory ability as well as fast, objective, reproducible, and portable data for epidemiological characterization of N. gonorrhoeae. Regarding putative antigenic epitopes of PorB for Genetic Systems monoclonal antibodies (MAbs), some of the previous findings were confirmed, but new findings were also observed. For several of the MAbs, however, the precise amino acid residues of PorB critical for single-MAb reactivity were difficult to identify. In addition, repeated serovar determination of 108 N. gonorrhoeae isolates revealed discrepancies for 34 isolates, mostly due to nonreproducible reactivity with single MAbs. Thus, the prospects of a genetic typing system with congruent translation of the serovar determination seem to be limited. In conclusion, analysis of short highly variable regions of the porB gene could form the basis for a fast molecular epidemiological tool for the examination of emergence and transmission of N. gonorrhoeae strains within the community.

Unemo, M., P. Olcen, et al. (2002). "Molecular Epidemiology of Neisseria gonorrhoeae: Sequence Analysis of the porB Gene Confirms Presence of Two Circulating Strains." J. Clin. Microbiol. **40**(10): 3741-3749.

http://jcm.asm.org/cgi/content/abstract/40/10/3741

The phenotypic and genotypic characteristics of Neisseria gonorrhoeae strains fluctuate over time both locally and globally, and highly discriminative and precise characterization of the strains is essential. Conventional characterization of N. gonorrhoeae strains for epidemiological purposes is mostly based on phenotypic methods, which have some inherent limitations. In the present study sequence analysis of porB1b gene sequences was used for examination of the genetic relationships among N. gonorrhoeae strains. Substantial genetic heterogeneity was identified in the porB genes of serovar IB-2 isolates (8.1% of the nucleotide sites were polymorphic) and serovar IB-3 isolates (5.2% of the nucleotide sites were polymorphic) as well as between isolates of different serovars. The highest degree of diversity was identified in the gene segments encoding the surface-exposed loops of the mature PorB protein. Phylogenetic analysis of the porB1b gene sequences confirmed previous findings that have indicated the circulation of one N. gonorrhoeae strain each of serovar IB-2 and serovar IB-3 in the Swedish community. These strains caused the majority of the cases in two domestic core groups comprising homosexual men and young heterosexuals, respectively, and were also detected in other patients. The phylogenetic analyses of porB gene sequences in the present study showed congruence, but not complete identity, with previous results obtained by pulsed-field gel electrophoresis of the same isolates. In conclusion, porB gene sequencing can be used as a molecular epidemiological tool for examination of genetic relationships among emerging and circulating N. gonorrhoeae strains, as well as for confirmation or discrimination of clusters of gonorrhea cases.

van der Zee, A., H. Verbakel, et al. (2002). "Novel PCR-Probe Assay for Detection of and Discrimination

between Legionella pneumophila and Other Legionella Species in Clinical Samples." J. Clin. Microbiol. **40**(3): 1124-1125.

http://jcm.asm.org

van Doornum, G. J. J., J. Guldemeester, et al. (2003). "Diagnosing Herpesvirus Infections by Real-Time Amplification and Rapid Culture." J. Clin. Microbiol. **41**(2): 576-580.

http://jcm.asm.org/cgi/content/abstract/41/2/576

Procedures using real-time technique were developed to demonstrate the presence of herpes simplex virus type 1 (HSV-1) and HSV-2, varicella zoster virus (VZV), and cytomegalovirus (CMV) in miscellaneous clinical specimens. The assays were compared to rapid culture using centrifugation followed by detection with monoclonal antibodies. A total of 711 consecutive samples were collected from different patient groups. Throat swabs were obtained from transplant patients; dermal or oral specimens were collected from patients suspected for VZV or HSV infection. Genital specimens were taken from patients who attended the Clinic for Sexually Transmitted Diseases at the Dijkzigt Hospital Rotterdam presenting with symptoms of a primary genital ulcer. Nucleic acid extraction was carried out using a MagnaPure LC instrument. The amplification steps were performed on the ABI Prism 7700 sequence detection system. To monitor the process of extraction and amplification, a universal control consisting of seal herpesvirus type 1 (PhHV-1) was added to the clinical specimens. By culture 127 of 668 (19%) samples were positive for HSV-1, 72 of 668 (10.8%) specimens were positive for HSV-2, and 17 of 366 (4.6%) were positive for VZV. Using real-time amplification the numbers of positive specimens were 143 of 668 (21.4%), 97 of 668 (14.5%), and 27 of 366 (7.4%), respectively. Eighty-six specimens were tested for CMV, 12 (14.0%) were positive by culture, and 17 (19.8%) were positive by real-time PCR. The clinical data of the patients with discrepant results were reviewed thoroughly. In all cases the patients with only real-time PCR-positive results could be considered as truly infected. We concluded that the real-time amplification technique is suitable for the detection of human herpesvirus infection. It offers a semiquantitative and reliable assay with a quick result that is more sensitive than rapid culture, especially for the diagnosis of HSV-2 and VZV infections.

van Elden, L. J. R., A. M. van Loon, et al. (2003). "Applicability of a Real-Time Quantitative PCR Assay for Diagnosis of Respiratory Syncytial Virus Infection in Immunocompromised Adults." J. Clin. Microbiol. **41**(9): 4378-4381.

http://jcm.asm.org/cgi/content/abstract/41/9/4378

Respiratory syncytial virus (RSV) accounts for the majority of respiratory virus infections, producing high mortality rates in immunocompromised patients with hematologic malignancies. The available methods for the rapid detection of RSV by antigen detection or PCR either lack sensitivity, require complex laboratory manipulation, or have not been evaluated in this patient population. To assess the applicability of a TaqMan-based real-time PCR technique for the detection of RSV A and B in immunocompromised adults, we developed a rapid, sensitive detection method that simultaneously detects RSV A and B and can be applied in routine diagnostics. The specificity of the assay was assessed using a panel of reference strains of other respiratory viruses and RSV. Electron microscopy-counted stocks of RSV A and B were used to develop a quantitative PCR format. Eleven copies of viral RNA could be detected for RSV A strain Long, and 14 copies could be detected for RSV B strain 9320, corresponding to 50% tissue culture infective doses of 0.86 and 0.34, respectively. The assay was evaluated on 411 combined

nose and throat swabs derived from immunocompromised adults with or without signs of respiratory tract infection. The diagnostic efficacy of the TaqMan PCR determined on the clinical samples showed that this real-time PCR technique was substantially more sensitive than the combination of conventional viral culture and shell vial culture. None of the clinical specimens derived from patients without signs of respiratory illness were found to be positive for RSV by real-time TaqMan PCR.

Wadowsky, R. M., E. A. Castilla, et al. (2002). "Evaluation of Chlamydia pneumoniae and Mycoplasma pneumoniae as Etiologic Agents of Persistent Cough in Adolescents and Adults." J. Clin. Microbiol. **40**(2): 637-640.

http://jcm.asm.org/cgi/content/abstract/40/2/637

Chlamydia pneumoniae and Mycoplasma pneumoniae were evaluated as agents of persistent cough in adolescents and adults (n = 491). Tests of 473 respiratory specimens by culture or PCR or both identified four episodes (0.8%) of M. pneumoniae-associated illness and no episodes of C. pneumoniae illness, suggesting that these bacteria do not frequently cause persistent cough.

Walker, E. S., F. Levy, et al. (2004). "A Decline in Mupirocin Resistance in Methicillin-Resistant Staphylococcus aureus Accompanied Administrative Control of Prescriptions." J. Clin. Microbiol. **42**(6): 2792-2795.

http://jcm.asm.org/cgi/content/abstract/42/6/2792

Susceptibility to mupirocin was assessed in methicillin-resistant Staphylococcus aureus isolates selected from eras corresponding to differences in usage rate and prescription policies at a Veterans Affairs medical center. The eras studied encompassed from the time of introduction of the drug to its widespread use, through recommended judicious use, and finally to subsequent stringent administrative control. Prescriptions declined from 3.0 to 0.1 per 1,000 patient days. Precipitous declines first in the numbers of isolates with high-level resistance (from 31% to 4%) and then in those with low-level resistance (from 26% to 10%) accompanied prescription control.

Waltz, T. L., S. Marras, et al. (2005). "Development of a Molecular-Beacon Assay To Detect the G1896A Precore Mutation in Hepatitis B Virus-Infected Individuals." J. Clin. Microbiol. **43**(1): 254-258.

http://jcm.asm.org/cgi/content/abstract/43/1/254

The 1896 precore (PC) mutation is the most frequent cause of hepatitis B virus e-antigen (HBeAg)-negative chronic hepatitis B virus (HBV) infection. Detection of the 1896 PC mutation has application in studies monitoring antiviral therapy and the natural history of the disease. Identification of this mutation is usually performed by direct sequencing, which is both costly and laborious. The aim of this study was to develop a rapid, high-throughput assay to detect the 1896 PC mutation using real-time PCR and molecular-beacon technology. The assay was initially standardized on oligonucleotide targets and plasmids containing the wild-type (WT) and PC mutation and then tested on plasma samples from children with HBV DNA of >106 copies/ml. Nine individuals were HBeAg negative and suspected to harbor HBeAg mutations, while 12 children were HBeAg positive and selected as controls. Ninety percent (19 of 21) of plasma samples tested with molecular beacons were in complete agreement with sequencing results. The remaining 10% (2 of 21) of samples were identified as heterogeneous mixtures of WT and

mutant virus by molecular beacons, though sequencing found only a homogeneous mutant in both cases. Overall, the 1896 PC mutation was detected by this assay in 55.5% of the children with HBeAg-negative infection. In summary, this assay is a rapid, sensitive, and specific technique that effectively discriminates WT from 1896 PC mutant HBV and may be useful in clinical and epidemiological studies.

Wang, C.-Y. J., J. J. Giambrone, et al. (2002). "Detection of Duck Hepatitis B Virus DNA on Filter Paper by PCR and SYBR Green Dye-Based Quantitative PCR." J. Clin. Microbiol. **40**(7): 2584-2590.

http://jcm.asm.org/cgi/content/abstract/40/7/2584

Duck hepatitis B virus (DHBV) belongs to the Hepadnaviridae family, which includes human Hepatitis B virus (HBV) and Woodchuck hepatitis virus. It is widely distributed in wild and domestic ducks due to congenital transmission. HBV is a worldwide health problem, with carriers at risk of developing cirrhosis and liver cancer. Medical staff and scientists working with HBV must be vaccinated because of its contagious nature. DHBV is a safe surrogate for HBV because of their similarities. Collection of serum and blood samples on filter paper has been used to screen for metabolic disorders, genetic diseases, and viral infection and for evolutionary studies of the genome. In this study, DHBV from serum and blood dried on filters was detected by PCR. A 0.1-{micro}l sample was sufficient for detection. The immobilization potential of filter papers for DHBV was examined, and the highest yield of PCR products was observed with Whatman paper. Dried serum was stable under different storage temperatures for 4 weeks, but the yields of PCR products decreased when the temperature was [>=]4{degrees}C. The optimal condition for storage was -70{degrees}C. A newly developed quantitative PCR based on monitoring the amplification by measuring the increase in fluorescence caused by the binding of SYBR green dye to double-stranded products was applied herein. DHBV genomic DNA cloned in a plasmid was used for the generation of standard DHBV DNA for quantitative PCR. It validated results from PCR in terms of the copy number of DHBV particles. The specificity of PCR was demonstrated by melting curve analysis, and the differentiation of two DHBV isolates amplified from dried serum was demonstrated based on their melting temperatures determined by GC contents and sequence. It was easier and simpler than other PCR-based DNA techniques. The use of serum dried on filters allows samples from distant field for which cold storage and transportation are a problem to be mailed to the diagnostic laboratory. Samples can be archived for comparison and used as a source of DNA for cloning and sequencing.

Wang, G., C. G. Clark, et al. (2002). "Detection in Escherichia coli of the Genes Encoding the Major Virulence Factors, the Genes Defining the O157:H7 Serotype, and Components of the Type 2 Shiga Toxin Family by Multiplex PCR." J. Clin. Microbiol. **40**(10): 3613-3619.

http://jcm.asm.org/cgi/content/abstract/40/10/3613

Strains of Shiga toxin-producing Escherichia coli (STEC) have been associated with outbreaks of diarrhea, hemorrhagic colitis, and hemolytic-uremic syndrome in humans. Most clinical signs of disease arise as a consequence of the production of Shiga toxin 1 (Stx1), Stx2 or combinations of these toxins. Other major virulence factors include enterohemorrhagic E. coli hemolysin (EHEC hlyA), and intimin, the product of the eaeA gene that is involved in the attaching and effacing adherence phenotype. In this study, a series of multiplex-PCR assays were developed to detect the eight most-important E. coli genes associated with virulence, two that define the serotype and therefore the identity of the organism, and a built-in gene detection control. Those genes detected were stx1, stx2, stx2c, stx2d, stx2e, stx2f, EHEC hlyA, and eaeA, as well as rfbE, which encodes the E. coli O157 serotype; fliC, which encodes the E. coli flagellum H7 serotype; and the E. coli 16S rRNA, which was included as an internal control. A total of 129 E. coli strains, including 81

that were O157:H7, 10 that were O157:non-H7, and 38 that were non-O157 isolates, were investigated. Among the 129 samples, 101 (78.3%) were stx positive, while 28 (21.7%) were lacked stx. Of these 129 isolates, 92 (71.3%) were EHEC hlyA positive and 96 (74.4%) were eaeA positive. All STEC strains were identified by this procedure. In addition, all Stx2 subtypes, which had been initially identified by PCR-restriction fragment length polymorphism, were identified by this method. A particular strength of the assay was the identification of these 11 genes without the need to use restriction enzyme digestion. The proposed method is a simple, reliable, and rapid procedure that can detect the major virulence factors of E. coli while differentiating O157:H7 from non-O157 isolates.

Wang, J.-Y., L.-N. Lee, et al. (2004). "Performance Assessment of a Nested-PCR Assay (the RAPID BAP-MTB) and the BD ProbeTec ET System for Detection of Mycobacterium tuberculosis in Clinical Specimens." J. Clin. Microbiol. **42**(10): 4599-4603.

http://jcm.asm.org/cgi/content/abstract/42/10/4599

The performance of a nested PCR-based assay (the RAPID BAP-MTB; AsiaGen, Taichung, Taiwan) and the BD ProbeTec ET (DTB) system (Becton Dickinson, Sparks, Md.) for detection of Mycobacterium tuberculosis was evaluated with 600 consecutive clinical samples. These samples, including 552 respiratory specimens and 48 nonrespiratory specimens, were collected from 333 patients treated at National Taiwan University Hospital from September to October 2003. The results of both assays were compared to the gold standard of combined culture results and clinical diagnosis. The overall sensitivity and specificity of the RAPID BAP-MTB assay for respiratory specimens were 66.7% and 97.2%, respectively, and for the DTB assay they were 56.7% and 95.3%, respectively. The positive and negative predictive values for the RAPID BAP-MTB were 74.1% and 96.0%, respectively, and for the DTB assay they were 59.6% and 94.7%, respectively. For smear-negative samples, the sensitivity of the RAPID BAP-MTB and DTB assays was 57.1% and 40.5%, respectively. The RAPID BAP-MTB assay produced 14 false-positive results in 14 samples, including one of the six samples yielding Mycobacterium abscessus, one of the six samples yielding Mycobacterium avium intracellulare, one sample from a patient with a history of pulmonary tuberculosis with complete treatment, and three samples from three patients with a previous diagnosis of tuberculosis who were under treatment at the time of specimen collection. Among the 48 nonrespiratory specimens, the RAPID BAP-MTB assay was positive in one biopsy sample from a patient with lumbar tuberculous spondylitis and one pus sample from a patient with tuberculous cervical lymphadenopathy. Our results showed that the RAPID BAP-MTB assay is better than the DTB assay for both respiratory specimens and nonrespiratory specimens. The overall time for processing this assay is only 5 h. In addition, its diagnostic accuracy in smear-negative samples is as high as in smear-positive samples.

Weidmann, M., U. Meyer-Konig, et al. (2003). "Rapid Detection of Herpes Simplex Virus and Varicella-Zoster Virus Infections by Real-Time PCR." J. Clin. Microbiol. **41**(4): 1565-1568.

http://jcm.asm.org/cgi/content/abstract/41/4/1565

The herpes simplex viruses types 1 and 2 (HSV-1 and HSV-2) and varicella-zoster virus (VZV) can cause life-threatening infections of the central nervous system and lead to severe infections in immunocompromised subjects and newborns. In these cases, rapid diagnosis is crucial. We developed three different real-time PCR assays based on TaqMan chemistry for the LightCycler instrument to detect HSV-1, HSV-2, and VZV. When the TaqMan assays were compared to our in-house nested PCR assays, the test systems had equal sensitivities of [<=]10 plasmid copies per assay. When clinical samples were investigated by TaqMan PCR to detect HSV-1, HSV-2, and VZV DNA, 95, 100, and 96% of the samples determined to be positive by nested PCR,

respectively, were positive by the real-time PCR assays. The specificities of all PCR assays were almost 100%. Furthermore, the TaqMan PCR assays could be performed within 2.5 h, whereas nested PCR results were available after 9 h. In addition to offering more rapid results, the TaqMan PCR assays appear to be less expensive than nested PCR assays due to less hands-on time. In summary, TaqMan PCR is an excellent alternative to conventional nested PCR assays for the rapid detection of HSV-1, HSV-2, and VZV in clinical samples.

Weidmann, M., P. Schmidt, et al. (2005). "Identification of Genetic Evidence for Dobrava Virus Spillover in Rodents by Nested Reverse Transcription (RT)-PCR and TaqMan RT-PCR." J. Clin. Microbiol. **43**(2): 808-812.

http://jcm.asm.org/cgi/content/abstract/43/2/808

A survey of 158 rodents caught in the Czech Republic identified Dobrava virus sequences closely related to that of the Dobrava virus type strain in Apodemus sylvaticus and Mus musculus rodents. The identity of A. sylvaticus was unequivocally confirmed by random amplified polymorphic DNA analysis. The data seem to indicate hantavirus spillover from Apodemus flavicollis to other rodents.

Wisselink, H. J., J. J. Joosten, et al. (2002). "Multiplex PCR Assays for Simultaneous Detection of Six Major Serotypes and Two Virulence-Associated Phenotypes of Streptococcus suis in Tonsillar Specimens from Pigs." J. Clin. Microbiol. **40**(8): 2922-2929.

http://jcm.asm.org/cgi/content/abstract/40/8/2922

Multiplex PCR assays for the detection and identification of various Streptococcus suis strains in tonsillar specimens from pigs were developed and evaluated. In two separate reactions, five distinct DNA targets were amplified. Three targets, based on the S. suis capsular polysaccharide (cps) genes specific for serotypes 1 (and 14), 7, and 9, were amplified in multiplex PCR I. Two other targets, based on the serotype 2- (and 1/2-) specific cps gene and the epf gene, encoding the EF proteins of virulent serotype 2 and highly virulent serotype 1 strains, were amplified in multiplex PCR II. To identify false-negative results, firefly luciferase (luc) DNA and primers based on the luc gene were included in the assay. The multiplex PCR assays were evaluated with tonsillar specimens from pigs infected with S. suis strains. The results obtained with the PCR assays were compared with the results obtained with a bacteriological examination. Most (94%) of the results obtained with multiplex PCR assays were confirmed by the bacteriological examination. The PCR method seems to be more sensitive compared to the bacteriological method, since the remaining 6% of the samples were positive by PCR and negative by bacteriological examination. These results indicate that the PCR method is highly specific for the detection of S. suis strains most frequently involved in clinical disease in infected pig herds. The serotypes found by PCR in tonsillar specimens from diseased pigs were compared with the serotypes of the strains isolated from the affected tissues of the same pigs. The results showed that there is significant association between carriership and clinical illness for S. suis serotype 9 and EF-positive serotype 2 strains and not for serotype 7 and EF-negative serotype 2 (or 1/2) strains.

Woo, P. C. Y., K. T. K. Chong, et al. (2003). "AFLMP1 Encodes an Antigenic Cell Wall Protein in Aspergillus flavus." J. Clin. Microbiol. **41**(2): 845-850.

http://jcm.asm.org/cgi/content/abstract/41/2/845

We have previously reported the cloning and characterization of the MP1 gene in Penicillium marneffei and the AFMP1 gene in Aspergillus fumigatus and their use for serodiagnosis of penicilliosis and aspergilloma and invasive aspergillosis, respectively. In this study, we describe the cloning of the AFLMP1 gene, which encodes the homologous antigenic cell wall protein in Aspergillus flavus, the most common Aspergillus species associated with human disease in our locality and in other Asian countries and the second most common Aspergillus species associated with human disease in Western countries. AFLMP1 codes for a protein, Aflmp1p, of 273 amino acid residues, with a few sequence features that are present in Mp1p and Afmp1p, the homologous antigenic cell wall proteins in P. marneffei and A. fumigatus, respectively, as well as several other cell wall proteins of Saccharomyces cerevisiae and Candida albicans. It contains a serine- and threonine-rich region for O glycosylation, a signal peptide, and a putative glycosylphosphatidylinositol attachment signal sequence. Specific anti-Aflmp1p antibody was generated with recombinant Aflmp1p protein purified from Escherichia coli to allow further characterization of Aflmp1p. Indirect immunofluorescence analysis indicated that Aflmp1p is present on the surface of the hyphae of A. flavus. Finally, it was observed that patients with aspergilloma and invasive aspergillosis due to A. flavus develop a specific antibody response against Aflmp1p. This suggested that the recombinant protein and its antibody may be useful for serodiagnosis in patients with aspergilloma or invasive aspergillosis, and the protein may represent a good cell surface target for host humoral immunity.

Yam, W. C., K. H. Chan, et al. (2003). "Evaluation of Reverse Transcription-PCR Assays for Rapid Diagnosis of Severe Acute Respiratory Syndrome Associated with a Novel Coronavirus." J. Clin. Microbiol. **41**(10): 4521-4524.

http://jcm.asm.org/cgi/content/abstract/41/10/4521

The reverse transcription (RT)-PCR protocols of two World Health Organization (WHO) severe acute respiratory syndrome (SARS) network laboratories (WHO SARS network laboratories at The University of Hong Kong [WHO-HKU] and at the Bernhard-Nocht Institute in Hamburg, Germany [WHO-Hamburg]) were evaluated for rapid diagnosis of a novel coronavirus (CoV) associated with SARS in Hong Kong. A total of 303 clinical specimens were collected from 163 patients suspected to have SARS. The end point of both WHO-HKU and WHO-Hamburg RT-PCR assays was determined to be 0.1 50% tissue culture infective dose. Using seroconversion to CoV as the "gold standard" for SARS CoV diagnosis, WHO-HKU and WHO-Hamburg RT-PCR assays exhibited diagnostic sensitivities of 61 and 68% (nasopharyngeal aspirate specimens), 65 and 72% (throat swab specimens), 50 and 54% (urine specimens), and 58 and 63% (stool specimens), respectively, with an overall specificity of 100%. For patients confirmed to have SARS CoV and from whom two or more respiratory specimens were collected, testing the second specimen increased the sensitivity from 64 and 71% to 75 and 79% for the WHO-HKU and WHO-Hamburg RT-PCR assays, respectively. Testing more than one respiratory specimen will maximize the sensitivity of PCR assays for SARS CoV.

Yamasaki, H., M. Nakao, et al. (2002). "DNA Differential Diagnosis of Human Taeniid Cestodes by Base Excision Sequence Scanning Thymine-Base Reader Analysis with Mitochondrial Genes." J. Clin. Microbiol. **40**(10): 3818-3821.

http://jcm.asm.org/cgi/content/abstract/40/10/3818

For DNA differential diagnosis of human Taenia cestodes, a base excision sequence scanning

thymine-base method using the cytochrome c oxidase subunit I and cytochrome b genes as targets was used. The characteristic thymine-base peak profiles provide four distinct types, unique for T. saginata, T. asiatica, and two genotypes of T. solium. This approach provides a useful tool for the identification and diagnosis of human taeniid cestodes without DNA sequencing if nucleotide sequence databases are available.

Yang, S., S. Lin, et al. (2002). "Quantitative Multiprobe PCR Assay for Simultaneous Detection and Identification to Species Level of Bacterial Pathogens." J. Clin. Microbiol. **40**(9): 3449-3454.

http://jcm.asm.org/cgi/content/abstract/40/9/3449

We describe a novel adaptation of the TaqMan PCR assay which potentially allows for highly sensitive detection of any eubacterial species with simultaneous species identification. Our system relies on a unique multiprobe design in which a single set of highly conserved sequences encoded by the 16S rRNA gene serves as the primer pair and is used in combination with both an internal highly conserved sequence, the universal probe, and an internal variable region, the species-specific probe. A pre-PCR ultrafiltration step effectively decontaminates or removes background DNA. The TaqMan system described reliabAly detected 14 common bacterial species with a detection limit of 50 fg. Further, highly sensitive and specific pathogen detection was demonstrated with a prototype species-specific probe designed to detect Staphylococcus aureus. This assay has broad potential in the clinical arena for rapid and specific diagnosis of infectious diseases.

Yera, H., M. E. Bougnoux, et al. (2003). "Mycetoma of the Foot Caused by Fusarium solani: Identification of the Etiologic Agent by DNA Sequencing." J. Clin. Microbiol. **41**(4): 1805-1808.

http://jcm.asm.org/cgi/content/abstract/41/4/1805

We report a case of Fusarium solani mycetoma of the foot that could not be diagnosed by culture, but was correctly identified after amplification and sequence analysis of fungal internal transcribed spacers 1 and 2 and 5.8S ribosomal DNA regions.

Yoshida, T., T. Deguchi, et al. (2002). "Quantitative Detection of Mycoplasma genitalium from First-Pass Urine of Men with Urethritis and Asymptomatic Men by Real-Time PCR." J. Clin. Microbiol. **40**(4): 1451-1455.

http://jcm.asm.org/cgi/content/abstract/40/4/1451

We developed a TaqMan-based real-time PCR assay for quantifying Mycoplasma genitalium. This assay is able to specifically quantify concentrations of the M. genitalium 16S rRNA gene ranging from 107 to 10 copies/reaction. Using the TaqMan assay, we quantified the M. genitalium 16S rRNA gene in first-pass urine of men with urethritis and asymptomatic men who were positive for M. genitalium by PCR- and phylogeny-based assay. Of 130 men with gonococcal urethritis (GU), five were positive for M. genitalium. The mycoplasma load for each specimen was <5 x 10 copies/ml. Of 84 men with chlamydial non-GU (CNGU), seven were positive for M. genitalium. One man had an M. genitalium load of <5 x 10 copies/ml, and six men had loads ranging from 1.1 x 107 to 2.7 x 102 copies/ml. Of 86 men with nonchlamydial NGU (NCNGU), 17 were positive for M. genitalium. The mycoplasma loads for these men ranged from 3.3 x 106 to 2.3 x 102 copies/ml. Of 76 asymptomatic men, only two were positive for M. genitalium. For these

men, the loads were 2 x 102 and <5 x 10 copies/ml. The patients with NGU had significantly higher concentrations of M. genitalium in their first-pass urine than did men with GU ($P < 0.01$) or asymptomatic men ($P < 0.05$). In addition, M. genitalium loads were significantly higher in men with NCNGU than those in asymptomatic men ($P < 0.05$). The quantitative assessment of M. genitalium loads by the TaqMan assay will provide useful information for understanding the pathogenicity of this mycoplasma in the urogenital tract.

Yoshida, T., S.-I. Maeda, et al. (2002). "Phylogeny-Based Rapid Identification of Mycoplasmas and Ureaplasmas from Urethritis Patients." J. Clin. Microbiol. **40**(1): 105-110.

http://jcm.asm.org/cgi/content/abstract/40/1/105

Some strains of mycoplasmas and ureaplasmas (family Mycoplasmataceae) are associated with nongonococcal urethritis (NGU) or other genitourinary infections. We have developed a rapid and reliable method of identifying the presence and prevalence of mycoplasmas and ureaplasmas in men with NGU. This method is based on the amplification of a part of the 16S rRNA gene by PCR and phylogenetic analysis. A portion of the 16S rRNA gene from 15 prototype strains was amplified with a set of common primers, and their nucleotides were sequenced. The nucleotide sequence of the V4 and V5 regions was analyzed by the neighbor-joining method. The 15 prototype strains were grouped into three distinct clusters, allowing us to clearly segregate the strains into distinct lineages. To determine the prevalence of these pathogens among patients with NGU, this protocol was tested with 148 urine samples. Amplifications were observed for 42 samples, and their nucleotide sequences were analyzed along with those of the 15 prototype strains. The phylogenetic tree thus constructed indicated that 15 of the 42 formed a cluster with Mycoplasma genitalium. Among the remaining specimens, 2 formed a cluster with Mycoplasma hominis, 19 with Ureaplasma urealyticum, and 5 with Ureaplasma parvum; the remaining sample contained both M. genitalium and U. urealyticum. This phylogeny-based identification of mycoplasmas and ureaplasmas provides not only a powerful tool for rapid diagnosis but also the basis for etiological studies of these pathogens.

Zhang, W., M. Bielaszewska, et al. (2002). "Identification, Characterization, and Distribution of a Shiga Toxin 1 Gene Variant (stx1c) in Escherichia coli Strains Isolated from Humans." J. Clin. Microbiol. **40**(4): 1441-1446.

http://jcm.asm.org/cgi/content/abstract/40/4/1441

By using sequence analysis of Shiga toxin 1 (Stx 1) genes from human and ovine Stx-producing Escherichia coli (STEC) strains, we identified an Stx1 variant in STEC of human origin that was identical to the Stx1 variant from ovine STEC, but demonstrated only 97.1 and 96.6% amino acid sequence identity in its A and B subunits, respectively, to the Stx1 encoded by bacteriophage 933J. We designated this variant "Stx1c" and developed stxB1 restriction fragment length polymorphism and stx1c-specific PCR strategies to determine the frequency and distribution of stx1c among 212 STEC strains isolated from humans. stx1c was identified in 36 (17.0%) of 212 STEC strains, 19 of which originated from asymptomatic subjects and 16 of which were from patients with uncomplicated diarrhea. stx1c was most frequently (in 23 STEC strains [63.9%]) associated with stx2d, but 12 (33.3%) of the 36 STEC strains possessed stx1c only. A single STEC strain possessed stx1c together with stx2 and was isolated from a patient with hemolytic-uremic syndrome. All 36 stx1c-positive STEC strains were eae negative and belonged to 10 different serogroups, none of which was O157, O26, O103, O111, or O145. Stx1c was produced by all stx1c-containing STEC strains, but reacted weakly with a commercial immunoassay. We conclude that STEC strains harboring the stx1c variant account for a significant proportion of human STEC isolates. The procedures developed in this study now allow the determination of the

frequency of STEC strains harboring stx1c among clinical STEC isolates and their association with human disease in prospective studies.

Zhou, W., W. Du, et al. (2004). "Detection of gyrA and parC Mutations Associated with Ciprofloxacin Resistance in Neisseria gonorrhoeae by Use of Oligonucleotide Biochip Technology." J. Clin. Microbiol. **42**(12): 5819-5824.

http://jcm.asm.org/cgi/content/abstract/42/12/5819

An oligonucleotide biochip that specifically detects point mutations in the gyrA and parC genes of Neisseria gonorrhoeae was designed and subsequently evaluated with 87 untreated clinical specimens. The susceptibilities of the N. gonorrhoeae strains were tested to determine the prevalence of ciprofloxacin-resistant strains in Anhui Province, People's Republic of China. Conventional DNA sequencing was also performed to identify mutations in gyrA and parC and to confirm the biochip data. The study demonstrates that all of the point mutations in the gyrA and parC genes of N. gonorrhoeae were easily discriminated by use of the oligonucleotide biochip. Fifteen different alteration patterns involved in the formation of ciprofloxacin resistance were identified by the biochip assay. Double mutations in both Ser91 and Asp95 of the GyrA protein were seen in all nonsensitive isolates. Double mutations in Ser91 and Asp95 of GyrA plus mutation of Glu91 or Ser87 of the ParC protein lead to significant high-level resistance to ciprofloxacin in N. gonorrhoeae isolates. The results obtained by use of the oligonucleotide biochip were identical to those obtained by use of DNA sequencing. In conclusion, the oligonucleotide biochip technology has potential utility for the rapid and reliable identification of point mutations in the drug resistance genes of N. gonorrhoeae.

Zink, A. R., C. Sola, et al. (2003). "Characterization of Mycobacterium tuberculosis Complex DNAs from Egyptian Mummies by Spoligotyping." J. Clin. Microbiol. **41**(1): 359-367.

http://jcm.asm.org/cgi/content/abstract/41/1/359

Bone and soft tissue samples from 85 ancient Egyptian mummies were analyzed for the presence of ancient Mycobacterium tuberculosis complex DNA (aDNA) and further characterized by spoligotyping. The specimens were obtained from individuals from different tomb complexes in Thebes West, Upper Egypt, which were used for upper social class burials between the Middle Kingdom (since ca. 2050 BC) and the Late Period (until ca. 500 BC). A total of 25 samples provided a specific positive signal for the amplification of a 123-bp fragment of the repetitive element IS6110, indicating the presence of M. tuberculosis DNA. Further PCR-based tests for the identification of subspecies failed due to lack of specific amplification products in the historic tissue samples. Of these 25 positive specimens, 12 could be successfully characterized by spoligotyping. The spoligotyping signatures were compared to those in an international database. They all show either an M. tuberculosis or an M. africanum pattern, but none revealed an M. bovis-specific pattern. The results from a Middle Kingdom tomb (used exclusively between ca. 2050 and 1650 BC) suggest that these samples bear an M. africanum-type specific spoligotyping signature. The samples from later periods provided patterns typical for M. tuberculosis. This study clearly demonstrates that spoligotyping can be applied to historic tissue samples. In addition, our results do not support the theory that M. tuberculosis originated from the M. bovis type but, rather, suggest that human M. tuberculosis may have originated from a precursor complex probably related to M. africanum.

J. Environ. Qual. (1)

Siddique, T., B. C. Okeke, et al. (2003). "Enrichment and Isolation of Endosulfan-Degrading Microorganisms." J. Environ. Qual. **32**(1): 47-54.

http://jeq.scijournals.org/cgi/content/abstract/32/1/47

Endosulfan (6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,3,4-benzo-dioxathiepin-3-oxide) is a cyclodiene organochlorine currently used as an insecticide all over the world and its residues are posing a serious environmental threat. This study reports the isolation and identification of enriched microorganisms, capable of degrading endosulfan. Enrichment was achieved by using the insecticide as either the sole source of carbon or sulfur in parallel studies. Two strains each of fungi (F1 and F4) and bacteria (BF2 and B4) were selected using endosulfan as a sole carbon source. A Pandoraea species (Lin-3) previously isolated in our laboratory using lindane ({gamma}-HCH) as a carbon source was also screened for endosulfan degradation. F1 and F4 (Fusarium ventricosum) degraded {alpha}-endosulfan by as much as 82.2 and 91.1% and {beta}-endosulfan by 78.5 and 89.9%, respectively, within 15 d of incubation. Bacterial strains B4 and Lin-3 degraded {alpha}-endosulfan up to 79.6 and 81.8% and {beta}-endosulfan up to 83.9 and 86.8%, respectively, in 15 d. Among the bacterial strains isolated by providing endosulfan as a sulfur source, B4s and F4t degraded {alpha}-endosulfan by as much as 70.4 and 68.5% and {beta}-endosulfan by 70.4 and 70.8%, respectively, after 15 d. Degradation of the insecticide occurred concomitant with bacterial growth reaching an optical density (OD600) of 0.366 and 0.322 for B4 and Lin-3, respectively. High OD600 was also noted with the other bacterial strains utilizing endosulfan as a sulfur source. Fungal and bacterial strains significantly decreased the pH of the nutrient culture media while growing on endosulfan. The results of this study suggest that these novel strains are a valuable source of potent endosulfan-degrading enzymes for use in enzymatic bioremediation.

J. Exp. Biol. (3)

Lundby, C., H. Pilegaard, et al. (2004). "Acclimatization to 4100 m does not change capillary density or mRNA expression of potential angiogenesis regulatory factors in human skeletal muscle." J. Exp. Biol. **207**(22): 3865-3871.

http://jeb.biologists.org/cgi/content/abstract/207/22/3865

Increased skeletal muscle capillary density would be a logical adaptive mechanism to chronic hypoxic exposure. However, animal studies have yielded conflicting results, and human studies are sparse. Neoformation of capillaries is dependent on endothelial growth factors such as vascular endothelial growth factor (VEGF), a known target gene for hypoxia inducible factor 1 (HIF-1). We hypothesised that prolonged exposure to high altitude increases muscle capillary density and that this can be explained by an enhanced HIF-1{alpha} expression inducing an increase in VEGF expression. We measured mRNA levels and capillary density in muscle biopsies from vastus lateralis obtained in sea level residents (SLR; N=8) before and after 2 and 8 weeks of exposure to 4100 m altitude and in Bolivian Aymara high-altitude natives exposed to

approximately 4100 m altitude (HAN; N=7). The expression of HIF-1{alpha} or VEGF mRNA was not changed with prolonged hypoxic exposure in SLR, and both genes were similarly expressed in SLR and HAN. In SLR, whole body mass, mean muscle fibre area and capillary to muscle fibre ratio remained unchanged during acclimatization. The capillary to fibre ratio was lower in HAN than in SLR (2.4{+/-}0.1 vs 3.6{+/-}0.2; P<0.05). In conclusion, human muscle VEGF mRNA expression and capillary density are not significantly increased by 8 weeks of exposure to high altitude and are not increased in Aymara high-altitude natives compared with sea level residents.

Seron, T. J., J. Hill, et al. (2004). "A GPI-linked carbonic anhydrase expressed in the larval mosquito midgut." J. Exp. Biol. **207**(26): 4559-4572.

http://jeb.biologists.org/cgi/content/abstract/207/26/4559

We have previously described the first cloning and partial characterization of carbonic anhydrase from larval Aedes aegypti mosquitoes. Larval mosquitoes utilize an alkaline digestive environment in the lumen of their anterior midgut, and we have also demonstrated a critical link between alkalization of the gut and carbonic anhydrase(s). In this report we further examine the nature of the previously described carbonic anhydrase and test the hypothesis that its pattern of expression is consistent with a role in gut alkalization. Additionally we take advantage of the recently published genome of the mosquito Anopheles gambiae to assess the complexity of the carbonic anhydrase gene family in these insects. We report here that the previously described carbonic anhydrase from Aedes aegypti is similar to mammalian CA IV in that it is a GPI-linked peripheral membrane protein. In situ hybridization analyses identify multiple locations of carbonic anhydrase expression in the larval mosquito. An antibody prepared against a peptide sequence specific to the Aedes aegypti GPI-linked carbonic anhydrase labels plasma membranes of a number of cell types including neuronal cells and muscles. A previously undescribed subset of gut muscles is specifically identified by carbonic anhydrase immunohistochemistry. Bioinformatic analyses using the Ensembl automatic analysis pipeline show that there are at least 14 carbonic anhydrase genes in the Anopheles gambiae genome, including a homologue to the GPI-linked gene product described herein. Therefore, as in mammals which similarly possess numerous carbonic anhydrase genes, insects require a large family of these genes to handle the complex metabolic pathways influenced by carbonic anhydrases and their substrates.

Zhao, Y., H. Sun, et al. (2005). "Lifespan extension and elevated hsp gene expression in Drosophila caused by histone deacetylase inhibitors." J. Exp. Biol. **208**(4): 697-705.

http://jeb.biologists.org/cgi/content/abstract/208/4/697

The heat shock proteins (Hsps) play a positive role in lifespan determination, and histone acetylation has been shown to be involved in transcription of hsp genes in Drosophila. To further determine if hsp22 and hsp70 expression is correlated with lifespan, and if histone acetylation participates in this process, RNA levels for hsp22 and hsp70 were analyzed throughout the lifespan in the long-lived and short-lived iso-female lines. The results showed that hsp22 and hsp70 RNA levels were higher in long-lived line than in short-lived line and that the long-lived flies responded more rapidly to heat but were more tolerant to high temperature. Moreover, we investigated the influences of histone acetylation modification on longevity and on hsp gene expression by using histone deacetylase (HDAC) inhibitors TSA and BuA. The results demonstrated that both inhibitors were able to extend the lifespan and promote hsp22 and hsp70 expression. However, the optimal concentrations of these inhibitors, and probably the mechanisms of their actions, vary with the genetic background. In addition, we showed that HDAC inhibitors caused the hyperacetylation of core histone H3, implicating the involvement of chromatin modulation in hsp gene transcription. These data suggested a close correlation among

histone acetylation, hsp gene expression and longevity in D. melanogaster.

J. Exp. Bot. (4)

Nishikawa, F., M. Kato, et al. (2003). "Ascorbate metabolism in harvested broccoli." J. Exp. Bot. **54**(392): 2439-2448.

http://jxb.oupjournals.org/cgi/content/abstract/54/392/2439

The ascorbate content declined rapidly in broccoli (Brassica oleracea L. var. italica) florets, but not in the stem tissue, during post-harvest senescence. Ascorbate peroxidase (APX), ascorbate oxidase (AO), L-galactono-1,4-lactone dehydrogenase (GLDH), monodehydroascorbate reductase (MDAR), dehydroascorbate reductase (DHAR), and glutathione reductase (GR) were investigated in gene expression after harvest in both florets and the stem tissue of broccoli. Cytosolic gene expressions (BO-APX 1, BO-APX 2, BO-AO, BO-MDAR 2, and BO-GR) were stimulated actively in broccoli florets after harvest. By contrast, it was observed that mRNA levels of chloroplastic APX, BO-sAPX and BO-tbAPX, had decreased by 12 h after harvest in broccoli florets, suggesting that the active oxygen species (AOS) scavenging system in chloroplasts was largely abolished in florets during the early hours of the post-harvest period. In addition, gene expressions in GLDH and other chloroplastic enzymes such as BO-MDAR 1 and BO-DHAR decreased rapidly within 24 h after harvest. Ethylene treatment had no effect on the ascorbate level and the expression of all genes investigated. The expressions of BO-GLDH and chloroplastic genes (BO-sAPX, BO-tbAPX, BO-MDAR 1, and BO-DHAR) mRNA were suppressed by treatment with methyl jasmonate (MJ) and abscisic acid (ABA) and were accompanied by the acceleration of ascorbate degradation. These data suggest that ascorbate metabolism tends to be inactivated in chloroplasts by transcriptional regulation, but not in the cytosol, when ascorbate decreases under stress conditions.

Nishikawa, F., M. Kato, et al. (2005). "Effect of sucrose on ascorbate level and expression of genes involved in the ascorbate biosynthesis and recycling pathway in harvested broccoli florets." J. Exp. Bot. **56**(409): 65-72.

http://jxb.oupjournals.org/cgi/content/abstract/56/409/65

The relationship between sucrose (Suc) and ascorbate (AA) metabolism was investigated in harvested broccoli (Brassica oleracea L. var. italica) florets. Decreases in both Suc and AA content were observed in broccoli florets 48 h after all the leaves were excised, but none were observed when the plants were kept intact or with leaves attached in a room at 20 {degrees}C. In harvested broccoli plants without leaves and roots, continuous absorption of a 10% (w/v) Suc solution from the cut surface of the stem suppressed the degreening of sepals and the loss of AA content in florets. The expression of the genes related to AA metabolism in chloroplasts and its biosynthesis were up-regulated by Suc feeding in broccoli florets. These data suggest that a decline in Suc leads to considerable damage not only to AA biosynthesis but also to the hydrogen peroxide-scavenging system in chloroplasts. In addition, the cessation of the Suc supply from leaves can be the main factor of AA degradation in harvested broccoli florets.

Paolocci, F., T. Bovone, et al. (2005). "Light and an exogenous transcription factor qualitatively and quantitatively affect the biosynthetic pathway of condensed tannins in Lotus corniculatus leaves." J. Exp. Bot. **56**(414): 1093-1103.

http://jxb.oupjournals.org/cgi/content/abstract/56/414/1093

The effects of increasing light and of a heterologous bHLH transcription factor on the accumulation of condensed tannins (CT) were investigated in leaves of Lotus corniculatus, a model legume species which accumulates these secondary metabolites in leaves as well as reproductive tissues. Light and expression of the transgene increased the level of CT in a synergistic way. To monitor how the changes in accumulation of condensed tannins were achieved, the level of expression of four key genes in the flavonoid pathway was estimated by real-time RT-PCR analysis. Early genes of the pathway (PAL and CHS) were affected less in their expression and so appeared to be less involved in influencing the final level of CT than later genes in the pathway (DFR and ANS). Steady-state levels of DFR and ANS transcripts showed a strong positive correlation with CT and these genes might be considered the first rate-limiting steps in CT biosynthesis in Lotus leaves. However, additional factors mediated by light are limiting CT accumulation once these genes are up-regulated by the transgene. Therefore, the increment of the steady-state mRNA level for DFR and ANS might not be sufficient to up-regulate condensed tannins in leaves. The real-time RT-PCR approach adopted showed that members within the CHS and DFR gene families are differentially regulated by the exogenous bHLH gene and light. This finding is discussed in relation to the approaches for controlling CT biosynthesis and for studying the expression profile of multi-gene families.

Yang, X., B. E. Scheffler, et al. (2004). "SOR1, a gene associated with bioherbicide production in sorghum root hairs." J. Exp. Bot. **55**(406): 2251-2259.

http://jxb.oupjournals.org/cgi/content/abstract/55/406/2251

Sorghum [Sorghum bicolor (L.) Moench] roots exude a potent bioherbicide known as sorgoleone, which is produced in living root hairs and is phytotoxic to broadleaf and grass weeds at concentrations as low as 10 {micro}M. Differential gene expression was studied in sorghum (S. bicolorxS. sudanense) cv. SX17 between roots with abundant root hairs and those without root hairs using a modified differential display approach. A differentially expressed gene, named SOR1, was cloned by using Rapid Amplification of the 5' ends of cDNA (5'-RACE). Real-time PCR analysis of multiple tissues of sorghum SX17 revealed that the SOR1 transcript level in root hairs was more than 1000 times higher than that of other tissues evaluated, including immature leaf, mature leaf, mature stem, panicle, and roots with hairs removed. Semi-quantitative RT-PCR revealed that SOR1 was expressed in the sorgoleone-producing roots of sorghum SX17, shattercane [S. bicolor (L.) Moench], and johnsongrass [S. halepense (L.) Pers.], but not in the shoots of sorghum or in the roots of sweet corn (Zea mays L.) Summer Flavor 64Y', in which sorgoleone production was not detected by HPLC analysis. Similarity searches indicated that SOR1 probably encodes a novel desaturase, which might be involved in the formation of a unique and specific double bonding pattern within the long hydrocarbon tail of sorgoleone.

Akatsuka, Y., T. Nishida, et al. (2003). "Identification of a Polymorphic Gene, BCL2A1, Encoding Two Novel Hematopoietic Lineage-specific Minor Histocompatibility Antigens." J. Exp. Med. **197**(11): 1489-1500.

http://www.jem.org/cgi/content/abstract/197/11/1489

We report the identification of two novel minor histocompatibility antigens (mHAgs), encoded by two separate single nucleotide polymorphisms on a single gene, BCL2A1, and restricted by human histocompatibility leukocyte antigen (HLA)-A*2402 (the most common HLA-A allele in Japanese) and B*4403, respectively. Two cytotoxic T lymphocyte (CTL) clones specific for these mHAgs were first isolated from two distinct recipients after hematopoietic cell transplantation. Both clones lyse only normal and malignant cells within the hematopoietic lineage. To localize the gene encoding the mHAgs, two-point linkage analysis was performed on the CTL lytic patterns of restricting HLA-transfected B lymphoblastoid cell lines obtained from Centre d'Etude du Polymorphisme Humain. Both CTL clones showed a completely identical lytic pattern for 4 pedigrees and the gene was localized within a 3.6-cM interval of 15q24.3-25.1 region that encodes at least 46 genes. Of those, only BCL2A1 has been reported to be expressed in hematopoietic cells and possess three nonsynonymous nucleotide changes. Minigene transfection and epitope reconstitution assays with synthetic peptides identified both HLA-A*2402- and B*4403-restricted mHAg epitopes to be encoded by distinct polymorphisms within BCL2A1.

Bosma, G. C., J. Kim, et al. (2002). "DNA-dependent Protein Kinase Activity Is Not Required for Immunoglobulin Class Switching." J. Exp. Med. **196**(11): 1483-1495.

http://www.jem.org/cgi/content/abstract/196/11/1483

Class switch recombination (CSR), similar to V(D)J recombination, is thought to involve DNA double strand breaks and repair by the nonhomologous end-joining pathway. A key component of this pathway is DNA-dependent protein kinase (DNA-PK), consisting of a catalytic subunit (DNA-PKcs) and a DNA-binding heterodimer (Ku70/80). To test whether DNA-PKcs activity is essential for CSR, we examined whether IgM+ B cells from scid mice with site-directed H and L chain transgenes were able to undergo CSR. Although B cells from these mice were shown to lack DNA-PKcs activity, they were able to switch from IgM to IgG or IgA with close to the same efficiency as B cells from control transgenic and nontransgenic scid/+ mice, heterozygous for the scid mutation. We conclude that CSR, unlike V(D)J recombination, can readily occur in the absence of DNA-PKcs activity. We suggest nonhomologous end joining may not be the (primary or only) mechanism used to repair DNA breaks during CSR.

Bottino, C., R. Castriconi, et al. (2003). "Identification of PVR (CD155) and Nectin-2 (CD112) as Cell Surface Ligands for the Human DNAM-1 (CD226) Activating Molecule." J. Exp. Med. **198**(4): 557-567.

http://www.jem.org/cgi/content/abstract/198/4/557

Human natural killer (NK) cells express a series of activating receptors and coreceptors that are involved in recognition and killing of target cells. In this study, in an attempt to identify the cellular ligands for such triggering surface molecules, mice were immunized with NK-susceptible target cells. On the basis of a functional screening, four mAbs were selected that induced a partial down-regulation of the NK-mediated cytotoxicity against the immunizing target cells. As revealed by biochemical analysis, three of such mAbs recognized molecules of [~]70 kD. The other mAb

reacted with two distinct molecules of [~]65 and 60 kD, respectively. Protein purification followed by tryptic digestion and mass spectra analysis, allowed the identification of the 70 kD and the 65/60 kD molecules as PVR (CD155) and Nectin-2 {delta}/{alpha} (CD112), respectively. PVR-Fc and Nectin-2-Fc soluble hybrid molecules brightly stained COS-7 cells transfected with the DNAM-1 (CD226) construct, thus providing direct evidence that both PVR and Nectin-2 represent specific ligands for the DNAM-1 triggering receptor. Finally, the surface expression of PVR or Nectin-2 in cell transfectants resulted in DNAM-1-dependent enhancement of NK-mediated lysis of these target cells. This lysis was inhibited or even virtually abrogated upon mAb-mediated masking of DNAM-1 (on NK cells) or PVR or Nectin-2 ligands (on cell transfectants).

de Stahl, T. D., J. Dahlstrom, et al. (2003). "A Role for Complement in Feedback Enhancement of Antibody Responses by IgG3." J. Exp. Med. **197**(9): 1183-1190.

http://www.jem.org/cgi/content/abstract/197/9/1183

IgG1, IgG2a, and IgG2b, passively administered with soluble Ags, enhance specific Ab responses. The effect of IgG3 in this type of feedback regulation has not been studied previously. We immunized mice with trinitrophenyl (TNP)-coupled carrier proteins (bovine serum albumin [BSA] or ovalbumin [OVA]) alone or complexed to monoclonal TNP-specific IgG3. The carrier-specific Ab responses were enhanced by several hundred-fold by IgG3. Enhancement was significantly impaired in mice depleted of complement factor C3 and in mice lacking complement receptors 1 and 2 (Cr2-/-). In contrast, mice lacking the common Fc-receptor gamma chain (FcR{gamma}-/-), resulting in reduced expression of Fc{gamma}RI and lack of Fc{gamma}RIII, and mice lacking Fc{gamma}RIIB (Fc{gamma}RIIB-/-), responded equally well to immunization with IgG3-complexed Ag as wild-type controls. These findings demonstrate that IgG3 can induce feedback enhancement and that IgG3, in analogy with IgM, uses the complement system for this function.

Gonzalez-Aseguinolaza, G., L. Van Kaer, et al. (2002). "Natural Killer T Cell Ligand {alpha}-Galactosylceramide Enhances Protective Immunity Induced by Malaria Vaccines." J. Exp. Med. **195**(5): 617-624.

http://www.jem.org/cgi/content/abstract/195/5/617

The important role played by CD8+ T lymphocytes in the control of parasitic and viral infections, as well as tumor development, has raised the need for the development of adjuvants capable of enhancing cell-mediated immunity. It is well established that protective immunity against liver stages of malaria parasites is primarily mediated by CD8+ T cells in mice. Activation of natural killer T (NKT) cells by the glycolipid ligand, {alpha}-galactosylceramide ({alpha}-GalCer), causes bystander activation of NK, B, CD4+, and CD8+ T cells. Our study shows that coadministration of {alpha}-GalCer with suboptimal doses of irradiated sporozoites or recombinant viruses expressing a malaria antigen greatly enhances the level of protective anti-malaria immunity in mice. We also show that coadministration of {alpha}-GalCer with various different immunogens strongly enhances antigen-specific CD8+ T cell responses, and to a lesser degree, Th1-type responses. The adjuvant effects of {alpha}-GalCer require CD1d molecules, V{alpha}14 NKT cells, and interferon {gamma}. As {alpha}-GalCer stimulates both human and murine NKT cells, these findings should contribute to the design of more effective vaccines against malaria and other intracellular pathogens, as well as tumors.

Goodyear, C. S. and G. J. Silverman (2003). "Death by a B Cell Superantigen: In Vivo VH-targeted Apoptotic Supraclonal B Cell Deletion by a Staphylococcal Toxin." J. Exp. Med. **197**(9): 1125-1139.

http://www.jem.org/cgi/content/abstract/197/9/1125

Amongst the many ploys used by microbial pathogens to interfere with host immune responses is the production of proteins with the properties of superantigens. These properties enable superantigens to interact with conserved variable region framework subdomains of the antigen receptors of lymphocytes rather than the complementarity determining region involved in the binding of conventional antigens. To understand how a B cell superantigen affects the host immune system, we infused protein A of Staphylococcus aureus (SpA) and followed the fate of peripheral B cells expressing B cell receptors (BCRs) with VH regions capable of binding SpA. Within hours, a sequence of events was initiated in SpA-binding splenic B cells, with rapid down-regulation of BCRs and coreceptors, CD19 and CD21, the induction of an activation phenotype, and limited rounds of proliferation. Apoptosis followed through a process heralded by the dissipation of mitochondrial membrane potential, the induction of the caspase pathway, and DNA fragmentation. After exposure, B cell apoptotic bodies were deposited in the spleen, lymph nodes, and Peyer's patches. Although in vivo apoptosis did not require the Fas death receptor, B cells were protected by interleukin (IL)-4 or CD40L, or overexpression of Bcl-2. These studies define a pathway for BCR-mediated programmed cell death that is VH region targeted by a superantigen.

Guy-Grand, D., O. Azogui, et al. (2003). "Extrathymic T Cell Lymphopoiesis: Ontogeny and Contribution to Gut Intraepithelial Lymphocytes in Athymic and Euthymic Mice." J. Exp. Med. **197**(3): 333-341.

http://www.jem.org/cgi/content/abstract/197/3/333

In the absence of thymopoiesis, T lymphocytes are nevertheless present, mainly in the gut epithelium. Ontogeny of the extrathymic pathway and the extent of its involvement in euthymic mice are controversial. These questions have been addressed by assessing the expression of recombinase activating gene (RAG) through the use of green fluorescent protein RAG2 transgenic mouse models. In athymic mice, T lymphopoiesis occurs mainly in the mesenteric lymph node and less in the Peyer's patches. Ontogenic steps of this lymphopoiesis resemble those of thymopoiesis, but with an apparent bias toward {gamma}{delta} T cell production and with a paucity of oligoclonal {alpha}{beta} T cells possibly resulting from a deficit in positive selection. Whether in athymic or euthymic mice, neither T intraepithelial lymphocytes (IEL) nor cryptopatch cells (reported to contain precursors of IEL) displayed fluorescence indicating recent RAG protein synthesis. Newly made T cells migrate from the mesenteric node into the thoracic duct lymph to reach the gut mucosa. In euthymic mice, this extrathymic pathway is totally repressed, except in conditions of severe lymphocytic depletion. Thus, in normal animals, all gut T IEL, including CD8{alpha}{alpha}+ cells, are of thymic origin, CD8{alpha}{alpha}+ TCR{alpha}{beta}+ IEL being the likely progeny of double negative NK1-1- thymocytes, which show polyclonal V{alpha} and V{beta} repertoires.

Hawn, T. R., A. Verbon, et al. (2003). "A Common Dominant TLR5 Stop Codon Polymorphism Abolishes Flagellin Signaling and Is Associated with Susceptibility to Legionnaires' Disease." J. Exp. Med. **198**(10): 1563-1572.

http://www.jem.org/cgi/content/abstract/198/10/1563

Although Toll-like receptors (TLRs) are critical mediators of the immune response to pathogens, the influence of polymorphisms in this gene family on human susceptibility to infection is poorly understood. We demonstrated recently that TLR5 recognizes flagellin, a potent inflammatory stimulus present in the flagellar structure of many bacteria. Here, we show that a common stop codon polymorphism in the ligand-binding domain of TLR5 (TLR5392STOP) is unable to mediate flagellin signaling, acts in a dominant fashion, and is associated with susceptibility to pneumonia caused by Legionella pneumophila, a flagellated bacterium. We also show that flagellin is a principal stimulant of proinflammatory cytokine production in lung epithelial cells. Together, these observations suggest that TLR5392STOP increases human susceptibility to infection through an unusual dominant mechanism that compromises TLR5's essential role as a regulator of the lung epithelial innate immune response.

Jung, Y.-J., R. LaCourse, et al. (2002). "Virulent but not Avirulent Mycobacterium tuberculosis Can Evade the Growth Inhibitory Action of a T Helper 1-dependent, Nitric Oxide Synthase 2-independent Defense in Mice." J. Exp. Med. **196**(7): 991-998.

http://www.jem.org/cgi/content/abstract/196/7/991

Control of infection with virulent Mycobacterium tuberculosis (Mtb) in mice is dependent on the generation of T helper (Th)1-mediated immunity that serves, via secretion of interferon (IFN)-{gamma} and other cytokines, to upregulate the antimycobacterial function of macrophages of which the synthesis of inducible nitric oxide synthase (NOS)2 is an essential event. As a means to understanding the basis of Mtb virulence, the ability of gene-deleted mice incapable of making NOS2 (NOS2-/-), gp91Phox subunit of the respiratory burst NADPH-oxidase complex (Phox-/-), or either enzyme (NOS2/Phox-/-), to control airborne infection with the avirulent R1Rv and H37Ra strains of Mtb was compared with their ability control infection with the virulent H37Rv strain. NOS2-/-, Phox-/-, and NOS2/Phox-/- mice showed no deficiency in ability to control infection with either strain of avirulent Mtb. By contrast, NOS2-/- mice, but not Phox-/- mice, were incapable of controlling H37Rv infection and died early from neutrophil-dominated lung pathology. Control of infection with avirulent, as well as virulent Mtb, depended on the synthesis of IFN-{gamma}, and was associated with a substantial increase in the synthesis in the lungs of mRNA for IFN-{gamma} and NOS2, and with production of NOS2 by macrophages at sites of infection. The results indicate that virulent, but not avirulent, Mtb can overcome the growth inhibitory action of a Th1-dependent, NOS2-independent mechanism of defense.

Khan, I. A., M. Moretto, et al. (2002). "Treatment with Soluble Interleukin-15R{alpha} Exacerbates Intracellular Parasitic Infection by Blocking the Development of Memory CD8+ T Cell Response." J. Exp. Med. **195**(11): 1463-1470.

http://www.jem.org/cgi/content/abstract/195/11/1463

Interferon (IFN)-{gamma}-producing CD8+ T cells are important for the successful resolution of the obligate intracellular parasite Toxoplasma gondii by preventing the reactivation or controlling a repeat infection. Previous reports from our laboratory have shown that exogenous interleukin (IL)-15 treatment augments the CD8+ T cell response against the parasite. However, the role of endogenous IL-15 in the proliferation of activated/memory CD8+ T cells during toxoplasma or any other infection is unknown. In this study, we treated T. gondii immune mice with soluble IL-15 receptor {alpha} (sIL-15R{alpha}) to block the host endogenous IL-15. The treatment markedly reduced the ability of the immune animals to control a lethal infection. CD8+ T cell activities in the sIL-15R{alpha}-administered mice were severely reduced as determined by IFN-{gamma} release and target cell lysis assays. The loss of CD8+ T cell immunity due to sIL-15R{alpha} treatment was further demonstrated by adoptive transfer experiments. Naive recipients transferred with

CD44hi activated/memory CD8+ T cells and treated with sIL-15R{alpha} failed to resist a lethal T. gondii infection. Moreover, sIL-15R{alpha} treatment of the recipients blocked the ability of donor CD44hi activated/memory CD8+ T cells to replicate in response to T. gondii challenge. To our knowledge, this is the first demonstration of the important role of host IL-15 in the development of antigen-specific memory CD8+ T cells against an intracellular infection.

McDonald, J. P., E. G. Frank, et al. (2003). "129-derived Strains of Mice Are Deficient in DNA Polymerase {iota} and Have Normal Immunoglobulin Hypermutation." J. Exp. Med. **198**(4): 635-643.

http://www.jem.org/cgi/content/abstract/198/4/635

Recent studies suggest that DNA polymerase {eta} (pol{eta}) and DNA polymerase {iota} (pol{iota}) are involved in somatic hypermutation of immunoglobulin variable genes. To test the role of pol{iota} in generating mutations in an animal model, we first characterized the biochemical properties of murine pol{iota}. Like its human counterpart, murine pol{iota} is extremely error-prone when catalyzing synthesis on a variety of DNA templates in vitro. Interestingly, when filling in a 1 base-pair gap, DNA synthesis and subsequent strand displacement was greatest in the presence of both pols {iota} and {eta}. Genomic sequence analysis of Poli led to the serendipitous discovery that 129-derived strains of mice have a nonsense codon mutation in exon 2 that abrogates production of pol{iota}. Analysis of hypermutation in variable genes from 129/SvJ (Poli-/-) and C57BL/6J (Poli+/+) mice revealed that the overall frequency and spectrum of mutation were normal in pol{iota}-deficient mice. Thus, either pol{iota} does not participate in hypermutation, or its role is nonessential and can be readily assumed by another low-fidelity polymerase.

Migliaccio, A. R., R. A. Rana, et al. (2003). "GATA-1 as a Regulator of Mast Cell Differentiation Revealed by the Phenotype of the GATA-1low Mouse Mutant." J. Exp. Med. **197**(3): 281-296.

http://www.jem.org/cgi/content/abstract/197/3/281

Here it is shown that the phenotype of adult mice lacking the first enhancer (DNA hypersensitive site I) and the distal promoter of the GATA-1 gene (neo{Delta}HS or GATA-1low mutants) reveals defects in mast cell development. These include the presence of morphologically abnormal alcian blue+ mast cells and apoptotic metachromatic- mast cell precursors in connective tissues and peritoneal lavage and numerous (60-70% of all the progenitors) "unique" trilineage cells committed to erythroid, megakaryocytic, and mast pathways in the bone marrow and spleen. These abnormalities, which were mirrored by impaired mast differentiation in vitro, were reversed by retroviral-mediated expression of GATA-1 cDNA. These data indicate an essential role for GATA-1 in mast cell differentiation.

Ochsenbein, A. F., S. R. Riddell, et al. (2004). "CD27 Expression Promotes Long-Term Survival of Functional Effector-Memory CD8+ Cytotoxic T Lymphocytes in HIV-infected Patients." J. Exp. Med. **200**(11): 1407-1417.

http://www.jem.org/cgi/content/abstract/200/11/1407

Human immunodeficiency virus (HIV)-specific CD8+ T cells persist in high frequencies in HIV-infected patients despite impaired CD4+ T helper response to the virus, but, unlike other differentiated effector cytotoxic T lymphocytes, most continue to express the tumor necrosis

factor receptor family member CD27. Because the ligand for CD27 (CD70) is also overexpressed in HIV-infected hosts, we examined the nature of expression and potential functional consequences of CD27 expression on HIV-specific CD8+ T cells. Analysis of CD27+ and CD27- T cells derived from the same HIV-specific clone revealed that retention of CD27 did not interfere with acquisition of effector functions, and that after T cell receptor stimulation, CD27+ cells that concurrently were triggered via CD27 exhibited more resistance to apoptosis, interleukin 2 production, and proliferation than CD27- T cells. After transfer back into an HIV-infected patient, autologous HIV-specific CD27- T cells rapidly disappeared, but CD27+ T cells derived from the same clone persisted at high frequency. Our findings suggest that the CD27-CD70 interaction in HIV infection may provide CD27+ CD8+ T cells with a survival advantage and compensate for limiting or absent CD4+ T help to maintain the CD8 response.

Oh-hora, M., S. Johmura, et al. (2003). "Requirement for Ras Guanine Nucleotide Releasing Protein 3 in Coupling Phospholipase C-{gamma}2 to Ras in B Cell Receptor Signaling." J. Exp. Med. **198**(12): 1841-1851.

http://www.jem.org/cgi/content/abstract/198/12/1841

Two important Ras guanine nucleotide exchange factors, Son of sevenless (Sos) and Ras guanine nucleotide releasing protein (RasGRP), have been implicated in controlling Ras activation when cell surface receptors are stimulated. To address the specificity or redundancy of these exchange factors, we have generated Sos1/Sos2 double- or RasGRP3-deficient B cell lines and determined their ability to mediate Ras activation upon B cell receptor (BCR) stimulation. The BCR requires RasGRP3; in contrast, epidermal growth factor receptor is dependent on Sos1 and Sos2. Furthermore, we show that BCR-induced recruitment of RasGRP3 to the membrane and the subsequent Ras activation are significantly attenuated in phospholipase C-{gamma}2-deficient B cells. This defective Ras activation is suppressed by the expression of RasGRP3 as a membrane-attached form, suggesting that phospholipase C-{gamma}2 regulates RasGRP3 localization and thereby Ras activation.

Stehlik, C., L. Fiorentino, et al. (2002). "The PAAD/PYRIN-Family Protein ASC Is a Dual Regulator of a Conserved Step in Nuclear Factor {kappa}B Activation Pathways." J. Exp. Med. **196**(12): 1605-1615.

http://www.jem.org/cgi/content/abstract/196/12/1605

Apoptosis-associated speck-like protein containing a Caspase recruitment domain (ASC) belongs to a large family of proteins that contain a Pyrin, AIM, ASC, and death domain-like (PAAD) domain (also known as PYRIN, DAPIN, Pyk). Recent data have suggested that ASC functions as an adaptor protein linking various PAAD-family proteins to pathways involved in nuclear factor (NF)-{kappa}B and pro-Caspase-1 activation. We present evidence here that the role of ASC in modulating NF-{kappa}B activation pathways is much broader than previously suspected, as it can either inhibit or activate NF-{kappa}B, depending on cellular context. While coexpression of ASC with certain PAAD-family proteins such as Pyrin and Cryopyrin increases NF-{kappa}B activity, ASC has an inhibitory influence on NF-{kappa}B activation by various proinflammatory stimuli, including tumor necrosis factor (TNF){alpha}, interleukin 1{beta}, and lipopolysaccharide (LPS). Elevations in ASC protein levels or of the PAAD domain of ASC suppressed activation of I{kappa}B kinases in cells exposed to pro-inflammatory stimuli. Conversely, reducing endogenous levels of ASC using siRNA enhanced TNF- and LPS-induced degradation of the IKK substrate, I{kappa}B{alpha}. Our findings suggest that ASC modulates diverse NF-{kappa}B induction pathways by acting upon the IKK complex, implying a broad role for this and similar proteins containing PAAD domains in regulation of inflammatory responses.

Sugita, S. and J. W. Streilein (2003). "Iris Pigment Epithelium Expressing CD86 (B7-2) Directly Suppresses T Cell Activation In Vitro via Binding to Cytotoxic T Lymphocyte-associated Antigen 4." J. Exp. Med. **198**(1): 161-171.

http://www.jem.org/cgi/content/abstract/198/1/161

A monolayer of pigment epithelium (PE) lines the iris PE (IPE), ciliary body PE, and retina PE of the inner eye, an immune-privileged site. These neural crest-derived epithelial cells participate in ocular immune privilege through poorly defined molecular mechanisms. Murine PE cells cultured from different ocular tissues suppress T cell activation by differing mechanisms. In particular, IPE cells suppress primarily via direct cell to cell contact. By examining surface expression of numerous candidate molecules (tumor necrosis factor receptor [TNFR]1, TNFR2, CD36, CD40, CD47, CD80, CD86, PD-L1, CD95 ligand, and type I interferon receptor), we report that IPE cells uniquely express on their surface the costimulatory molecule CD86. When IPE were blocked with anti-CD86 or were derived from CD80/CD86 (but not CD80) knockout (KO) mice, the cells displayed reduced capacity to suppress T cell activation. IPE also failed to suppress activation of T cells in the presence of cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) immunoglobulin or if the T cells were obtained from CTLA-4 (but not CD28) KO mice. We conclude that iris pigment epithelial cells constitutively express cell surface CD86, which enables the cells to contact inhibit T cells via direct interaction with CTLA-4. Thus, ocular immune privilege is achieved in part by subversion of molecules that are usually used for conventional immune costimulation.

Yu, P., Y. Lee, et al. (2005). "Intratumor depletion of CD4+ cells unmasks tumor immunogenicity leading to the rejection of late-stage tumors." J. Exp. Med. **201**(5): 779-791.

http://www.jem.org/cgi/content/abstract/201/5/779

Tumor environment can be critical for preventing the immunological destruction of antigenic tumors. We have observed a selective accumulation of CD4+CD25+ T cells inside tumors. In a murine fibrosarcoma Ld-expressing Ag104, these cells made up the majority of tumor-infiltrating lymphocytes at the late stage of tumor progression, and their depletion during the effector phase, rather than priming phase, successfully enhanced antitumor immunity. We show here that CD4+CD25+ T cells suppressed the proliferation and interferon-{gamma} production of CD8+ T cells in vivo at the local tumor site. Blockade of the effects of IL-10 and TGF-{beta} partially reversed the suppression imposed by the CD4+ cells. Furthermore, local depletion of CD4+ cells inside the tumor resulted in a change of cytokine milieu and led to the eradication of well-established highly aggressive tumors and the development of long-term antitumor memory. Therefore, CD4+CD25+ T cells maintained an environment in the tumor that concealed the immunogenicity of tumor cells to permit progressive growth of antigenic tumors. Our study illustrates that the suppression of antitumor immunity by regulatory T cells occurs predominantly at the tumor site, and that local reversal of suppression, even at a late stage of tumor development, can be an effective treatment for well-established cancers.

Zippelius, A., M. J. Pittet, et al. (2002). "Thymic Selection Generates a Large T Cell Pool Recognizing a Self-Peptide in Humans." J. Exp. Med. **195**(4): 485-494.

http://www.jem.org/cgi/content/abstract/195/4/485

The low frequency of self-peptide-specific T cells in the human preimmune repertoire has so far precluded their direct evaluation. Here, we report an unexpected high frequency of T cells specific for the self-antigen Melan-A/MART-1 in CD8 single-positive thymocytes from human histocompatibility leukocyte antigen-A2 healthy individuals, which is maintained in the peripheral blood of newborns and adults. Postthymic replicative history of Melan-A/MART-1-specific CD8 T cells was independently assessed by quantifying T cell receptor excision circles and telomere length ex vivo. We provide direct evidence that the large T cell pool specific for the self-antigen Melan-A/MART-1 is mostly generated by thymic output of a high number of precursors. This represents the only known naive self-peptide-specific T cell repertoire directly accessible in humans.

J. Gen. Virol. (28)

Chen, X., W. J. Zhang, et al. (2002). "Comparative analysis of the complete genome sequences of Helicoverpa zea and Helicoverpa armigera single-nucleocapsid nucleopolyhedroviruses." J. Gen. Virol. **83**(3): 673-684.

http://vir.sgmjournals.org/cgi/content/abstract/83/3/673

The complete nucleotide sequence of Helicoverpa zea single-nucleocapsid nucleopolyhedrovirus (HzSNPV) has been determined (130869 bp) and compared to the nucleotide sequence of Helicoverpa armigera (Ha) SNPV. These two genomes are very similar in their nucleotide (97% identity) and amino acid (99% identity) sequences. The coding regions are much more conserved than the non-coding regions. In HzSNPV/HaSNPV, the 63 open reading frames (ORFs) present in all baculoviruses sequenced so far are much more conserved than other ORFs. HzSNPV has four additional small ORFs compared with HaSNPV, one of these (Hz42) being in a correct transcriptional context. The major differences between HzSNPV and HaSNPV are found in the sequence and organization of the homologous regions (hrs) and the baculovirus repeat ORFs (bro genes). The sequence identity between the HzSNPV and HaSNPV hrs ranges from 90% (hr1) to almost 100% (hr5) and the hrs differ in the presence/absence of one or more type A and/or B repeats. The three HzSNPV bro genes differ significantly from those in HaSNPV and may have been acquired independently in the ancestral past. The sequence data suggest strongly that HzSNPV and HaSNPV are variants of the same virus species, a conclusion that is supported by the physical and biological data.

Chisaka, H., E. Morita, et al. (2002). "A transgenic mouse model for non-immune hydrops fetalis induced by the NS1 gene of human parvovirus B19." J. Gen. Virol. **83**(2): 273-281.

http://vir.sgmjournals.org/cgi/content/abstract/83/2/273

Human parvovirus B19 (B19) infection during pregnancy is associated with the adverse foetal outcome known as non-immune hydrops fetalis (NIHF). Although B19 is known to infect erythroid-lineage cells in vivo as well as in vitro, the mechanism leading to the occurrence of NIHF is not clear. To investigate the possible involvement of the B19 non-structural protein NS1 in NIHF, three independent lines of transgenic mice were generated that expressed NS1 under the control of the Cre-loxP system and the GATA1 promoter. Two of the three lines expressed NS1 in erythroid-lineage cells. Most of the transgenic mice died at the embryonic stage, some of which

developed hydropic changes caused by severe anaemia at embryonic day 15{middle dot}5 (E15{middle dot}5). Histological examination of embryos at E15{middle dot}5 showed significantly fewer erythropoietic islands in the liver parenchyma, whereas their hearts showed no abnormal signs, such as cardiomegaly and apoptotic cells. The NS1-transgenic mouse lines established here provide an animal model for human NIHF and suggest that NS1 plays a crucial role in the adverse outcome associated with intrauterine B19 infection in humans.

Gubser, C. and G. L. Smith (2002). "The sequence of camelpox virus shows it is most closely related to variola virus, the cause of smallpox." J. Gen. Virol. **83**(4): 855-872.

http://vir.sgmjournals.org/cgi/content/abstract/83/4/855

Camelpox virus (CMPV) and variola virus (VAR) are orthopoxviruses (OPVs) that share several biological features and cause high mortality and morbidity in their single host species. The sequence of a virulent CMPV strain was determined; it is 202182 bp long, with inverted terminal repeats (ITRs) of 6045 bp and has 206 predicted open reading frames (ORFs). As for other poxviruses, the genes are tightly packed with little non-coding sequence. Most genes within 25 kb of each terminus are transcribed outwards towards the terminus, whereas genes within the centre of the genome are transcribed from either DNA strand. The central region of the genome contains genes that are highly conserved in other OPVs and 87 of these are conserved in all sequenced chordopoxviruses. In contrast, genes towards either terminus are more variable and encode proteins involved in host range, virulence or immunomodulation. In some cases, these are broken versions of genes found in other OPVs. The relationship of CMPV to other OPVs was analysed by comparisons of DNA and predicted protein sequences, repeats within the ITRs and arrangement of ORFs within the terminal regions. Each comparison gave the same conclusion: CMPV is the closest known virus to variola virus, the cause of smallpox.

Hacker, C. V., C. M. Brasier, et al. (2005). "A double-stranded RNA from a Phytophthora species is related to the plant endornaviruses and contains a putative UDP glycosyltransferase gene." J. Gen. Virol. **86**(5): 1561-1570.

http://vir.sgmjournals.org/cgi/content/abstract/86/5/1561

A new dsRNA was isolated from a Phytophthora isolate from Douglas fir. Sequence analysis showed the dsRNA to consist of 13 883 bp and to contain a single open reading frame with the potential to encode a polyprotein of 4548 aa. This polyprotein contained amino acid sequence motifs characteristic of virus RNA-dependent RNA polymerases (RdRps) in its C-terminal region and motifs characteristic of RNA helicases in its N-terminal region. These sequence motifs were related to corresponding motifs in plant viruses in the genus Endornavirus. In phylogenetic trees constructed from the RdRp and helicase motifs of a range of ssRNA and dsRNA viruses, the Phytophthora RdRp and helicase sequences clustered with those of the plant endornaviruses with good bootstrap support. The properties of the Phytophthora dsRNA are consistent with its being classified as the first non-plant member of the genus Endornavirus, for which we propose the name phytophthora endornavirus 1 (PEV1). A region between the RdRp and helicase domains of the PEV1 protein had significant amino acid sequence similarity to UDP glycosyltransferases (UGTs). Two sequence motifs were identified, one characteristic of all UGTs and the other characteristic of sterol UGTs. The PEV1 UGT would be the first for an RNA virus, although ecdysteroid UGT genes have been found in many baculoviruses. The PEV1 UGT was only distantly related to baculovirus ecdysteroid UGTs, which belong to a family distinct from the sterol UGTs.

Hatchette, T. F., D. Walker, et al. (2004). "Influenza A viruses in feral Canadian ducks: extensive reassortment in nature." J. Gen. Virol. **85**(8): 2327-2337.

http://vir.sgmjournals.org/cgi/content/abstract/85/8/2327

The current dogma of influenza accepts that feral aquatic birds are the reservoir for influenza A viruses. Although the genomic information of human influenza A viruses is increasing, little of this type of data is available for viruses circulating in feral waterfowl. This study presents the genetic characterization of 35 viruses isolated from wild Canadian ducks from 1983 to 2000, as the first attempt at a comprehensive genotypic analysis of influenza viruses isolated from feral ducks. This study demonstrates that influenza virus genes circulating in Canadian ducks have achieved evolutionary stasis. The majority of these duck virus genes are clustered in distinct North American clades; however, some H6 and H9 genes are clustered with those from Eurasian viruses. Genes appeared to reassort in a random fashion. None of the genotypes identified remained present throughout all of the years examined and most PA and PB2 genes that crossed over into swine were clustered in one phylogenetic grouping. Additionally, matrix genes were identified that branch very early in the evolutionary tree. These findings demonstrate the diversity of the influenza virus gene pool in Canadian ducks, and suggest that genes which cluster in specific phylogenetic groupings in the PB2 and PA genes can be used for markers of viruses with the potential for crossing the species barrier. A more comprehensive study of this important reservoir is needed to provide further insight into the genomic composition of viruses that crossover the species barrier, which would be a useful component to pandemic planning.

Huy, T. T.-T., H. Ushijima, et al. (2004). "Genotype C of hepatitis B virus can be classified into at least two subgroups." J. Gen. Virol. **85**(2): 283-292.

http://vir.sgmjournals.org/cgi/content/abstract/85/2/283

A genomic characterization of hepatitis B virus (HBV) was done for 56 pre-S1/pre-S2 genes and 10 full-length HBV genotype C isolates from five Asian countries. Phylogenetic analysis of the pre-S1/pre-S2 genes revealed two major groups within genotype C: one for isolates from southeast Asia including Vietnam, Myanmar and Thailand (named HBV/C1) and the other for isolates from Far East Asia including Japan, Korea and China (named HBV/C2). This finding was confirmed by phylogenetic analysis based on the full-length sequence of 32 HBV genotype C isolates, including 22 from database entries. Two isolates from Okinawa, the island off the southern end of Japan, formed a different branch. Specific amino acid sequence changes were identified in the large S protein (amino acids 51, 54, 60, 62 and 73) and P protein (amino acids 231, 233, 236, 248, 252 and 304). Our results indicate that genotype C of HBV can be classified into at least two subgroups.

Imlach, W., C. A. McCaughan, et al. (2002). "Orf virus-encoded interleukin-10 stimulates the proliferation of murine mast cells and inhibits cytokine synthesis in murine peritoneal macrophages." J. Gen. Virol. **83**(5): 1049-1058.

http://vir.sgmjournals.org/cgi/content/abstract/83/5/1049

Orf virus (ORFV) is the type species of the parapoxvirus genus and produces cutaneous pustular lesions in sheep, goats and humans. The genome encodes a polypeptide with remarkable homology to interleukin-10 (IL-10), particularly ovine IL-10, and also to IL-10-like proteins

encoded by Epstein-Barr virus (EBV) and equine herpesvirus. IL-10 is a pleiotropic cytokine that can exert either immunostimulatory or immunosuppressive effects on many cell types. We have expressed and purified C-terminal FLAG and His6-tagged versions of ORFV-IL-10 and shown that ORFV-IL-10 costimulates murine mast cells (MC/9) and inhibits tumour necrosis factor-{alpha} synthesis in activated mouse peritoneal macrophages. Our results demonstrate that although ORFV-IL-10 is structurally similar to EBV-IL-10 it has evolved a different spectrum of activities. EBV-IL-10 does not stimulate the proliferation of thymocytes or mast cells whereas ORFV-IL-10 has both of these activities. Recent studies show that the critical difference in molecular structure of human IL-10 and EBV-IL-10, which may be the basis of their functional differences, is linked to a single amino acid substitution. Consistent with the activity spectrum reported here for ORFV-IL-10, the viral gene encodes the critical amino acid seen in human IL-10. Although the ORFV-IL-10 gene has clearly undergone significant evolutionary change at the nucleotide level compared with ovine IL-10, it has largely retained the polypeptide structure and functional characteristics of its ovine counterpart, suggesting that mutations of the gene to a potentially more potent immunosuppressive form may compromise the co-existence of host and virus.

Kaverin, N. V., I. A. Rudneva, et al. (2002). "Structure of antigenic sites on the haemagglutinin molecule of H5 avian influenza virus and phenotypic variation of escape mutants." J. Gen. Virol. **83**(10): 2497-2505.

http://vir.sgmjournals.org/cgi/content/abstract/83/10/2497

To elucidate the structure of the antigenic sites of avian H5 influenza virus haemagglutinin (HA) we analysed escape mutants of a mouse-adapted variant of the H5N2 strain A/Mallard/Pennsylvania/10218/84. A panel of five anti-H5 monoclonal antibodies (mAbs) was used to select 16 escape mutants. The mutants were tested by ELISA and haemagglutination inhibition with this panel of anti-H5 mAbs and the HA genes of the mutants were sequenced. The sequencing demonstrated that the amino acid changes were grouped in two antigenic sites. One corresponded to site A in the H3 HA. The other contained areas that are separated in the amino acid sequence but are topographically close in the three-dimensional structure and partially overlap in the reactions with mAbs. This site corresponds in part to site B in the H3 structure; it also includes a region not involved in site B that partially overlaps site Sa in the H1 HA and an antigenic area in H2 HA. Mutants with the amino acid change K152N, as well as those with the change D126N, showed reduced lethality in mice. The substitution D126N, creating a new glycosylation site, was accompanied by an increase in the sensitivity of the mutants to normal mouse serum inhibitors. Several amino acid changes in the H5 escape mutants occurred at the positions of reported changes in H2 drift variants. This coincidence suggests that the antigenic sites described and analysed here may be important for drift variation if H5 influenza virus ever appears as a pathogen circulating in humans.

Kuntz-Simon, G., G. Le Gall-Recule, et al. (2002). "Muscovy duck reovirus {sigma}C protein is atypically encoded by the smallest genome segment." J. Gen. Virol. **83**(5): 1189-1200.

http://vir.sgmjournals.org/cgi/content/abstract/83/5/1189

Although muscovy duck reovirus (DRV) shares properties with the reovirus isolated from chicken, commonly named avian reovirus (ARV), the two virus species are antigenically different. Similar to the DRV {sigma}B-encoded gene (1201 bp long) previously identified, the three other double-stranded RNA small genome segments of DRV have been cloned and sequenced. They were 1325, 1191 and 1124 bp long, respectively, and contained conserved terminal sequences common to ARVs. They coded for single expression products, except the smallest (S4), which

contained two overlapping open reading frames (ORF1 and ORF2). BLAST analyses revealed that the proteins encoded by the 1325 and 1191 bp genes shared high identity levels with ARV {sigma}A and {sigma}NS, respectively, and to a lesser extent with other orthoreovirus counterparts. No homology was found for the S4 ORF1-encoded p10 protein. The 29{middle dot}4 kDa product encoded by S4 ORF2 appeared to be 25% identical to ARV S1 ORF3-encoded {sigma}C, a cell-attachment oligomer inducing type-specific neutralizing antibodies. Introduction of large gaps in the N-terminal part of the DRV protein was necessary to improve DRV and ARV {sigma}C amino acid sequence alignments. However, a leucine zipper motif was conserved and secondary structure analyses predicted a three-stranded {alpha}-helical coiled-coil feature at this amino portion. Thus, despite extensive sequence divergence, DRV {sigma}C was suggested to be structurally and probably functionally related to ARV {sigma}C. This work provides evidence for the diversity of the polycistronic S class genes of reoviruses isolated from birds and raises the question of the relative classification of DRV in the Orthoreovirus genus.

Lipatov, A. S., S. Andreansky, et al. (2005). "Pathogenesis of Hong Kong H5N1 influenza virus NS gene reassortants in mice: the role of cytokines and B- and T-cell responses." J. Gen. Virol. **86**(4): 1121-1130.

http://vir.sgmjournals.org/cgi/content/abstract/86/4/1121

The severity of disease caused in humans by H5N1 influenza viruses remains unexplained. The NS gene of Hong Kong H5N1/97 viruses was shown to contribute to high pathogenicity of reassortants in a pig model. However, the molecular pathogenesis and host immune response underlying this phenomenon remain unclear. Here, in a mouse model, H1N1 A/Puerto Rico/8/34 (PR/8) reassortants that contained the H5N1/97 NS gene, the H5N1/01 NS gene, or an altered H5N1/97 NS gene encoding a Glu92[-&gt]Asp substitution in NS1 was studied. The pathogenicity of reassortant viruses, the induction of cytokines and chemokine CXCL1 (KC) in the lungs and specific B- and T-cell responses was characterized. In mice infected with reassortant virus containing the H5N1/97 NS gene, the mouse lethal dose (50 %) and lung virus titres were similar to those of PR/8, which is highly pathogenic to mice. This reassortant virus required two more days than PR/8 to be cleared from the lungs of infected mice. Reassortants containing the altered H5N1/97 NS gene or the H5N1/01 NS gene demonstrated attenuated pathogenicity and lower lung titres in mice. Specific B- and T-cell responses were consistent with viral pathogenicity and did not explain the delayed clearance of the H5N1/97 NS reassortant. The reassortant induced elevated pulmonary concentrations of the inflammatory cytokines IL1{alpha}, IL1{beta}, IL6, IFN-{gamma} and chemokine KC, and decreased concentrations of the anti-inflammatory cytokine IL10. This cytokine imbalance is reminiscent of the clinical findings in two humans who died of H5N1/97 infection and may explain the unusual severity of the disease.

Love, A., V. Molnegren, et al. (2004). "Evolution of hepatitis C virus variants following blood transfusion from one infected donor to several recipients: a long-term follow-up." J. Gen. Virol. **85**(2): 441-450.

http://vir.sgmjournals.org/cgi/content/abstract/85/2/441

Variants of hepatitis C virus (HCV) from a single infected blood donor and 13 viraemic recipients who were traced were examined by sequencing and cloning to determine the extent of virus diversity in hypervariable region 1. Serum-derived viral isolates were studied from the donor when his HCV infection was discovered in 1993, in his recipients that year (0{middle dot}3-5 years post-transfusion) and 5 years later in the donor and six viraemic recipients who were still alive. Viral variants of broad diversity were readily demonstrated in the baseline samples of the donor (nucleotide p-distance 0{middle dot}130), but significantly less (P<0{middle dot}00003) diversity

was observed in the recipients' first samples (p-distances within recipients 0{middle dot}003-0{middle dot}062). In the first blood samples of the recipients, many of the viral variants identified were closely related to a strain variant from the donor. In follow-up samples drawn 5 years later from the donor and six recipients, the p-distance among donor clones had increased (0{middle dot}172, P<0{middle dot}0005) compared with the recipients, who displayed significantly narrower quasispecies (0{middle dot}011-0{middle dot}086). A common finding was that recipients of blood components processed from the same donation differed substantially in persisting HCV infectious sequence. Markedly few changes leading to changes of amino acids had occurred during follow-up in four of six recipients. These results question the significance of the development of viral variants as a necessary phenomenon in the evolution of HCV and pathogenesis of the disease.

May, J. S., H. M. Coleman, et al. (2004). "Forced lytic replication impairs host colonization by a latency-deficient mutant of murine gammaherpesvirus-68." J. Gen. Virol. **85**(1): 137-146.

http://vir.sgmjournals.org/cgi/content/abstract/85/1/137

A regulated switch between latent and lytic gene expression is common to all known herpesviruses. However, the effects on host colonization of altering this switch are largely unknown. We deregulated the transcription of the gene encoding the major lytic transactivator of murine gammaherpesvirus-68, ORF50, by inserting a new and powerful promoter element in its 5' untranslated region. In vitro, the mutant virus (M50) transcribed ORF50 at a high level and showed more rapid lytic spread in permissive fibroblast cultures, but in vivo, the M50 virus showed a severe deficit in latency establishment, with no sign of the infectious mononucleosis-like illness normally associated with wild-type infection. Although a low level of M50 viral DNA was detectable by PCR in spleens, replication-competent virus could not be recovered beyond 10 days post-infection. The M50 virus was also attenuated in immunocompromised mice. Thus a gammaherpesvirus unable to shut off lytic cycle gene expression showed severely restricted host colonization.

Mittelholzer, C., K.-O. Hedlund, et al. (2003). "Molecular characterization of a novel astrovirus associated with disease in mink." J. Gen. Virol. **84**(11): 3087-3094.

http://vir.sgmjournals.org/cgi/content/abstract/84/11/3087

Pre-weaning diarrhoea is a well-known problem in mink farming in Europe, causing morbidity that varies between farms, regions and season. Different causalities for the disease have been proposed, but only most recently has a novel astrovirus been identified as an important risk factor. In this report, the molecular characterization, origin and evolution of this novel astrovirus of mink are discussed. The polyadenylated, positive-stranded RNA genome was sequenced and found to contain 6610 nt, organized into three ORFs and two short UTRs. A ribosomal frameshift sequence links the 5' two ORFs, containing sequence motifs for a serine protease (ORF1a) and an RNA-dependent RNA polymerase (ORF1b). The structural proteins are encoded by ORF2 and, presumably, are expressed as a polyprotein precursor to be cleaved into the mature capsid proteins. These results indicate that mink astrovirus (MiAstV) has all of the features typical of members of the Astroviridae. Phylogenetic analyses revealed that MiAstV is distantly related to established astroviruses, showing less than 67 % similarity at the nucleotide level with its closest relative, ovine astrovirus, and even lower identities at the predicted amino acid level. Nevertheless, sequence analysis of MiAstV isolates from geographically distinct Swedish and Danish farms showed much less diversity. This suggests either the spread in the mink population of a virus that has evolved a long time ago or the recent introduction of an ancient virus into a new host species.

Mork, C., P. Hershberger, et al. (2004). "Isolation and characterization of a rhabdovirus from starry flounder (Platichthys stellatus) collected from the northern portion of Puget Sound, Washington, USA." J. Gen. Virol. **85**(2): 495-505.

http://vir.sgmjournals.org/cgi/content/abstract/85/2/495

The initial characterization of a rhabdovirus isolated from a single, asymptomatic starry flounder (Platichthys stellatus) collected during a viral survey of marine fishes from the northern portion of Puget Sound, Washington, USA, is reported. Virions were bullet-shaped and approximately 100 nm long and 50 nm wide, contained a lipid envelope, remained stable for at least 14 days at temperatures ranging from -80 to 5 {degrees}C and grew optimally at 15 {degrees}C in cultures of epithelioma papulosum cyprini (EPC) cells. The cytopathic effect on EPC cell monolayers was characterized by raised foci containing rounded masses of cells. Pyknotic and dark-staining nuclei that also showed signs of karyorrhexis were observed following haematoxylin and eosin, May-Grunwald Giemsa and acridine orange staining. PAGE of the structural proteins and PCR assays using primers specific for other known fish rhabdoviruses, including Infectious hematopoietic necrosis virus, Viral hemorrhagic septicemia virus, Spring viremia of carp virus, and Hirame rhabdovirus, indicated that the new virus, tentatively termed starry flounder rhabdovirus (SFRV), was previously undescribed in marine fishes from this region. In addition, sequence analysis of 2678 nt of the amino portion of the viral polymerase gene indicated that SFRV was genetically distinct from other members of the family Rhabdoviridae for which sequence data are available. Detection of this virus during a limited viral survey of wild fishes emphasizes the void of knowledge regarding the diversity of viruses that naturally infect marine fish species in the North Pacific Ocean.

Negri, D. R. M., S. Baroncelli, et al. (2004). "Protective efficacy of a multicomponent vector vaccine in cynomolgus monkeys after intrarectal simian immunodeficiency virus challenge." J. Gen. Virol. **85**(5): 1191-1201.

http://vir.sgmjournals.org/cgi/content/abstract/85/5/1191

We investigated the protective efficacy of a systemic triple vector (DNA/rSFV/rMVA)-based vaccine against mucosal challenge with pathogenic simian immunodeficiency virus (SIV) in cynomolgus monkeys. Animals were immunized at week 0 with DNA (intradermally), at weeks 8 and 16 with recombinant Semliki Forest virus (rSFV, subcutaneously) and finally, at week 24, with recombinant modified vaccinia virus Ankara strain (rMVA, intramuscularly). Both DNA and recombinant viral vectors expressed a wide range of SIV proteins (Gag, Pol, Tat, Rev, Env and Nef). This immunization strategy elicited cell-mediated rather than humoral responses that were especially increased following the last boost. Upon intrarectal challenge with pathogenic SIVmac251, three of the four vaccinated monkeys dramatically abrogated virus load to undetectable levels up to 41 weeks after challenge. A major contribution to this vaccine effect appeared to be the T-cell-mediated immune response to vaccine antigens (Gag, Rev, Tat, Nef) seen in the early phase of infection in three of the four vaccinated monkeys. Indeed, the frequency of T-cells producing antigen-induced IFN-{gamma} mirrored virus clearance in the vaccinated and protected monkeys. These results, reminiscent of the efficacy of live attenuated virus vaccines, suggest that vaccination with a combination of many viral antigens can induce a robust and stable vaccine-induced immunity able to abrogate virus replication.

Nicot, F., F. Legrand-Abravanel, et al. (2005). "Heterogeneity of hepatitis C virus genotype 4 strains

circulating in south-western France." J. Gen. Virol. **86**(1): 107-114.

http://vir.sgmjournals.org/cgi/content/abstract/86/1/107

Hepatitis C virus (HCV) is a major cause of liver disease. Knowledge of HCV variability is crucial for clinical and epidemiological analysis. HCV genotype 4 (HCV-4) has become increasingly prevalent in European countries, including France, in recent years. The present study investigates the heterogeneity of HCV-4 in south-western France by phylogenetic analysis of NS5B sequences from 166 patients. The E2 region of 90 strains was also analysed. Genotype 4 accounts for 7{middle dot}4 % of HCV infections in this area. Analysis of the NS5B region revealed 12 subtypes and the NS5B and E2 phylogeny data were congruent, except for one strain. The epidemiological data indicated two main groups of patients. One included intravenous drug users (IVDUs) of French origin, who were infected by homogeneous strains of subtypes 4a or 4d. The second group comprised non-IVDU patients who were infected with heterogeneous strains. This group was subdivided into patients of French origin, who were infected with eight subtypes, and patients from non-European countries (Central Africa or the Middle East), who were mainly infected with 4f, 4k, 4r and other subtypes; they showed the greatest genetic heterogeneity. This study of a large cohort of patients shows the great diversity of HCV-4 strains, and that these subtypes have spread differently.

Ortin, A., C. Cousens, et al. (2003). "Characterization of enzootic nasal tumour virus of goats: complete sequence and tissue distribution." J. Gen. Virol. **84**(8): 2245-2252.

http://vir.sgmjournals.org/cgi/content/abstract/84/8/2245

The complete genome sequence of a new isolate of enzootic nasal tumour virus (ENTV-2), associated with enzootic nasal adenocarcinoma (ENA) of goats, was determined. The genome exhibits a genetic organization characteristic of{beta} -retroviruses. ENTV-2 is closely related to the retrovirus (ENTV-1) associated with enzootic adenocarcinoma of sheep, and to jaagsiekte retrovirus. The main sequence differences between these viruses reside in orfX, the U3 LTR, two small regions in gag and the transmembrane (TM) region of env. Sequence analysis of the TM region of env from several sheep and goats naturally affected by ENA suggested that ENTV-1 and ENTV-2 are distinct viruses rather than geographical variants. Although both viruses transform secretory epithelial cells of the ethmoid turbinate, the study of their tissue distribution using specific PCRs showed that ENTV-2 establishes a disseminated lymphoid infection whereas ENTV-1 is mainly confined to the tumour.

Ozbun, M. A. (2002). "Infectious human papillomavirus type 31b: purification and infection of an immortalized human keratinocyte cell line." J. Gen. Virol. **83**(11): 2753-2763.

http://vir.sgmjournals.org/cgi/content/abstract/83/11/2753

Human papillomaviruses (HPVs) are aetiological agents of human malignancies, most notably cervical cancers. The life-cycles of HPVs are dependent on epithelial differentiation, and this has impeded many basic studies of HPV biology. The organotypic (raft) culture system supports epithelial differentiation such that infectious virions are synthesized in raft tissues from epithelial cells that replicate extrachromosomal HPV genomes. The CIN-612 9E cell line maintains episomal copies of HPV type 31b (HPV31b), an HPV type associated with cervical cancers. Many previous studies, including our own, have focused on characterizing the later stages of the HPV31b life-cycle in CIN-612 9E raft tissues. In this study, we have used the raft system to generate large numbers of HPV31b viral DNA (vDNA)-containing particles. We found a

biologically contained homogenization system to be efficient at virion extraction from raft epithelial tissues. We also determined that vDNA-containing particles could be directly quantified from density-gradient fractions. Using an RT-PCR assay, the presence of newly synthesized, spliced HPV31b transcripts was detected following HPV31b infection of the immortalized HaCaT epithelial cell line. Spliced E6 and E1{wedge}E4 RNAs were detected using a single round of RT-PCR from cells infected with a dose as low as 1{middle dot}0 vDNA-containing particle per cell. Spliced E1*I,E2 transcripts were found in cells infected with an HPV31b dose as low as 10 vDNA-containing particles per cell. Infectivity was blocked by HPV31 antiserum, but was not affected by DNase I. This work lays a foundation for a detailed analysis of the early events in HPV infection.

Pignatelli, S., P. Dal Monte, et al. (2003). "Human cytomegalovirus glycoprotein N (gpUL73-gN) genomic variants: identification of a novel subgroup, geographical distribution and evidence of positive selective pressure." J. Gen. Virol. **84**(3): 647-655.

http://vir.sgmjournals.org/cgi/content/abstract/84/3/647

Human cytomegalvirus (HCMV) ORF UL73 is a polymorphic locus, encoding the viral glycoprotein gpUL73-gN, a component of the gC-II envelope complex. The previously identified gN genomic variants, denoted gN-1, gN-2, gN-3 and gN-4, were further investigated in this work by analysing a large panel of HCMV clinical isolates collected from all over the world (223 samples). Sequencing and phylogenetic analysis confirmed the existence of the four gN genotypes, but also allowed the identification of a novel subgroup belonging to the gN-3 genotype, which was designated gN-3b. The number of non-synonymous (dN) and synonymous (dS) nucleotide substitutions and their ratio (dN/dS) were estimated among the gN genotypes to evaluate the possibility of positive selection. Results showed that the four variants evolved by neutral (random) selection, but that the gN-3 and gN-4 genotypes are maintained by positive selective pressure. The 223 HCMV clinical isolates were subdivided according to their geographical origin, and four main regions of gN prevalence were identified: Europe, China, Australia and Northern America. The gN variants were found to be widespread and represented within the regions analysed without any significant difference, and no new genotype was detected. Finally, for clinical and epidemiological purposes, a rapid and low-cost method for genetic grouping of the HCMV clinical isolates was developed based on the RFLP revealed by SacI, ScaI and SalI digestion of the PCR-amplified UL73 sequence. This technique enabled us to distinguish all four gN genomic variants and also their subtypes.

Sabouri, A. H., M. Saito, et al. (2005). "Differences in viral and host genetic risk factors for development of human T-cell lymphotropic virus type 1 (HTLV-1)-associated myelopathy/tropical spastic paraparesis between Iranian and Japanese HTLV-1-infected individuals." J. Gen. Virol. **86**(3): 773-781.

http://vir.sgmjournals.org/cgi/content/abstract/86/3/773

Human T-cell lymphotropic virus type 1 (HTLV-1)-associated myelopathy/tropical spastic paraparesis (HAM/TSP) is a neurological disease observed only in 1-2 % of infected individuals. HTLV-1 provirus load, certain HLA alleles and HTLV-1 tax subgroups are reported to be associated with different levels of risk for HAM/TSP in Kagoshima, Japan. Here, it was determined whether these risk factors were also valid for HTLV-1-infected individuals in Mashhad in northeastern Iran, another region of endemic HTLV-1 infection. In Iranian HTLV-1-infected individuals (n=132, 58 HAM/TSP patients and 74 seropositive asymptomatic carriers), although HLA-DRB1*0101 was associated with disease susceptibility in the absence of HLA-A*02 (P=0{middle dot}038; odds ratio=2{middle dot}71) as observed in Kagoshima, HLA-A*02 and HLA-Cw*08 had no effect on either the risk of developing HAM/TSP or HTLV-1 provirus load. All

Iranian subjects possessed tax subgroup A sequences, and the protective effects of HLA-A*02 were observed only in Kagoshima subjects with tax subgroup B but not in those with tax subgroup A. Both the prevalence of HTLV-1 subgroups and the host genetic background may explain the different risks levels for HAM/TSP development in these two populations.

Scobie, L., S. Taylor, et al. (2004). "Characterization of germline porcine endogenous retroviruses from Large White pig." J. Gen. Virol. **85**(8): 2421-2428.

http://vir.sgmjournals.org/cgi/content/abstract/85/8/2421

Porcine endogenous retroviruses (PERV) are of concern when the microbiological safety aspects of xenotransplantation are considered. Four unique isolates of PERV B have been identified previously from a lambda library constructed from genomic DNA from a Large White pig. This study shows that none of these isolates are replication competent when transfected into permissive human or pig cells in vitro, and the removal of flanking genomic sequences does not confer a human tropic replication competent (HTRC) phenotype on these PERV proviruses. Analysis of the envelope sequences revealed that PERV B demonstrated high similarity to the envelope sequences derived from replication-competent PERV, indicating that lack of replication competence does not appear to be attributable to this region of the provirus. These data complement recent findings that HTRC PERV are recombinants between the PERV A and PERV C subgroups, and that these recombinants are not present in the germline of miniature swine. Together, these results indicate that these individual PERV B proviruses are unlikely to give rise to HTRC PERV.

Simon, S., H. Li, et al. (2003). "The vascular lesions of a cow and bison with sheep-associated malignant catarrhal fever contain ovine herpesvirus 2-infected CD8+ T lymphocytes." J. Gen. Virol. **84**(8): 2009-2013.

http://vir.sgmjournals.org/cgi/content/abstract/84/8/2009

Malignant catarrhal fever (MCF) is a herpesvirus disease syndrome of ruminants. The microscopic pathology of MCF is characterized by lymphoid proliferation and infiltration, necrotizing vasculitis and epithelial necrosis. Because previous attempts to detect viral antigen or nucleic acids in lesions have been unsuccessful, the pathogenesis of the lesions in acute MCF has been speculated to involve mechanisms of autoimmunity and lymphocyte dysregulation. In this study, the vascular lesions in the brains of a cow and a bison with acute MCF were evaluated by in situ PCR and immunohistochemistry. The results demonstrated that the predominant infiltrating cell type in these lesions was CD8+ T lymphocytes and that large numbers of these cells were infected with ovine herpesvirus 2. The lesions also contained macrophages, but no detectable CD4+ or B lymphocytes.

Sun, Z. F., C. T. Larsen, et al. (2004). "Genetic identification of avian hepatitis E virus (HEV) from healthy chicken flocks and characterization of the capsid gene of 14 avian HEV isolates from chickens with hepatitis-splenomegaly syndrome in different geographical regions of the United States." J. Gen. Virol. **85**(3): 693-700.

http://vir.sgmjournals.org/cgi/content/abstract/85/3/693

Avian hepatitis E virus (HEV), a novel virus identified from chickens with hepatitis-splenomegaly

(HS) syndrome, is genetically and antigenically related to human HEV. Recently, it was found that avian HEV antibody is also prevalent in healthy chickens. A prospective study was done on a known seropositive but healthy chicken farm to identify avian HEV isolates from healthy chickens. Fourteen chickens were randomly selected, tagged and monitored under natural conditions for 19 weeks. All 14 chickens were seronegative at the beginning of the study at 12 weeks of age. By 21 weeks of age, all 14 chickens had seroconverted to avian HEV antibody. None of the chickens had any sign of HS syndrome. Partial helicase gene and capsid gene sequences of avian HEV isolates recovered from a healthy chicken were determined and found to share 75-97 % nucleotide sequence identity with the corresponding regions of avian HEV isolates from chickens with HS syndrome. Thus far, only one strain of avian HEV from a chicken with HS syndrome has been genetically characterized for its capsid gene, therefore the capsid gene region of an additional 14 isolates from chickens with HS syndrome were also characterized. The capsid genes of avian HEV isolates from chickens with HS syndrome were found to be heterogeneic, sharing 76-100 % nucleotide sequence identity with each other. This study indicates that avian HEV is enzootic in chicken flocks and spreads subclinically among chickens in the United States and that the virus is heterogeneic.

Tan, J. and G. A. Tannock (2005). "Role of viral load in the pathogenesis of chicken anemia virus." J. Gen. Virol. **86**(5): 1327-1333.

http://vir.sgmjournals.org/cgi/content/abstract/86/5/1327

The pathogenesis of strain 3711 of the chicken anemia virus (CAV), propagated in chickens, and two preparations of strain 3711 that had been adapted to grow to high titre in cells of the MDCC-MSB1 line were studied in chicken embryos and/or chickens. Highest viral loads in infected chickens, as measured by a microplate DNA-hybridization assay, were detected in the thymus, clotted blood and pancreas, and the lowest in the duodenum. The CAV DNA copy number in the organs of chicken embryos was significantly lower than in chickens. Route of infection was an important determinant of the course of disease in chickens, with clinical signs appearing earlier in birds infected by the intramuscular than those infected by the oral route; there was a direct relationship between viral load in particular organs and the extent of clinical signs. No reduction in the pathogenicity for chickens was noted for strain 3711 after 65 or 129 passages in the MDCC-MSB1 cell line.

Tomioka, Y., K. Ochiai, et al. (2004). "Genome sequence analysis of the avian retrovirus causing so-called fowl glioma and the promoter activity of the long terminal repeat." J. Gen. Virol. **85**(3): 647-652.

http://vir.sgmjournals.org/cgi/content/abstract/85/3/647

So-called fowl glioma is a retroviral infectious disease caused by avian leukosis virus subgroup A (ALV-A). We determined the complete nucleotide sequence of the virus genome. The full-length sequence was consistent with a genetic organization typical of a replication-competent type C retrovirus lacking viral oncogenes. The coding sequences were well conserved with those of replication-competent viruses, but the 3' noncoding regions including LTR were most related to those of replication-defective sarcoma viruses. The U3 region of the LTR had a few deletions and several point mutations compared to that of other ALVs. The promoter activities of the LTRs of glioma-inducing ALV and ALV-A standard strain, RAV-1, were equivalent in chick embryo fibroblasts (CEF), while that of glioma-inducing ALV was significantly lower than that of RAV-1 in human astrocytic cells. These subtle differences of the promoter activity of the LTR may be related to the induction of glial neoplasm.

van der Wilk, F., A. M. Dullemans, et al. (2002). "Nucleotide sequence and genomic organization of an ophiovirus associated with lettuce big-vein disease." J. Gen. Virol. **83**(11): 2869-2877.

http://vir.sgmjournals.org/cgi/content/abstract/83/11/2869

The complete nucleotide sequence of an ophiovirus associated with lettuce big-vein disease has been elucidated. The genome consisted of four RNA molecules of approximately 7{middle dot}8, 1{middle dot}7, 1{middle dot}5 and 1{middle dot}4 kb. Virus particles were shown to contain nearly equimolar amounts of RNA molecules of both polarities. The 5'- and 3'-terminal ends of the RNA molecules are largely, but not perfectly, complementary to each other. The virus genome contains seven open reading frames. Database searches with the putative viral products revealed homologies with the RNA-dependent RNA polymerases of rhabdoviruses and Ranunculus white mottle virus, and the capsid protein of Citrus psorosis virus. The gene encoding the viral polymerase appears to be located on the RNA segment 1, while the nucleocapsid protein is encoded by the RNA3. No significant sequence similarities were observed with other viral proteins. In spite of the morphological resemblance with species in the genus Tenuivirus, the ophioviruses appear not to be evolutionary closely related to this genus nor any other viral genus.

van Munster, M., A. M. Dullemans, et al. (2002). "Sequence analysis and genomic organization of Aphid lethal paralysis virus: a new member of the family Dicistroviridae." J. Gen. Virol. **83**(12): 3131-3138.

http://vir.sgmjournals.org/cgi/content/abstract/83/12/3131

The complete nucleotide sequence of the genomic RNA of an aphid-infecting virus, Aphid lethal paralysis virus (ALPV), has been determined. The genome is 9812 nt in length and contains two long open reading frames (ORFs), which are separated by an intergenic region of 163 nt. The first ORF (5' ORF) is preceded by an untranslated leader sequence of 506 nt, while an untranslated region of 571 nt follows the second ORF (3' ORF). The deduced amino acid sequences of the 5' ORF and 3' ORF products respectively showed similarity to the non-structural and structural proteins of members of the newly recognized genus Cripavirus (family Dicistroviridae). On the basis of the observed sequence similarities and identical genome organization, it is proposed that ALPV belongs to this genus. Phylogenetic analysis showed that ALPV is most closely related to Rhopalosiphum padi virus, and groups in a cluster with Drosophila C virus and Cricket paralysis virus, while the other members of this genus are more distantly related. Infectivity experiments showed that ALPV can not only infect aphid species but is also able to infect the whitefly Trialeurodes vaporariorum, extending its host range to another family of the order Hemiptera.

van Munster, M., A. M. Dullemans, et al. (2003). "A new virus infecting Myzus persicae has a genome organization similar to the species of the genus Densovirus." J. Gen. Virol. **84**(1): 165-172.

http://vir.sgmjournals.org/cgi/content/abstract/84/1/165

The genomic sequence of a new icosahedral DNA virus infecting Myzus persicae has been determined. Analysis of 5499 nt of the viral genome revealed five open reading frames (ORFs) evenly distributed in the 5' half of both DNA strands. Three ORFs (ORF1-3) share the same strand, while two other ORFs (ORF4 and ORF5) are detected in the complementary sequence. The overall genomic organization is similar to that of species from the genus Densovirus. ORFs 1-3 most likely encode the non-structural proteins, since their putative products contain

conserved replication motifs, NTP-binding domains and helicase domains similar to those found in the NS-1 protein of parvoviruses. The deduced amino acid sequences from ORFs 4 and 5 show sequence similarities with the structural proteins of the members of the genus Densovirus. These data indicate that this virus is a new species of the genus Densovirus in the family Parvoviridae. The virus was tentatively named Myzus persicae densovirus.

J. Med. Microbiol. (24)

Afset, J. E., K. Bergh, et al. (2003). "High prevalence of atypical enteropathogenic Escherichia coli (EPEC) in Norwegian children with diarrhoea." J. Med. Microbiol. **52**(11): 1015-1019.

http://jmm.sgmjournals.org/cgi/content/abstract/52/11/1015

The aim of the present study was to investigate the relative contribution of enteropathogenic Escherichia coli (EPEC) as a cause of infectious diarrhoea in Norwegian children. Data from faecal specimens from children <2 years old with diarrhoea during the year 2001 were analysed. E. coli isolates with the attaching and effacing genotype (eae+) were examined for the presence of the bundle-forming pilus (bfpA) and Shiga toxin genes by PCR, and for genetic relatedness by PFGE. During the 1-year period, 598 specimens from 440 patients <2 years old were analysed. Potential enteric pathogens were identified in 124 patients (28.2 %). EPEC was the most frequently identified agent (44 patients), followed by rotavirus (41 patients), Campylobacter jejuni (17 patients) and adenovirus (17 patients). All other agents were detected in five patients or less. Only one of the eae+ E. coli isolates was classified as typical EPEC (bfpA+). Among the 43 isolates that were classified as atypical EPEC (bfpA-), eight strains belonged to EPEC serogroups, whereas the majority of strains (n = 35) were not agglutinated by EPEC antisera. None of the EPEC isolates were genetically related. This study demonstrates that atypical EPEC of non-EPEC serogroups is highly prevalent among Norwegian children with diarrhoea.

Afset, J. E., L. Bevanger, et al. (2004). "Association of atypical enteropathogenic Escherichia coli (EPEC) with prolonged diarrhoea." J. Med. Microbiol. **53**(11): 1137-1144.

http://jmm.sgmjournals.org/cgi/content/abstract/53/11/1137

The aim of the present case control study was to investigate the prevalence of atypical enteropathogenic Escherichia coli (EPEC) and its possible role in causing diarrhoea among children < 5 years of age in Norway. Stool specimens received in the laboratory from children with suspected gastroenteritis (n = 251) were, in addition to routine testing, analysed for the presence of EPEC by PCR of the eae, bfpA and stx genes. Specimens from healthy children (n = 210) recruited from Maternal and Child Health Centres were analysed for EPEC only. EPEC isolates (eae+, stx-) were classified as typical (bfpA+) or atypical (bfpA-), and were tested for O: K serogroup. Information on duration of diarrhoea was recorded in a questionnaire and from referral forms. Atypical EPEC was diagnosed in 37 patients (14.7 %) compared to 21 (10.0 %) of the healthy controls [Odds ratio (OR) = 1.4, P = 0.3]. Only three isolates, all from patients, belonged to EPEC serogroups. One patient had typical EPEC. Twenty (22.5 %) of 89 patients with diarrhoea lasting [>=]14 days had atypical EPEC. The association between atypical EPEC and prolonged diarrhoea (OR = 2.1, P = 0.04) was caused by a high prevalence among female patients (40.6 %). In conclusion, atypical EPEC was found to be slightly more prevalent in

patients than controls, without any overall significant association with diarrhoea. However, a significant association was observed with diarrhoea lasting 14 days or more, a finding that may indicate a role for atypical EPEC in prolonged disease.

Ahmad, S., Z. Khan, et al. (2004). "Isolation and molecular identification of Candida dubliniensis from non-human immunodeficiency virus-infected patients in Kuwait." J. Med. Microbiol. **53**(7): 633-637.

http://jmm.sgmjournals.org/cgi/content/abstract/53/7/633

Candida dubliniensis is an emerging pathogen capable of causing oropharyngeal, vaginal and bloodstream infections. Although C. dubliniensis is similar to Candida albicans in several phenotypic characteristics, it differs from it with respect to epidemiology, certain virulence factors and the ability to develop resistance to fluconazole rapidly. In this study, the first seven isolations of C. dubliniensis from Kuwait are described, all originating from non-human immunodeficiency virus (HIV)-infected patients. The isolates were initially identified by the Vitek 2 yeast identification system, positive germ tube test, production of rough colonies and chlamydospores on Staib agar and by their inability to assimilate xylose, trehalose or methyl {alpha}-D-glucoside. The species identity of the isolates was subsequently confirmed by specific amplification of rDNA targeting the internally transcribed spacer 2 (ITS2), restriction endonuclease digestion of the amplified DNA and direct DNA sequencing of the ITS2. Using the E-test method, the MICs of C. dubliniensis test isolates were in the range 0.125-0.75 {micro}g ml-1 for fluconazole, 0.002-0.75 {micro}g ml-1 for itraconazole, 0.006-0.125 {micro}g ml-1 for ketoconazole, 0.002-0.5 {micro}g ml-1 for amphotericin B and 0.002-0.016 {micro}g ml-1 for voriconazole. Two of the isolates were resistant to 5-flucytosine (>32 {micro}g ml-1), but none against fluconazole. The study reinforces the current view that C. dubliniensis has a much wider geographical and epidemiological distribution.

Al-Soud, W. A., M. Bennedsen, et al. (2003). "Assessment of PCR-DGGE for the identification of diverse Helicobacter species, and application to faecal samples from zoo animals to determine Helicobacter prevalence." J. Med. Microbiol. **52**(9): 765-771.

http://jmm.sgmjournals.org/cgi/content/abstract/52/9/765

Helicobacter species are fastidious bacterial pathogens that are difficult to culture by standard methods. A PCR-denaturing gradient gel electrophoresis (PCR-DGGE) technique for detection and identification of different Helicobacter species was developed and evaluated. The method involves PCR detection of Helicobacter DNA by genus-specific primers that target 16S rDNA and subsequent differentiation of Helicobacter PCR products by use of DGGE. Strains are identified by comparing mobilities of unknown samples to those determined for reference strains; sequence analysis can also be performed on purified amplicons. Sixteen DGGE profiles were derived from 44 type and reference strains of 20 Helicobacter species, indicating the potential of this approach for resolving infection of a single host by multiple Helicobacter species. Some more highly related species were not differentiated whereas in highly heterogeneous species, sequence divergence was observed and more than one PCR-DGGE profile was obtained. Application of the PCR-DGGE method to DNA extracted from faeces of zoo animals revealed the presence of Helicobacter DNA in 13 of 16 samples; a correlation was seen between the mobility of PCR products in DGGE analysis and DNA sequencing. In combination, this indicated that zoo animals are colonized by a wide range of different Helicobacter species; seven animals appeared to be colonized by multiple Helicobacter species. By this approach, presumptive identifications were made of Helicobacter bilis and Helicobacter hepaticus in a Nile crocodile, Helicobacter cinaedi in a baboon and a red panda, and Helicobacter felis in a wolf and a Taiwan beauty snake. All of these PCR products ([~]400 bp) showed 100 % sequence similarity to 16S rDNA sequences of

the mentioned species. These results demonstrate the potential of PCR-DGGE-based analysis for identification of Helicobacter species in complex ecosystems, such as the gastrointestinal tract, and could contribute to a better understanding of the ecology of helicobacters and other pathogens with a complex aetiology.

Clarke, L., J. E. Moore, et al. (2003). "Development of a diagnostic PCR assay that targets a heat-shock protein gene (groES) for detection of Pseudomonas spp. in cystic fibrosis patients." J. Med. Microbiol. **52**(9): 759-763.

http://jmm.sgmjournals.org/cgi/content/abstract/52/9/759

Laboratory detection of Pseudomonas spp., in particular Pseudomonas aeruginosa, remains an important assay in the management of patients with cystic fibrosis (CF). As the groES and groEL genes of P. aeruginosa have now been cloned and their nucleotide sequences determined, the aim of this study was to develop a novel PCR assay for the detection of Pseudomonas spp. from patients with CF by employing conserved primer regions of the groE heat-shock protein domain gene. A PCR assay was designed that targeted a 536 bp region of the groE gene to detect Pseudomonas spp. PCR amplification of genomic DNA from extracted organisms generated an amplicon of the expected size (approx. 536 bp) for all P. aeruginosa (n = 60), Pseudomonas putida, Pseudomonas fluorescens and Pseudomonas stutzeri isolates examined, but did not produce a positive amplicon for several other genera and species that are commonly isolated from the sputum of CF patients. RFLP analysis of the amplicons of all P. aeruginosa isolates demonstrated a single RFLP type that consisted of three bands at approximately 80, 190 and 250 bp; direct sequencing of the amplicons demonstrated the presence of a single sequence type, indicating the highly conserved nature of this region. In addition, the assay successfully produced a positive signal from primary non-selective plates of three known P. aeruginosa culture-positive CF patients, but was unable to generate a signal in a further six CF patients who had no history of infection with P. aeruginosa or other Pseudomonas spp. This assay is recommended to detect the presence of Pseudomonas spp., including P. aeruginosa, from primary culture plates that originate from laboratory analysis of CF patients' sputum, particularly at review, in those patients with no previous history of Pseudomonas infection or those who appear to be transiently colonized by this organism. Employment of such molecular methodologies, in conjunction with routine clinical sputum cultures, may provide improved information on the microbial status of CF patients, which will aid clinicians in both optimum patient management in terms of antibiotic regimes and CF centre infection-control practices.

Fry, N. K., O. Tzivra, et al. (2004). "Laboratory diagnosis of pertussis infections: the role of PCR and serology." J. Med. Microbiol. **53**(6): 519-525.

http://jmm.sgmjournals.org/cgi/content/abstract/53/6/519

This study reports on practical laboratory aspects of pertussis diagnosis. PCR assays were applied to respiratory specimens obtained during a large study of infants (less than 5 months old) admitted to paediatric intensive care units (n = 122), children (less than 15 years old) admitted to paediatric wards (n = 16) and their household contacts (n = 320). Estimation of antibodies to pertussis toxin and culture for Bordetella pertussis were attempted on specimens from the same patients, where available, and the overall utility of the diagnostic PCR assays was assessed by comparison to these results. A PCR assay for the human mitochondrial cytochrome oxidase (HMCO) gene was used for quality control of the extracted samples and an internal process control (IPC) was included in each sample to test for PCR inhibition. Four of 458 samples were considered unsuitable (three HMCO negative, one IPC negative) and excluded from further analyses. Positive PCR results were considered valid if they were either (i) positive for both of two

B. pertussis gene targets (pertussis toxin S1 promoter and the insertion element IS481), i.e. consensus PCR positive, or (ii) repeatably positive in only one assay. Using these criteria, 52 of 454 (11.5 %) samples were considered as PCR positive for B. pertussis. Six of 356 samples were culture-positive for B. pertussis, 1/88 infants, 3/14 children and 2/254 contacts, giving an overall isolation rate of 1.7 %. Using these data, PCR gave an almost fivefold increase in diagnostic yield compared with culture (McNemar's test; P < 0.0001). Sera from 9/111 infants, 5/10 children and 14/210 contacts were positive. Serology and PCR results showed a high level of agreement (113/121) for infants and children. PCR demonstrated a significant improvement in diagnostic yield over culture. Serological testing also resulted in a significant increase in diagnostic yield compared to culture alone. PCR is a useful technique, but validity of results must be assured by careful control. Rapid diagnosis of B. pertussis infection particularly in infants by PCR, together with serological assays, can enhance surveillance systems for pertussis in all age groups.

Goto, T., H. Nagamune, et al. (2002). "Rapid identification of Streptococcus intermedius by PCR with the ily gene as a species marker gene." J. Med. Microbiol. **51**(2): 178-186.

http://jmm.sgmjournals.org/cgi/content/abstract/51/2/178

Streptococcus intermedius belongs to the anginosus group of streptococci (AGS) and is associated with endogenous infections leading to abscesses in the oral cavity and at deep-seated sites, such as the brain and liver. Two other species, S. anginosus and S. constellatus, and some presently unnamed taxa, are also classified as AGS. Recently, S. constellatus subsp. pharyngis, a new subspecies with biochemical characteristics similar to S. intermedius, was described with the potential for causing confusion when trying to identify isolates of these two species routinely with commercial identification kits, such as Rapid ID32 Strep and Fluo-Card Milleri. To correctly identify S. intermedius, this study attempted to develop an accurate PCR identification system with the ily gene as a species marker. This approach relies on amplification of an 819-bp fragment of the ily gene and its 3'-flanking region and is shown here to be specific for S. intermedius strains among all other streptococcal species. Moreover, this PCR system was applicable in direct rapid PCR with whole bacterial cells and TaKaRa Z-TaqTM (TaKaRa), a highly efficient DNA polymerase, as the template and DNA amplification enzyme, respectively.

Jurstrand, M., J. S. Jensen, et al. (2005). "Detection of Mycoplasma genitalium in urogenital specimens by real-time PCR and by conventional PCR assay." J. Med. Microbiol. **54**(1): 23-29.

http://jmm.sgmjournals.org/cgi/content/abstract/54/1/23

A real-time LightCycler PCR (LC-PCR) with hybridization probes for detection of Mycoplasma genitalium in endocervical and first void urine specimens was developed and compared to a conventional PCR. The primers for both assays were identical and designed to amplify a 427 bp fragment of the 16S rRNA gene of M. genitalium. The LC-PCR assay had a detection limit of < 5 bacterial genomes per reaction when dilutions of genomic DNA from a type strain of M. genitalium were tested. First void urine from 398 men and first void urine and endocervical specimens from 301 women attending an STD clinic were analysed by LC-PCR and by the conventional PCR. Using the conventional PCR as reference, the LC-PCR had a specificity of 99.7 % and a sensitivity of 72.2 % for the detection of M. genitalium in first void urine samples from men. There was no significant difference in the performance of the LC-PCR assay compared to the conventional PCR when endocervical swabs were considered (58 and 65 %, respectively) or with a set of endocervical swab/urine specimens for which the LC-PCR assay detected 73 % of the infections (specificity = 98.6 % and sensitivity = 68.2 %) while the conventional PCR detected 85 % of the infections. With female urine specimens there was a significant difference between the two assays (38 and 73 %, respectively; P = 0.01 McNemar's

test). This illustrates the need to analyse both endocervical and urine specimens, because M. genitalium DNA was detected in only one of the two specimens in a great number of the M. genitalium-infected women. The lower sensitivity of the LC-PCR assay was probably caused by a combination of inhibition and limitations regarding the amount of template DNA. The LC-PCR assay was easy to perform and the simultaneous amplification and detection eliminated the need for further handling of PCR products. With improvement in sample preparation methods and increased volumes of the template DNA, the LC-PCR assay could be a useful routine diagnostic method.

Kobayashi, D., Y. Eishi, et al. (2002). "Gastric mucosal density of Helicobacter pylori estimated by real-time PCR compared with results of urea breath test and histological grading." J. Med. Microbiol. **51**(4): 305-311.

http://jmm.sgmjournals.org/cgi/content/abstract/51/4/305

The accuracy of the urea breath test (UBT) and histological grading for estimation of the density of Helicobacter pylori in gastric mucosa is not known. Real-time (TaqMan) PCR was used to estimate the total number of H. pylori genomes in biopsy samples. These values were compared with those obtained by the UBT and the histological grade obtained by the Sydney system. The UBT and endoscopy with antral and corporal biopsies were performed in 88 consecutive untreated patients with dyspepsia. Bacterial culture and the rapid urease test were done with fresh biopsy materials. TaqMan PCR and histological examination were done on serial paraffin sections of the biopsy samples. Of the five methods tested, TaqMan PCR had the highest sensitivity and specificity (both 100%) in the diagnosis of H. pylori infection. The mean density of H. pylori genomes for pairs of biopsy samples from individual patients was compared with the individual values obtained by the UBT; correlation between the results was significant. The density of H. pylori genomes was higher in histological grades 1, 2 and 3 than in grade 0, without significant differences between adjacent grades from 1 to 3. These results suggest that the severity of H. pylori infection of the stomach can be estimated by the UBT and that histopathologists might state whether the organism is present or absent, rather than making a quantitative statement as recommended in the Sydney system.

Kotlowski, R., A. Martin, et al. (2004). "One-tube cell lysis and DNA extraction procedure for PCR-based detection of Mycobacterium ulcerans in aquatic insects, molluscs and fish." J. Med. Microbiol. **53**(9): 927-933.

http://jmm.sgmjournals.org/cgi/content/abstract/53/9/927

The purpose of this study was to develop a simple procedure for cell lysis and DNA extraction for direct detection of Mycobacterium ulcerans in aquatic insects, gills and intestinal contents of fish, molluscs and human tissue samples using a nested PCR method specific for the insertion sequence IS2404. The simultaneous action of sodium N-lauroyl sarcosine, guanidinium isothiocyanate, chloroform and Tris-saturated phenol on mycobacteria, followed by a DNA purification method using mini-columns fitted with silica-cellulose membranes was successfully employed to extract DNA from cultured bacteria, environmental and human tissue samples. All specimens were collected from Buruli ulcer endemic regions. M. ulcerans DNA was detected in 11 of 57 aquatic insects, one of six molluscs and three of 15 fish, supporting the hypothesis that the fauna of major Buruli ulcer endemic foci in swampy terrain of tropical and subtropical regions can be a source of M. ulcerans infection.

Kulkarni, S. P., M. A. Jaleel, et al. (2005). "Evaluation of an in-house-developed PCR for the diagnosis of tuberculous meningitis in Indian children." J. Med. Microbiol. **54**(4): 369-373.

http://jmm.sgmjournals.org/cgi/content/abstract/54/4/369

Early and rapid detection of the causative organism is necessary in tuberculosis, particularly tuberculous meningitis, as the disease affects mainly children and if untreated or improperly treated can cause severe central nervous system disorders and can often be fatal. An in-house-developed PCR technique was developed for the detection of Mycobacterium tuberculosis DNA, in which the target for amplification was a 340 bp nucleotide sequence located within the 38 kDa protein gene. The test can detect as small an amount of DNA as 10 fg, which is equivalent to two to three organisms, and is highly specific. Amplified product was detected by ethidium bromide staining after electrophoresis and Southern hybridization. Evaluation of test sensitivity and specificity was carried out using acid-fast bacilli-positive sputum samples from patients with pulmonary tuberculosis and an equal number of non-tuberculosis patient samples as negative controls. In a double-masked study 30 cerebrospinal fluid samples from tuberculous meningitis patients and 30 samples from non-tuberculous meningitis patients were investigated. Out of the 30 samples 22 were positive by ethidium bromide-stained gel electrophoresis and 27 gave positive results by Southern hybridization. All of the 30 control samples showed negative results. The sensitivity of this PCR was 90 % and specificity, 100 %.

Leclerq, S., J. S. Harms, et al. (2002). "Induction of a Th1-type of immune response but not protective immunity by intramuscular DNA immunisation with Brucella abortus GroEL heat-shock gene." J. Med. Microbiol. **51**(1): 20-26.

http://jmm.sgmjournals.org/cgi/content/abstract/51/1/20

The immunogenicity and protective efficacy of a DNA vaccine encoding the GroEL heat-shock gene from Brucella abortus was tested in BALB/c mice immunised by intramuscular (i.m.) needle injection or epidermally by gene gun. The Brucella GroEL gene was amplified by PCR and cloned into two different mammalian expression vectors pCMV-link and pCMV-tPA. The D17 cell line was transfected with both constructs and GroEL transcripts were detected by Northern blot. To determine the level of protein synthesised, transfected cell lysates were then submitted to Western blot. The non-secreted form of the recombinant GroEL produced by the pCMV-link construct was detected in much greater amount than the secreted form of the protein produced by the pCMV-tPA construct. After immunisation, a strong anti-GroEL IgG response was detected in mice vaccinated by i.m. injection or gene gun only when the pCMV-link/GroEL plasmid was used. Regarding the pattern of immune response induced, i.m. needle injection raised a predominantly Th1 response with mostly IgG2a-specific anti-GroEL and high levels of IFN-{gamma} produced by splenic T cells. Gene gun immunisation induced a Th0 type of immune response in mice characterised by a high IgG1/IgG2a ratio, and IL-4 and interferon (IFN)-{gamma} production. Even though a distinct pattern of immune response was generated depending upon the immunisation route used, neither method engendered a significant level of protection with the GroEL DNA vaccine.

Leung, E. T.-Y., K.-M. Kam, et al. (2003). "Detection of katG Ser315Thr substitution in respiratory specimens from patients with isoniazid-resistant Mycobacterium tuberculosis using PCR-RFLP." J. Med. Microbiol. **52**(11): 999-1003.

http://jmm.sgmjournals.org/cgi/content/abstract/52/11/999

Mutations in the katG locus of catalase peroxidase in Mycobacterium tuberculosis (MTB) account for major isoniazid (INH) resistance. In the South China region, a collection of 906 respiratory specimens and 142 MTB isolates was used to evaluate the sensitivity and specificity of a PCR-RFLP method for the detection of INH resistance-associated mutations. Except for four catalase-negative MTB isolates, katG PCR for a 620-bp amplicon was successful for all purified MTB isolates. For respiratory specimens, diagnostic sensitivity and specificity of katG PCR was 85 and 100 %. Subsequent RFLP of the katG amplicons by MspI digestion identified that 51 % of INH-resistant MTB were associated with the Thr315 phenotype, and that codon 463 was a polymorphic site with no linkage to INH resistance. The Arg463 wild-type MTB isolates predominant in the Western world were replaced by isolates carrying Leu463 in the South China region. RFLP patterns of katG amplicons from respiratory specimens were identical to those of the corresponding MTB cultured colonies. This method has potential application for rapid diagnosis of INH resistance due to katG Ser315Thr mutation.

Liu, D., L. Pearce, et al. (2002). "PCR identification of dermatophyte fungi Trichophyton rubrum, T. soudanense and T. gourvilii." J. Med. Microbiol. **51**(2): 117-122.

http://jmm.sgmjournals.org/cgi/content/abstract/51/2/117

Diagnosis of dermatophytosis employing conventional laboratory procedures has been complicated by the slow growth and varied morphological features shown by dermatophytes. After analysis of the nucleotide base sequences of a 1.2-kb fragment amplified from a dermatophyte fungus Trichophyton rubrum by arbitrarily primed PCR with random primer OPD18, a pair of primers (TR1F and TR1R) was designed and evaluated for specific identification of T. rubrum. The sensitivity of the primers TR1F and TR1R was high, as a specific PCR band of c. 600 bp was detected from as little as 7 pg of T. rubrum DNA. By examining 92 dermatophyte strains and clinical isolates, it was found that this pair of primers reacted in PCR with T. rubrum, T. soudanense and T. gourvilii through formation of the specific fragment of 600 bp, but not with any other of the dermatophyte species or varieties, fungi, yeasts or bacteria tested. As T. rubrum is one of the most frequently isolated dermatophyte fungi, and T. soudanense and T. gourvilii are relatively uncommon in many parts of the world, these primers can be used for rapid, sensitive and specific identification and differentiation of T. rubrum from other fungi and micro-organisms.

Ma, L., G. Kutty, et al. (2003). "Characterization of variants of the gene encoding the p55 antigen in Pneumocystis from rats and mice." J. Med. Microbiol. **52**(11): 955-960.

http://jmm.sgmjournals.org/cgi/content/abstract/52/11/955

Variants of the p55 gene in rat-derived Pneumocystis carinii have been identified and its counterpart in mouse-derived P. carinii f. sp. muris has been cloned. By PCR amplification of P. carinii genomic DNA, five variants were identified that differed from each other in size and sequence, primarily in the number and size of encoded amino acid repeats. For P. carinii f. sp. muris, a single PCR fragment (471 bp) was obtained, which contained an incomplete ORF encoding a 157 aa protein that was most similar to a p55 variant in P. carinii, with nucleotide and amino acid sequence identity of 79 and 68 %, respectively. Southern blot analysis revealed the presence of more than one copy of the p55 gene in both Pneumocystis species. Thus, like other Pneumocystis antigens, p55 exhibits polymorphism that could potentially benefit the organism in host interactions.

Maegawa, T., T. Karasawa, et al. (2002). "Linkage between toxin production and purine biosynthesis in Clostridium difficile." J. Med. Microbiol. **51**(1): 34-41.

http://jmm.sgmjournals.org/cgi/content/abstract/51/1/34

The production of toxins A and B by Clostridium difficile was greatly enhanced under biotin-limited conditions, in which a 140-kDa protein was expressed strongly. Gene cloning revealed that this protein was a homologue of formylglycinamidine ribonucleotide synthetase (FGAM synthetase, EC 6.3.5.3), which is known as PurL in Escherichia coli and catalyses the fourth step of the de novo purine biosynthesis pathway. This enzyme consisted of a single polypeptide, although FGAM synthetases of gram-positive bacteria usually consist of two subunits. Inhibition of the enzymic activity of C. difficile PurL by O-diazoacetyl-L-serine (azaserine) resulted in enhanced toxin B production even in biotin-sufficient conditions. In contrast, blockade of the preceding step of the PurL catalysing step by sulfamethoxazole inhibited toxin B production almost completely. These results suggest that accumulation of formylglycinamide ribonucleotide (FGAR), a substrate of FGAM synthetase, enhances toxin production by C. difficile and depletion of FGAR reduces toxin production.

Paciorek, J. (2002). "Virulence properties of Escherichia coli faecal strains isolated in Poland from healthy children and strains belonging to serogroups O18, O26, O44, O86, O126 and O127 isolated from children with diarrhoea." J. Med. Microbiol. **51**(7): 548-571.

http://jmm.sgmjournals.org/cgi/content/abstract/51/7/548

Four hundred and twenty-seven Escherichia coli isolates from 427 cases of infantile diarrhoea in Poland, belonging to serogroups O18, O26, O44, O86, O126 and O127 and 150 E. coli isolates from 52 healthy children were examined for selected virulence properties. The presence of the plasmid pAA, a plasmid encoding enterohaemolysin, the genes encoding intimin (eae), bundle-forming pili (bfp), Shiga toxins I and II (stxI, stxII) and cytotoxic necrotising factor types 1 and 2 (cnf1, cnf2) was investigated by PCR. Adhesion to HEp-2 cell monolayers was also tested and selected strains were investigated for the presence of P-fimbriae and haemolytic activity. Typical enteropathogenic E. coli isolates (typical EPEC, strains possessing eae and bfp, but not stx) were not found. The particular classes of E. coli among 427 isolates from ill children were: atypical EPEC (eae+ bfp, stx-), 21.3%; Shiga toxin-producing E. coli (STEC), 0.7%; diffusely adherent E. coli (DAEC), 4%; enteroaggregative E. coli (EAEC), 16.9%; necrotoxic E. coli type 1 (NTEC1), 0.2%; and cell-detaching E. coli (CDEC), 29%. With the exception of STEC, all the above classes of E. coli were found among the isolates from healthy children which comprised: atypical EPEC 8.0%, DAEC 6.7%, EAEC 17.3%, NTEC1 14.0% and CDEC 40.0%. Cell detachment (CD) was significantly associated with 3-h haemolytic activity. There was also strong correlation between haemolytic activity (Hly) and the presence of P-fimbriae. No correlation was found between the presence of the cnf1 gene and CD, Hly or P-fimbriae.

Panelius, J., P. Lahdenne, et al. (2002). "Recombinant OspC from Borrelia burgdorferi sensu stricto, B. afzelii and B. garinii in the serodiagnosis of Lyme borreliosis." J. Med. Microbiol. **51**(9): 731-739.

http://jmm.sgmjournals.org/cgi/content/abstract/51/9/731

Genes for the outer-surface protein C (OspC) from three north European human isolates of Borrelia burgdorferi sensu stricto, B. afzelii and B. garinii were cloned and sequenced. Polyhistidine-tagged recombinant OspC (rOspC) proteins were produced in Escherichia coli and used, after biotinylation, as antigens on streptavidin-coated plates in enzyme-linked

immunosorbent assays (ELISA). In IgM ELISA, 30% (5/17) and 35% (6/17) of patients with erythema migrans (EM) in the acute or convalescent phase, respectively, reacted with one to three rOspCs. Of the patients, 53% (8/15) with neuroborreliosis (NB) and 53% (8/15) with Lyme arthritis (LA) had IgM antibodies to OspC. The immunoreactivity was stronger against rOspC from B. afzelii and B. garinii than against rOspC from B. burgdorferi sensu stricto. In early Lyme borreliosis (LB), rOspC and flagella performed equally well in detecting IgM antibodies. Cross-reactive antibodies to rOspC were observed in serum samples from patients with rheumatoid factor positivity and with syphilis or Epstein-Barr virus (EBV) infection. In IgM ELISA, thiocyanate in the serum dilution buffer reduced EBV-associated non-specific positive reactions. Of the patient sera examined in IgG ELISA, 30% (5/17) with EM in the acute phase, 35% (6/17) with EM in the convalescent phase, 33% (5/15) with NB and 60% (9/15) with LA were positive. Because of the heterogeneity of OspC, a polyvalent antigen with several OspC variants from at least B. afzelii and B. garinii is needed to improve the sensitivity of OspC ELISA in the serodiagnosis of LB in Europe.

Robey, M., E. Morgan, et al. (2002). "A new chromosomal locus associated with gut-modulated phenotypes in Salmonella enterica serotype Typhimurium." J. Med. Microbiol. **51**(3): 247-263.

http://jmm.sgmjournals.org/cgi/content/abstract/51/3/247

A cosmid DNA library had been constructed previously from 40-kb fragments of genomic DNA from a virulent invasive strain of Salmonella enterica serotype Typhimurium (TML) in an avirulent hypo-invasive Typhimurium strain (LT7). Selection of invasive clones from the library was attempted by iterative passage through a rabbit ileal organ culture. After the fourth passage, a clone, designated LT7(pHC20uu.2), was isolated. Exposure to both gut tissue and Caco-2 cells enhanced the growth, invasiveness for gut and Caco-2 cells, and flagellin expression of LT7(pHC20uu.2) although its invasiveness was less than that of strain TML. Expression of appendages (surface structures c. 60-70 nm diameter) was shown to play a role in but not to confer invasiveness, and was demonstrated in the absence of direct contact with eukaryotic cells. Exposure to gut tissue also affected the expression of several outer-membrane proteins (OMPs) in all four Salmonella strains - TML, LT7, LT7(pHC79), LT7(pHC20uu.2) - used in this work. As the genes involved in flagella, invasin and porin expression are distributed around the salmonella chromosome, it is possible that pHC20uu.2 encodes a pleiotropic regulator of genes involved in gastro-enteritic virulence and adaptation to the in-vivo gut environment. pHC20uu.2 mapped at c. centisome 25 on the salmonella chromosome close to, but distinct from, SPI-5.

Schmidt, D. and P.-M. Rath (2003). "Faster genetic identification of medically important aspergilli by using gellan gum as gelling agent in mycological media." J. Med. Microbiol. **52**(8): 653-655.

http://jmm.sgmjournals.org/cgi/content/abstract/52/8/653

Using gellan gum as a substitute for agar-agar in a mycological medium and sequencing of the ITS 1 and 2 regions resulted in an accurate identification of Aspergillus fumigatus, Aspergillus nidulans, Aspergillus terreus and Aspergillus ustus within 24 h of subculture.

Smith, S. I., C. Kirsch, et al. (2002). "Prevalence of Helicobacter pylori vacA, cagA and iceA genotypes in Nigerian patients with duodenal ulcer disease." J. Med. Microbiol. **51**(10): 851-854.

http://jmm.sgmjournals.org/cgi/content/abstract/51/10/851

Distinct virulence factors of Helicobacter pylori have been associated with clinical outcome of the infection; however, considerable variations have been reported from different geographic regions. Data on genotypes of African H. pylori isolates are sparse. The aim of this study was to determine the prevalence of specific genotypes of H. pylori in Nigerian patients with duodenal ulcer and non-ulcer dyspepsia. H. pylori was cultured from endoscopic biopsies obtained from 41 Nigerian patients (19 with duodenal ulcer, 22 with non-ulcer dyspepsia). The vacA alleles, cagA and iceA genotypes were determined by PCR. The vacA s1,m1 and s1,m2 genotypes were found in 26.3% and 22.7%, and in 73.7% and 72.7% of H. pylori isolates from patients with duodenal ulcer and non-ulcer dyspepsia, respectively. The iceA1 genotype was present in 94.7% and 86.4% of isolates from duodenal ulcer and non-ulcer dyspepsia patients, respectively. cagA+ infection was found predominantly (>90%) in Nigerian H. pylori isolates irrespective of the clinical diagnosis. In conclusion, vacA s1,m2, iceA1 and cagA+ are common genotypes of H. pylori isolated from Nigerian patients. As in several other developing countries there seems to be no association between these genotypes and duodenal ulcer disease.

Soki, J., E. Fodor, et al. (2004). "Molecular characterization of imipenem-resistant, cfiA-positive Bacteroides fragilis isolates from the USA, Hungary and Kuwait." J. Med. Microbiol. **53**(5): 413-419.

http://jmm.sgmjournals.org/cgi/content/abstract/53/5/413

Fifteen Bacteroides fragilis isolates from the USA, Hungary and Kuwait were examined for carbapenem resistance, for carbapenemase activity and, with the use of various PCR-based methods and nucleotide sequencing, for cfiA genes and activating insertion sequence (IS) elements. All the B. fragilis isolates were cfiA-positive, 10 of the cfiA genes being upregulated by IS elements that are already known. Of these 10, one was of a novel type (designated IS943) and two further ones (IS614B and IS614C) were suspected hybrids of IS612, IS614 and IS942. There were five cfiA-positive imipenem-resistant B. fragilis isolates with elevated imipenem MICs (minimal inhibitory concentration) that harboured no IS insertion upstream of the cfiA gene, but produced carbapenemase; these isolates might possess a novel activation mechanism. On the basis of the available phenotypic and genotypic evidence, the present data suggest that there are at least two cfiA activation mechanisms among B. fragilis isolates.

Uehara, N., A. Yagihashi, et al. (2003). "Human {beta}-defensin-2 induction in Helicobacter pylori-infected gastric mucosal tissues: antimicrobial effect of overexpression." J. Med. Microbiol. **52**(1): 41-45.

http://jmm.sgmjournals.org/cgi/content/abstract/52/1/41

The objective of this study was to understand more of the innate immune response to Helicobacter pylori by determining the expression of human {beta}-defensin-2 (hBD-2) in various gastric mucosal tissues and MKN45 gastric cancer cells with or without H. pylori. Semi-quantitative TaqMan RT-PCR and immunohistochemistry were carried out. The antimicrobial effects of a transfected hBD-2 gene against H. pylori were also evaluated. The results showed that hBD-2 was expressed in inflamed gastric mucosal tissues with H. pylori infection, but not in the absence of H. pylori infection. Expression was also detected in gastric cancers in patients with H. pylori infection. Expression was induced in the MKN45 gastric cancer cell line by H. pylori in a manner dependent on the abundance of bacteria. hBD-2-transfected 3T3J2-1 cells secreted hBD-2 protein into the culture medium and this protein inhibited growth of H. pylori completely. The results suggest that hBD-2 may be involved in the pathophysiology of H. pylori-induced gastritis.

Xu, J., J. R. Rao, et al. (2002). "Improved molecular identification of Thermoactinomyces spp. associated with mushroom worker's lung by 16S rDNA sequence typing." J. Med. Microbiol. **51**(12): 1117-1127.

http://jmm.sgmjournals.org/cgi/content/abstract/51/12/1117

Mushroom worker's lung (MWL) is a hypersensitivity pneumonitis or allergic alveolitis caused by a type III IgG-mediated immunopathogenic inflammatory reaction in the host due to the inhalation of several thermophilic organisms, including Thermoactinomyces spp. It is difficult to distinguish phenotypically the eight species of this genus; therefore, this study sought to develop an improved molecular means of identifying Thermoactinomyces spp. associated with MWL by partial 16S rDNA PCR amplification and direct sequencing. Hypervariable regions within the 16S rRNA gene, which could be employed as signature sequences of the eight individual species, were identified and employed with highly conserved flanking primers to allow initial PCR amplification, before direct DNA sequencing of the 16S rDNA amplicons. A novel 24-mer 16S rDNA oligonucleotide upstream primer was designed from in silico alignments of all Thermoactinomyces spp. and was employed in combination with downstream (reverse) 16S rDNA primers. This permitted the successful identification of all four isolates associated with mushroom workers' lung. The method may be useful in the identification of Thermoactinomyces spp. associated with allergic alveolitis or pneumonitis associated with occupational exposure in agricultural and horticultural environments.

J. Nutr. (9)

Auborn, K. J., S. Fan, et al. (2003). "Indole-3-Carbinol Is a Negative Regulator of Estrogen." J. Nutr. **133**(7): 2470S-2475.

http://www.nutrition.org/cgi/content/abstract/133/7/2470S

Studies increasingly indicate that dietary indole-3-carbinol (I3C) prevents the development of estrogen-enhanced cancers including breast, endometrial and cervical cancers. Epidemiological, laboratory, animal and translational studies support the efficacy of I3C. Whereas estrogen increases the growth and survival of tumors, I3C causes growth arrest and increased apoptosis and ameliorates the effects of estrogen. Our long-range goal is to best use I3C together with other nutrients to achieve maximum benefits for cancer prevention. This study examines the possibility that induction of growth arrest in response to DNA damage (GADD) in genes by diindolylmethane (DIM), which is the acid-catalyzed condensation product of I3C, promotes metabolically stressed cancer cells to undergo apoptosis. We evaluated whether genistein, which is the major isoflavonoid in soy, would alter the ability of I3C/DIM to cause apoptosis and decrease expression driven by the estrogen receptor (ER)-{alpha}. Expression of GADD was evaluated by real-time reverse transcription-polymerase chain reaction. Proliferation and apoptosis were measured by a mitochondrial function assay and by fluorescence-activated cell sorting analysis. The luciferase reporter assay was used to specifically evaluate expression driven by ER-{alpha}. The estrogen-sensitive MCF-7 breast cancer cell line was used for these studies. We show a synergistic effect of I3C and genistein for induction of GADD expression, thus increasing apoptosis, and for decrease of expression driven by ER-{alpha}. Because of the synergistic effect of I3C and genistein, the potential exists for prophylactic or therapeutic efficacy

of lower concentrations of each phytochemical when used in combination.

Cooney, C. A., A. A. Dave, et al. (2002). "Maternal Methyl Supplements in Mice Affect Epigenetic Variation and DNA Methylation of Offspring." J. Nutr. **132**(8): 2393S-2400.

http://www.nutrition.org/cgi/content/abstract/132/8/2393S

This study was designed to determine if maternal dietary methyl supplements increase DNA methylation and methylation-dependent epigenetic phenotypes in mammalian offspring. Female mice of two strains were fed two levels of dietary methyl supplement or control diet prior to and during pregnancy. Offspring of these mice vary in phenotype, which is epigenetically determined and affects health and 2-y survival. Phenotype and DNA methylation of a long terminal repeat (LTR) controlling expression of the agouti gene were assayed in the resulting offspring. Methyl supplements increase the level of DNA methylation in the agouti LTR and change the phenotype of offspring in the healthy, longer-lived direction. This shows that methyl supplements have strong effects on DNA methylation and phenotype and are likely to affect long-term health. Optimum dietary supplements for the health and longevity of offspring should be intensively investigated. This should lead to public policy guidance that teaches optimal, rather than minimal, dose levels of maternal supplements.

Finnell, R. H., O. Spiegelstein, et al. (2002). "DNA Methylation in Folbp1 Knockout Mice Supplemented with Folic Acid during Gestation." J. Nutr. **132**(8): 2457S-2461.

http://www.nutrition.org/cgi/content/abstract/132/8/2457S

Periconceptional folic acid supplementation has been shown to prevent up to 70% of neural tube and other birth defects in humans; however, the mechanism is still unknown. In this study, we tested whether defective intracellular folate transport, as achieved by inactivation of the murine folate-binding protein 1 (Folbp1), affects global DNA methylation in the liver and brain from gestational day (GD) 15 [IMG]f1.gif" BORDER="0"> embryos. Complete Folbp1 inactivation is embryolethal but can be reversed by maternal folinic acid (FA) supplementation, and thus we also tested the effect of FA supplementation on DNA methylation in Folbp1 fetuses. Overall, the extent of global DNA methylation seems to be similar across all genotypes in unsupplemented control Folbp1 mice; however, explicit conclusions regarding Folbp1-/- fetuses were not possible because only a single living unsupplemented fetus was viable at GD 15 [IMG]f1.gif" BORDER="0">. FA supplementation induced global DNA hypomethylation across all genotypes. FA-induced hypomethylation is most likely due to its ability to inhibit the enzyme glycine hydroxymethyltransferase, thereby inhibiting the homocysteine remethylation cycle necessary to regenerate S-adenosylmethionine, the methyl donor for DNA methyltransferases. Our hypothesis was that due to defective folate transport in Folbp1-/- embryos and fetuses, DNA would be hypomethylated, thereby altering the temporal expression of critical genes necessary for normal embryonic development. However, these results suggest that an extended examination of changes in DNA methylation prior to GD 15 [IMG]f1.gif" BORDER="0"> is required to unequivocally prove or disprove the hypothesis.

Kim, S., I. Sohn, et al. (2005). "Hepatic Gene Expression Profiles Are Altered by Genistein Supplementation in Mice with Diet-Induced Obesity." J. Nutr. **135**(1): 33-41.

http://www.nutrition.org/cgi/content/abstract/135/1/33

We reported previously that genistein enhances the expression of genes involved in fatty acid catabolism through activation of peroxisome proliferator-activated receptor (PPAR) {alpha} in HepG2 cells, suggesting that genistein holds great promise for therapeutic applications to lipid abnormalities such as obesity and hyperlipidemia in humans. In this study, we examined the changes in hepatic transcriptional profiles using cDNA microarrays in mice with high-fat diet (HFD)-induced obesity supplemented with genistein. C57BL/6J male mice (n = 10/group) were fed a low-fat diet (LFD), a HFD, or a HFD supplemented with 2 g/kg genistein (HFD+GEN) for 12 wk. Mice fed the HFD had abnormal lipid profiles and significantly greater body weight and visceral fat accumulation than the LFD-fed group. Genistein supplementation improved lipid profiles and hepatic steatosis and attenuated the increases in body weight and visceral fat in HFD-fed mice. The cDNA microarrays revealed marked alterations in the expression of 107 genes in the mice fed the HFD and/or the HFD+GEN. Of 97 transcripts altered in the HFD-fed group, 84 genes were normalized by genistein supplementation. However, several genes involved in fatty acid catabolism were not normalized but were still upregulated in the HFD+GEN-fed group, relative to the LFD-fed group. Furthermore, carnitine O-octanoyltransferase, which accelerates fatty acid oxidation, was not affected by the HFD, but was induced by genistein supplementation. These results are consistent with our previous study showing that genistein is an activator of PPAR {alpha} in vitro. This study showed beneficial effects of genistein supplementation in preventing the development of obesity and metabolic abnormalities in mice with diet-induced obesity. Our results also provide interesting information about the genes associated with the beneficial effects of genistein as well as the mechanisms underlying the development and maintenance of the obesity phenotype in vivo.

Liu, L. and Y.-Y. Yeh (2002). "S-Alk(en)yl Cysteines of Garlic Inhibit Cholesterol Synthesis by Deactivating HMG-CoA Reductase in Cultured Rat Hepatocytes." J. Nutr. **132**(6): 1129-1134.

http://www.nutrition.org/cgi/content/abstract/132/6/1129

The effects of water-soluble organosulfur compounds of garlic on hepatic cholesterol biosynthesis in cultured rat hepatocytes were studied. S-Alk(en)yl cysteines, i.e., S-allyl cysteine (SAC), S-ethyl cysteine (SEC) and S-propyl cysteine (SPC) inhibited cholesterol synthesis from [14C]acetate but not from [14C]mevalonate. The activity of 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase in the cells treated with SAC, SEC and SPC was 30-40% lower than that of the untreated cells. S-Alk(en)yl cysteines did not alter abundance of mRNA coded for HMG-CoA reductase or protein concentration of the enzyme. The ratio of expressed to total activity (E/T) of HMG-CoA reductase was then determined as an index of phosphorylation status of the enzyme. The E/T ratio was reduced 18-29% by SAC, SEC and SPC, resulting primarily from decreased expressed activity. The results suggest that S-alk(en)yl cysteines inhibit cholesterol synthesis by deactivating HMG-CoA reductase via enhanced phosphorylation, but not changing levels of mRNA or the amount of the enzyme. Additionally, of the three S-alk(en)yl cysteines tested, only SAC appears to further decrease the activity of HMG-CoA reductase by increasing sulfhydryl oxidation of the enzyme.

McCann, S. E., K. B. Moysich, et al. (2002). "The Risk of Breast Cancer Associated with Dietary Lignans Differs by CYP17 Genotype in Women." J. Nutr. **132**(10): 3036-3041.

http://www.nutrition.org/cgi/content/abstract/132/10/3036

Lignans are plant compounds metabolized in the gut to produce the phytoestrogens enterolactone and enterodiol. Reduced breast cancer risks associated with higher urinary lignan excretion may be related to competitive inhibition of endogenous estrogens. Evidence exists that associations with reproductive risk factors for breast cancer differ according to cytochrome

P450c17{alpha} (CYP17) genotype. Genetic variability in estrogen metabolism could affect lignan metabolism thereby modifying risk associations. We examined breast cancer risk, dietary lignans and CYP17 genotype among 207 women with primary, incident, histologically confirmed breast cancer and 188 controls frequency matched to cases by age and county of residence. Self-reported frequency of intake of 170 foods and beverages during the 2 y before the interview and other relevant data were collected by detailed in-person interviews. Dietary lignan intake was expressed as the sum of enterolactone and enterodiol production from foods. Odds ratios (OR) and 95% confidence intervals (CI) were estimated by unconditional logistic regression, adjusting for age, education and other breast cancer risk factors. Women in the highest tertile of dietary lignans tended to have reduced breast cancer risk (OR 0.45, 95% CI 0.20-1.01 and OR 0.59, 95% CI 0.28-1.27, pre- and postmenopausal women, respectively). Substantially reduced risks in the highest tertile of lignans were observed for premenopausal women with at least one A2 allele (OR 0.12, 95% CI 0.03-0.50). Our results suggest that CYP17 genotype may be important in modifying the effect on breast cancer risk of exogenous estrogens, particularly for premenopausal women.

Perez, M. J., A. Suarez, et al. (2002). "Dietary Nucleotide Supplementation Reduces Thioacetamide-Induced Liver Fibrosis in Rats." J. Nutr. **132**(4): 652-657.

http://www.nutrition.org/cgi/content/abstract/132/4/652

Dietary nucleotides reportedly promote functionality and repair in fibrotic liver. Liver fibrosis is characterized by an excessive accumulation of extracellular matrix components, which lead to the impairment of the hepatic function. The aim of this work was to evaluate the influence of dietary nucleotides on liver fibrosis induced by thioacetamide and to elucidate the mechanism by which nucleotides exert their protective effects. Rats consumed ad libitum 300 mg/L thioacetamide in drinking water and were pair-fed diets with (group TN) or without nucleotides (group TS) for 4 mo. Liver histology and extracellular matrix components, liver collagenase and prolyl 4-hydroxylase activities, and tissue inhibitor of metalloproteinases-1 were assessed. The degree of fibrosis was lower in group TN than in group TS. Group TN had lower hepatic concentration of hydroxyproline ($P < 0.05$), collagen type I ($P = 0.12$) and type III ($P = 0.20$), fibronectin ($P = 0.05$), laminin ($P = 0.11$) and desmin ($P = 0.07$), higher collagenolytic activity ($P < 0.05$), lower prolyl 4-hydroxylase activity ($P < 0.05$) and lower prolyl 4-hydroxylase ($P = 0.10$) and tissue inhibitor of metalloproteinase-1 ($P = 0.06$) expression than group TS. Moreover, expression of tissue inhibitor of the metalloproteinases-1 gene was lower in group TN than in group TS ($P < 0.05$). These data indicate that the reduction of liver fibrosis in nucleotide-supplemented rats may rely on the enhancement of collagenase activity and the reduction of collagen content and maturation.

Ten Bruggencate, S. J. M., I. M. J. Bovee-Oudenhoven, et al. (2005). "Dietary Fructooligosaccharides Increase Intestinal Permeability in Rats." J. Nutr. **135**(4): 837-842.

http://www.nutrition.org/cgi/content/abstract/135/4/837

We showed previously that fructooligosaccharides (FOS) decrease the resistance to salmonella infection in rats. However, the mechanism responsible for this effect is unclear. Therefore, we examined whether dietary FOS affects intestinal permeability before and after infection with Salmonella enterica serovar Enteritidis. Male Wistar rats were fed restricted quantities of a purified diet that mimicked the composition of a Western human diet. The diet was supplemented with 60 g/kg cellulose (control) or 60 g/kg FOS and with 4 mmol/kg of the intestinal permeability marker chromium EDTA (CrEDTA) ($n = 8$ or 10). After an adaptation period of 2 wk, rats were orally infected with 108 colony-forming units (cfu) of S. enteritidis. Mucin concentrations in intestinal contents and mucosa were measured fluorimetrically, as markers of mucosal irritation.

Intestinal permeability was determined by measuring urinary CrEDTA excretion. Translocation of salmonella was quantified by analysis of urinary nitric oxide metabolites with time. Before infection, FOS increased mucosal lactobacilli and enterobacteria in cecum and colon, but not in the ileum. However, FOS increased cytotoxicity of fecal water and intestinal permeability. Moreover, FOS increased fecal mucin excretion and mucin concentrations in cecal and colonic contents, and in cecal mucosa before infection. After infection, mucin excretion and intestinal permeability in the FOS groups increased even further in contrast to the control group. In addition, FOS increased translocation of salmonella to extraintestinal sites. Thus, FOS impairs the intestinal barrier in rats, as indicated by higher intestinal permeability. Whether these results can be extrapolated to humans requires further investigation.

Tryfonidou, M. A., J. J. Stevenhagen, et al. (2002). "Moderate Cholecalciferol Supplementation Depresses Intestinal Calcium Absorption in Growing Dogs." J. Nutr. **132**(9): 2644-2650.

http://www.nutrition.org/cgi/content/abstract/132/9/2644

Hormonal regulation of calcium (Ca) absorption was investigated in a cholecalciferol (vitamin D3)-supplemented group (hVitD) vs. a control group (cVitD) of growing Great Danes (100 vs. 12.5 {micro}g vitamin D3/kg diet). Although Ca intakes did not differ, fractional Ca absorption was significantly lower in the hVitD group than in the cVitD group. There were no differences in plasma concentrations of Ca, inorganic phosphate, parathyroid hormone, growth hormone or insulin-like growth factor I between groups. Plasma 25-hydroxycholecalciferol [25(OH)D3] concentrations were maintained in the hVitD dogs at the same levels as in the cVitD dogs due to increased turnover of 25(OH)D3 into 24,25-dihydroxycholecalciferol [24,25(OH)2D3] and 1,25-dihydroxycholecalciferol [1,25(OH)2D3]. In hVitD dogs, the greater plasma 24,25(OH)2D3 concentration and the enhanced metabolic clearance rate (MCR) of 1,25(OH)2D3 indicated upregulated 24-hydroxylase activity. The increased MCR of 1,25(OH)2D3 decreased plasma 1,25(OH)2D3 concentrations. In hVitD dogs, the greater production rate of 1,25(OH)2D3 was consistent with the 12.9-fold greater renal 1{alpha}-hydroxylase gene expression compared with cVitD dogs and compensated to a certain extent for the accelerated MCR of 1,25(OH)2D3. The moderately decreased plasma 1,25(OH)2D3 concentration can only partially explain the decreased Ca absorption in the hVitD dogs. Intestinal vitamin D receptor concentrations did not differ between groups and did not account for the decreased Ca absorption. We suggest that 24,25(OH)2D3 may downregulate Ca absorption.

J. Wildl. Dis. (6)

Divina, B. P., E. Wilhelmsson, et al. (2002). "Molecular identification and prevalence of Dictyocaulus spp. (Trichostrongyloidea: Dictyocaulidae) in Swedish semi-domestic and free-living cervids." J. Wildl. Dis. **38**(4): 769-775.

http://www.jwildlifedis.org/cgi/content/abstract/38/4/769

Lungs of 102 roe deer (Capreolus capreolus), 136 moose (Alces alces), 68 fallow deer (Dama dama), and six red deer (Cervus elaphus) were examined during hunting seasons from 16 September 1997 to 1 March 2000. The aim was to determine the species composition and prevalence of Dictyocaulus lungworms in these hosts in Sweden. Worms were identified following

polymerase chain reaction (PCR) amplification of the internal transcribed spacer of ribosomal DNA (ITS2), followed by hybridization with four species-specific oligonucleotides. In addition, 50 lungworms from five reindeer (Rangifer tarandus) from Norway were similarly analyzed. A total of 399 worms were recovered and analyzed representing a range of 29-128 worms per host species. All specimens from roe deer were identified as Dictyocaulus capreolus, whereas those from red deer and reindeer were identical with D. eckerti. From moose, 73 (81.1%) of the worms were identified as D. capreolus whereas 17 (18.9%) were D. eckerti. The ITS2 sequence of fallow deer lungworms differed significantly when compared with the ITS2 of D. viviparus, D. capreolus, and D. eckerti. This indicated that fallow deer in Sweden may be infected with a new genotype of Dictyocaulus spp. Consequently, a specific probe designed for the ITS2 from this Dictyocaulus sp. hybridized exclusively with samples from lungworms of fallow deer. Interestingly, no D. viviparus were found in any of these hosts. The prevalence of infection in each host was as follows: D. capreolus in roe deer (14.7%) and moose (10.6%); D. eckerti in moose (0.7%) and red deer (33.3%); and Dictyocaulus sp. in fallow deer (10.3%). Regardless of lungworm species, the overall prevalence of Dictyocaulus spp. in these hosts was 12.2%. Prevalence between male and female animals and among the different age groups did not differ significantly. Finally an enzyme linked immunosorbent assay (ELISA) specific for patent D. viviparus infection in cattle was utilized to analyze lung tissue fluids from infected animals. All samples from roe deer, red deer, and fallow deer were negative in the ELISA. However, three out of twelve (25%) samples from moose and 17 of 40 (43%) samples from cattle were positive. This indicated that moose anti-D. capreolus antibodies recognized the D. viviparus antigen and that anti-cattle immunoglobulin cross-reacted with moose antibodies.

Ketz-Riley, C. J., M. V. Reichard, et al. (2003). "An intraerythrocytic small piroplasm in wild-caught Pallas's cats (Otocolobus manul) from Mongolia." J. Wildl. Dis. **39**(2): 424-430.

http://www.jwildlifedis.org/cgi/content/abstract/39/2/424

During the quarantine examination of four Pallas's cats (Otocolobus manul) imported from Mongolia in October and December 2000, intraerythrocytic piroplasms were detected on Wright-Giemsa stained blood films that were morphologically indistinguishable from other small piroplasms of felids. Further characterization of this unknown organism via polymerase chain reaction amplification, sequencing of a portion of the 18S nuclear small subunit rRNA gene, and comparisons with orthologous sequences from other piroplasms, revealed similarity to Cytauxzoon felis. This is the first report of naturally occurring erythroparasitemia in Pallas's cats and the first documented case of naturally occurring piroplasm infections in a free-ranging felid from Mongolia.

Olsen, S. C., J. Rhyan, et al. (2004). "SAFETY OF BRUCELLA ABORTUS STRAIN RB51 IN BLACK BEARS." J. Wildl. Dis. **40**(3): 429-433.

http://www.jwildlifedis.org/cgi/content/abstract/40/3/429

In two studies conducted from October 1999 to March 2000 and December 2000 to April 2001, adult black bears (Ursus americanus) were orally inoculated with 1.4-3.1x1010 colony-forming units (CFU) of Brucella abortus strain RB51 (SRB51, n=12) or 2 ml of 0.15 M NaCl solution (saline, n=11). We did not detect a difference (P>0.05) in antibody titers to SRB51 in serum obtained before vaccination, at 8 wk after vaccination, or at necropsy at 21 or 23 wk after vaccination between SRB51-vaccinated and nonvaccinated bears. The SRB51 vaccine strain was recovered from tissues obtained at necropsy from one of six SRB51-vaccinated bears in study 1, but none of the six SRB51-vaccinated bears in study 2. Vaccination of black bears with SRB51 did not appear to influence (P>0.05) reproductive performance.

Rotstein, D. S., T. R. Schoeb, et al. (2002). "Detection by microsatellite analysis of early embryonic mortality in an alligator population in Florida." J. Wildl. Dis. **38**(1): 160-165.

http://www.jwildlifedis.org/cgi/content/abstract/38/1/160

In the 1980s, alligators (Alligator mississippiensis) of Lake Apopka (Florida, USA) underwent a population decline associated with decreased egg viability, effects that have been associated with endocrine-disrupting, persistent organochlorine pesticides. It is currently unknown whether the decreased egg viability is due to fertilization failure or early embryonic death. Therefore, we conducted a preliminary study to evaluate the use of microsatellite DNA loci to determine the fertilization status of nonviable eggs. Using microsatellite analysis, we compared genotypes from blasto-disks and embryos with the genotypes from females trapped at the nest. Four of five nonviable egg samples tested yielded evidence of fertilization. No evidence of unfertilized eggs was obtained, but amplifiable DNA could not be obtained from one entirely nonviable clutch. Thus, we demonstrate that early embryonic mortality in alligators can be detected by microsatellite analysis, but also suggest substantial effort is needed to improve the recovery of DNA and amplification of alligator microsatellite loci.

Schettler, E., J. Fickel, et al. (2003). "Newcastle disease virus and Chlamydia psittaci in free-living raptors from eastern Germany." J. Wildl. Dis. **39**(1): 57-63.

http://www.jwildlifedis.org/cgi/content/abstract/39/1/57

Organ samples from free-living raptors from the federal states of Berlin and Brandenburg in eastern Germany were tested for Newcastle disease virus (NDV; n = 331) and Chlamydia psittaci (n = 39) by polymerase chain reaction (PCR). In 18 individuals NDV nucleic acids were detected. These samples originated from barn owls (Tyto alba; n = 15, 28%), tawny owl (Strix aluco; n = 1, 5%), common buzzard (Buteo buteo, n = 1, 1%), and European kestrel (Falco tinnunculus; n = 1, 4%). In 29 (74%) of 39 samples C. psittaci was detected. Chlamydia psittaci is common in free-living birds of prey in the investigated area.

Stabel, J. R., M. V. Palmer, et al. (2003). "Immune responses after oral inoculation of weanling bison or beef calves with a bison or cattle isolate of Mycobacterium avium subsp. paratuberculosis." J. Wildl. Dis. **39**(3): 545-555.

http://www.jwildlifedis.org/cgi/content/abstract/39/3/545

Paratuberculosis is endemic in domestic and wild ruminants worldwide. We designed the following study to compare host immune responses and pathologic changes in beef calves and bison calves after challenge with either a cattle or bison (Bison bison) strain of Mycobacterium avium subsp. paratuberculosis. In the first part of the study, six bison and six beef calves were orally inoculated with a cattle isolate of M. avium subsp. paratuberculosis over a 2 wk period. In the second part, an additional six bison and six beef calves were similarly inoculated with a bison strain of M. avium subsp. paratuberculosis. Throughout each of the studies, blood and fecal samples were taken monthly for a 6 mo infection period. Tissue samples were obtained at necropsy for culture and histopathologic analyses. Results from this study demonstrated that bison calves were more susceptible to tissue colonization than beef calves after challenge with the cattle isolate and, conversely, that beef calves were more susceptible to the bison strain of M. avium subsp. paratuberculosis. Although lesions were minimal they were most apparent in the

jejunum and distal ileum. Interferon-gamma (IFN-gamma) responses were noted in some calves by 1 mo postinoculation and were sustained longer in beef calves after challenge with the bison isolate. Antibody was not detected in either beef or bison calves during the 6 mo infection period. These results indicate that the host response to strains of M. avium subsp. paratuberculosis may differ between ruminant species.

Journal of Archaeological Science (3)

Bathurst, R. R. and J. L. Barta (2004). "Molecular evidence of tuberculosis induced hypertrophic osteopathy in a 16th-century Iroquoian dog." Journal of Archaeological Science **31**(7): 917.

http://www.sciencedirect.com/science/article/B6WH8-4BH64WX-5/2/bea0b91b551e6b86e86de4613695e3e6

A fully articulated dog skeleton excavated from a 16th-century Neutral Iroquoian site in Ontario, Canada displays a distinctive osteological condition known as hypertrophic osteopathy (HPO). Ancient DNA (aDNA) analysis of the dog has isolated Mycobacterium tuberculosis complex DNA, linking the secondary condition of HPO to tuberculosis (TB) and representing the oldest known case of TB yet to be discovered in the domestic dog. We emphasize that dogs should be considered as potential reservoirs of TB into the Americas.

Yang, D. Y., A. Cannon, et al. (2004). "DNA species identification of archaeological salmon bone from the Pacific Northwest Coast of North America." Journal of Archaeological Science **31**(5): 619.

http://www.sciencedirect.com/science/article/B6WH8-4B53YBF-1/2/3bdc2f1e331ce325510afaa5d127bef6

This paper reports on the development and application of methods for using DNA analysis for species identification of archaeological salmon bone. Short fragments (less than 300 bp) of mitochondrial DNA from the control region (D-loop) and cytochrome B (CytB) gene were targeted for amplification using the polymerase chain reaction (PCR) technique. The method was used on more than 20 salmon bone samples (dated 7000 to 2000 BP) from the site of Namu on the central coast of British Columbia. Four species: coho, sockeye, pink and chum salmon were identified from the samples. The results are considered valid since systematic contaminations were not detected, multiple species and multiple DNA haplotypes of the same species were identified from the same set of bone samples, and the identified species are consistent with those inferred from other lines of evidence. The results demonstrate the applicability of the ancient DNA technique to species identification of even single salmon vertebrae from archaeological sites in the Pacific Northwest of North America.

Yang, D. Y., J. R. Woiderski, et al. (2005). "DNA analysis of archaeological rabbit remains from the American Southwest." Journal of Archaeological Science **32**(4): 567.

http://www.sciencedirect.com/science/article/B6WH8-4FFGJ5Y-2/2/f9bd7490eff97c81aaa6ff63d4b2a6ab

Ancient DNA analysis was carried out on 20 archaeological rabbit remains from an early Pueblo II period site in Colorado (circa 1000 A.D.) to explore the possibility of obtaining accurate rabbit genus and species identifications. The presence of abundant rabbit remains at archaeological sites in the American Southwest indicates the importance of rabbit species in the subsistence economy and ritual activities of early aboriginal populations. The study of these remains is hindered by the difficulty of accurate identification due to the fragmentary nature of the bones and the lack of genus- and species-specific morphological features.A short cytochrome b gene fragment was amplified and sequenced to produce a genetic profile for each bone sample. At the genus level, the DNA identifications were consistent with those based on the analysis of mandible morphology for the majority of specimens. When compared to species-specific reference DNA sequences, Lepus americanus and Lepus californicus samples were easily identified. Identification of an unexpected L. americanus (snowshoe hare) from the remains provided new information concerning hunting ranges or exchange between groups in the region. Sylvilagus nuttallii and Sylvilagus audubonii, however, could not be confidently differentiated at this point due to the difficulty in obtaining accurate species-specific reference sequences.The inability to obtain such reference sequences can be a serious problem for DNA species identification of non-domestic animals that lack population-level genetic data and have few sequences available in GenBank. The lack of the DNA data increases the possibility that inappropriate reference sequences could be applied, resulting in false species identification even when authentic DNA is retrieved and amplified from ancient remains.

Journal of Cereal Science (3)

Altenbach, S. B. and K. M. Kothari (2004). "Transcript profiles of genes expressed in endosperm tissue are altered by high temperature during wheat grain development." Journal of Cereal Science **40**(2): 115.

http://www.sciencedirect.com/science/article/B6WHK-4D34KBK-1/2/678e7ac2bdbe20c72e1e7982d7e37a83

Timing of transcript accumulation for genes involved in a variety of cellular processes was assessed by RT-PCR in endosperm from developing wheat grains grown under moderate (24/17 [deg]C day/night) and high (37/28 [deg]C day/night) temperature regimens. Under moderate temperatures, transcripts for proteins with storage functions were present at all five time points examined between 7 and 34 DPA, while transcripts for proteins involved in signal transduction, protein synthesis and metabolism were most abundant from 7 to 20 DPA. Transcripts for proteins that play roles in defense were present from 14 DPA, about the time that starch accumulation commenced, to 34 DPA. High temperatures advanced and compressed the timing of transcript accumulation during grain development. Comparisons of transcript profiles with the timing of key events in grain development identified genes whose transcripts were accumulated at equivalent stages under the two temperature regimens and might serve as markers for grain development. These comparisons also revealed a number of genes with transcript profiles that were shifted under high temperatures in a manner that was not consistent with developmental events. These genes may be involved in responses to high temperature that are distinct from effects on the timing of developmental processes.

Ikeda, T. M., N. Ohnishi, et al. (2005). "Identification of new puroindoline genotypes and their relationship

to flour texture among wheat cultivars." Journal of Cereal Science **41**(1): 1.

http://www.sciencedirect.com/science/article/B6WHK-4F0GBM3-1/2/03606ff2b31f35c0628089e05059f08e

Puroindoline genotypes (Pina and Pinb) and their encoded proteins related to grain hardness were studied in various common wheat cultivars from Australia, China, Japan, Korea and North America. Most of the hard wheats had the Pinb-D1b genotype with a glycine to serine mutation at position 46. In addition to the known Pina and Pinb genotypes, cultivars were found with Pina and Pinb double-null mutations (Pina-D1b/Pinb-D1h (t)) and a new Pinb frameshift mutation (designated Pinb-D1i (t)) within the region encoding a tryptophan-rich domain. This new Pinb frameshift mutation was found only in Chinese cultivars. Endosperm proteins encoded by Pina and Pinb in these cultivars were analysed by 2D-gel electrophoresis (IPGXSDS-PAGE). Cultivars with Pina and Pinb double-null mutations showed no PIN-a or PIN-b protein, and cultivars with Pinb-D1i (t) had no PIN-b protein. Surprisingly, cultivars with Pinb-D1b had severely reduced amounts of PIN-b and cultivars with Pinb-D1c showed no PIN-b proteins. Grain hardness among cultivars having mutated Pinb may be explained by the amount of PIN-b protein and not by the type of amino acid substitutions.

Maruyama-Funatsuki, W., K. Takata, et al. (2005). "Identification and characterization of a novel LMW-s glutenin gene of a Canadian Western Extra-Strong wheat." Journal of Cereal Science **41**(1): 47.

http://www.sciencedirect.com/science/article/B6WHK-4DVTG8K-1/2/9f88e65b09d5b9dcb9a32e9907ec8f36

A near-isogenic line (NIL) into which low-molecular-weight glutenin subunits (LMW-GSs) were introduced from a Canadian Western Extra-Strong wheat cultivar (Triticum aestivum L.), 'Glenlea', into a Japanese spring wheat cultivar, 'Harunoakebono', had a much better bread-making quality than does Harunoakebono. LMW-GSs associated with good bread-making quality of the NIL and the allelic LMW-GS in Harunoakebono were monitored by two-dimensional polyacrylamide gel electrophoresis in BC5F2 progenies derived from a cross between Harunoakebono and Glenlea. The results show that LMW-GSs associated with good bread-making quality consist of five LMW glutenin components that co-segregate in a segregating population and that the allelic LMW-GS derived from Harunoakebono is also composed of five LMW glutenin components that co-segregate. N-terminal amino acid sequences of the five LMW glutenin components start with serine residues (LMW-s). Reverse transcription-polymerase chains reactions using primers specific to an LMW glutenin gene were performed for Harunoakebono, the NIL and Glenlea and amplified a novel LMW-s glutenin gene for Glenlea and the NIL and another LMW-s glutenin gene for Harunoakebono. Genomic PCRs using primers designed on the basis of internal sequences of the genes, s-F1/s-R2, were performed for the segregating population and amplified two DNA fragments that correspond to the LMW-s glutenin genes in reverse transcription-polymerase chain reaction and are, respectively, linked to LMW glutenin components from Glenlea associated with good bread-making quality and the allelic components from Harunoakebono. The results suggest that a novel LMW-s glutenin gene encodes LMW glutenin components associated with good bread-making quality.

Journal of Clinical Forensic Medicine (1)

Chattopadhyay, P. and S. Guha "RAPD & FINS (mitochondrial 16s rRNA gene sequence) in analysis of food contamination: detection of food poisoning by Lizard." Journal of Clinical Forensic Medicine **In Press, Corrected Proof** http://www.sciencedirect.com/science/article/B6WHN-4DJ49HS-1/2/ac01b2bcf45336052a58c9df386af167

In poisoning, detection of the nature of causative agent is important for management of trauma and forensic investigation. Most of the methods in clinical toxicology are developed for detection of toxins and poisons. A random amplified polymorphic DNA (RAPD)-based method has been described for detection of species of animal from its morphologically unrecognizable fragments, recovered from food substances, consumption of which caused even death. Pre-mixed RAPD reaction beads and six RAPD primers were used in polymerase chain reaction analysis. Among six RAPD primers used, any one of them was sufficient in resolving this practical forensic situation. To enhance the probability values for matching in the present study of fixing identity of an animal, six set of market available RAPD primers were used. This is the first report of a forensic application of RAPD DNA typing in identification of charred skeleton remnants of Lizard species in food material. Furthermore unique amplicons were generated for different reptilian species, which can be used as species specific markers for species identification in forensic situation, however, no variations among individuals of same species were observed.

Journal of Clinical Microbiology (2)

Al-Soud, W. A. and P. Radstrom (2001). "Purification and Characterization of PCR-Inhibitory Components in Blood Cells." Journal of Clinical Microbiology **39**(2): 485.

http://jcm.asm.org/cgi/content/abstract/39/2/485

In a recent study, immunoglobulin G in human plasma was identified as a major inhibitor of diagnostic PCR (W. Abu Al-Soud, L. J. Jonsson, and P. Radstrom. J. Clin. Microbiol. 38:345-350, 2000). In this study, two major PCR inhibitors in human blood cells were purified using size exclusion and anion-exchange chromatographic procedures. Based on N-terminal amino acid sequencing and electrophoretic analysis of the purified polypeptides, hemoglobin and lactoferrin were identified as PCR-inhibitor components in erythrocytes and leukocytes, respectively. When different concentrations of hemoglobin or lactoferrin were added to PCR mixtures of 25 {micro}l containing 10 different thermostable DNA polymerases and 1 ng of Listeria monocytogenes DNA as template DNA, AmpliTaq Gold, Pwo, and Ultma were inhibited in the presence of [<=]1.3 {micro}g of hemoglobin and [<=]25 ng of lactoferrin, while rTth and Tli were found to resist inhibition of at least 100 {micro}g of hemoglobin. In addition, the quantitative effects of seven low-molecular-mass inhibitors, present in blood samples or degradation products of hemoglobin, on real-time DNA synthesis of rTth using the LightCycler Instrument were investigated. A reaction system based on a single-stranded poly(dA) template with an oligo(dT) primer annealed to the 3' end was used. It was found that the addition of 0.25 to 0.1 mg of bile per ml, 2.5 mM CaCl2, 0.25 mM EDTA, 5 {micro}M FeCl3, and 0.01 IU of heparin per ml reduced the fluorescence to approximately 76, 70, 46, 17, and 51%, respectively. Finally, the effects of nine amplification facilitators were studied in the presence of hemoglobin and lactoferrin. Bovine serum albumin (BSA) was the most efficient amplification facilitator, so that the addition of 0.4% (wt/vol) BSA allowed AmpliTaq Gold to amplify DNA in the presence of 20 instead of 1 {micro}g of hemoglobin and 500 instead of 5 ng of lactoferrin. Including 0.02% (wt/vol) gp32, a single-stranded-DNA binding protein, in the reaction mixture of AmpliTaq Gold was also found to reduce the inhibitory effects of hemoglobin and lactoferrin.

Hendolin, P. H., L. Paulin, et al. (2000). "Clinically Applicable Multiplex PCR for Four Middle Ear Pathogens." Journal of Clinical Microbiology **38**(1): 125.

http://jcm.asm.org/cgi/content/abstract/38/1/125

The multiplex PCR method for the detection of Alloiococcus otitidis, Haemophilus influenzae, Moraxella catarrhalis, and Streptococcus pneumoniae (P. H. Hendolin, A. Markkanen, J. Ylikoski, and J. J. Wahlfors, J. Clin. Microbiol. 35:2854-2858, 1997) in middle ear effusions (MEEs) was modified to be better suited for clinical use. To detect false-negative results, an internal amplification was added to the reaction, and to prevent carryover contamination, the dUTP-uracil-N-glycosidase system was incorporated into the procedure. Labor was minimized by using the heat-activatable AmpliTaq Gold polymerase in order to circumvent manual hot start and by detecting the amplification products on an automated sequencer. The performance of the improved protocol was verified with MEEs from patients with otitis media with effusion. In addition, a ligase detection reaction (LDR) was developed for confirmation of the PCR products. The modifications increased the reliability of the protocol and the hands-off time significantly. However, when two DNA extraction protocols were compared, gram-negative bacteria were detected more often in phenol-treated MEEs (94 versus 46%; $P < 0.001$), and gram-positive bacteria were detected more often in MEEs dissolved in sodium dodecyl sulfate-NaOH-chaotropic salt (83 versus 27%; $P < 0.001$). The LDR was found to be 100% specific. In all, the results demonstrate the feasibility of the rapid (7-h) multiplex PCR method for routine laboratory use.

Journal of Experimental Marine Biology and Ecology (7)

Beynon, C. M. and D. O. F. Skibinski (1996). "The evolutionary relationships between three species of mussel (Mytilus) based on anonymous DNA polymorphisms." Journal of Experimental Marine Biology and Ecology **203**(1): 1.

http://www.sciencedirect.com/science/article/B6T8F-3W2515R-1/2/9ed4f3bc90f62bda0889203d8785ebaa

Anonymous nuclear DNA polymorphisms were developed for the marine mussel Mytilus by cloning random segments of nuclear DNA, sequencing the ends, constructing primers to amplify the cloned segments, and restricting the PCR product. The technique of PCR RFLP analysis was applied, for three cloned segments, using genomic DNA preparations from the three closely related mussel species, Mytilus edulis L., Mytilus galloprovincialis (Lamarck) and Mytilus trossulus (Gould) sampled from European populations. M. trossulus was found to differ at the nucleotide level from the other two species which were closely similar. This result contrasts with that obtained for mtDNA where M. edulis and M. trossulus have higher resemblance, and for allozymes where genetic distance values between the species are similar. The contrasting results for mtDNA and nuclear polymorphisms can be attributed to extensive mtDNA gene flow between M. edulis and M. trossulus.

Hualkasin, W., P. Sirimontaporn, et al. (2003). "Molecular phylogenetic analysis of white prawns species and the existence of two clades in Penaeus merguiensis." Journal of Experimental Marine

Biology and Ecology **296**(1): 1.

http://www.sciencedirect.com/science/article/B6T8F-4950C78-1/2/65233e8030210dc362121fa4db215146

Closely related species, Penaeus merguiensis and Penaeus silasi from Thai waters, were genetically examined using variation observed in 558 base pairs (bp) of sequence from cytochrome oxidase subunit I (COI) gene of mtDNA. The sequence divergences of COI between P. merguiensis and other Penaeus species were 5.76-6.15% (P. silasi), 13.17-13.97% (Penaeus indicus), 16.43% (Penaeus vannamei), 16.63% (Penaeus monodon), and 18.37% (Penaeus japonicus). From the alignment reported that there were four clades on phylogenetic tree, the distinction of the two monophyletic clades was referred as P. merguiensis, one monophyletic clade within P. silasi and P. indicus. These results point toward the possibility of P. merguiensis being a complex of two cryptic species or a single species with strong phylogeographic subdivision.

Loomis, S. H. and M. Zinser (2001). "Isolation and identification of an ice-nucleating bacterium from the gills of the intertidal bivalve mollusc Geukensia demissa." Journal of Experimental Marine Biology and Ecology **261**(2): 225.

http://www.sciencedirect.com/science/article/B6T8F-436F9F7-7/2/b7a24ee14d84ba8b3044a308e6f00be8

In the fall, freeze tolerant intertidal invertebrates usually produce ice-nucleating proteins that are secreted into the hemolymph. These proteins help protect against freeze damage by insuring that ice formation is limited to extracellular spaces. Geukensia demissa, a freeze tolerant, salt marsh bivalve mollusc was examined for the presence of ice nucleating proteins. The ice-nucleating temperature (INT) of the hemolymph was not significantly different from artificial seawater of the same salinity indicating the lack of an ice nucleating protein in the hemolymph. The palial fluid did have an elevated INT, indicating the presence of an ice nucleator. The INT of the palial fluid was significantly reduced by boiling and filtration through a 0.45-[mu]m filter. High INT was also observed in the seawater associated with the bivalves, and was demonstrated in water samples collected from salt marshes but not sand and pebble beaches. Moreover, the INT of water samples collected from a salt marsh decreased in the summer. All of these data suggest that the ice-nucleating agents in the hemolymph and the seawater are ice-nucleating bacteria. One species of ice-nucleating bacteria, Pseudomonas fulva was isolated from the gills of Geukensia. These bacteria could perform the same function as hemolymph ice-nucleating proteins by limiting ice formation to extracellular compartments.

Rosel, P. E. and T. D. Kocher (2002). "DNA-based identification of larval cod in stomach contents of predatory fishes." Journal of Experimental Marine Biology and Ecology **267**(1): 75.

http://www.sciencedirect.com/science/article/B6T8F-44B4JBK-1/2/64503c5a72d037ed3aaa047af553da74

Predator-prey interactions play an influential role in determining the demographics of a population or species. In the Northwest Atlantic, Atlantic cod, Gadus morhua, once the basis of a lucrative commercial fishery, have not recovered despite regulations imposed on the fishery to reduce harvest rates. One possible reason for the lack of recovery is that high predation pressure on juvenile and larval stages, particularly from species such as herring and mackerel, may regulate the abundance of cod. However, traditional methods used to identify larval cod and haddock often

fail when applied to partially digested remains. Here, we described a DNA-based assay to identify the presence of digested cod remains from the stomachs of predatory fish species. After development, the assay was tested on two sets of field samples. Larval and juvenile cod were successfully detected in both tests.

Schein, V., A. L. F. Chitto, et al. (2005). "Effects of hypo- or hyperosmotic stress on gluconeogenesis, phosphoenolpyruvate carboxykinase activity, and gene expression in jaw muscle of the crab Chasmagnathus granulata: seasonal differences." Journal of Experimental Marine Biology and Ecology **316**(2): 203.

http://www.sciencedirect.com/science/article/B6T8F-4F08F01-1/2/3f1f931c39ba62f77cb346cbbbe15e20

In its habitat, Chasmagnathus granulata is exposed to many different environmental challenges according to the season of the year. For this reason, the investigation of the participation of the gluconeogenic pathway in the acclimation to hypo- and hyperosmotic conditions in summer and winter was considered interesting.By comparing the gluconeogenesis capacity, phosphoenolpyruvate carboxykinase (PEPCK) activity, and mRNA PEPCK gene expression values obtained in control crabs during summer and winter, we found two opposite tendencies: a decrease in winter and an increase in summer.The present results show that, in the C. granulata jaw muscle, PEPCK activity is divided almost equally between the cytosol and mitochondria in winter crabs. This distribution is different from the one found in muscle from summer crabs, in which most of the PEPCK activity (85%) takes place in the mitochondria. The data reported here show that the natural light/dark cycle typical for winter regulated the proportion of PEPCK activity in the cytosolic and mitochondrial fractions at the transcriptional level, with a marked decrease in the mitochondrial PEPCK activity and, as a result, in the gluconeogenic capability.The gluconeogenic activity decreased 48% after 24 h of hyperosmotic stress in summer. Furthermore, this treatment reverted the proportion of PEPCK activity in cytosolic and mitochondrial fractions: it decreased in the mitochondrial fraction and increased in the cytosolic one. However, at 72 h of hyperosmotic shock, the incorporation of label from alanine into glucose increased 45% as compared to a 24-h group. Hence, it is possible that the increase in gluconeogenic capacity after 72 h of hyperosmotic stress is due to the enhanced PEPCK gene expression at 24 h of osmotic stress.The present study shows that the rises in the incorporation of 14C-alanine into glucose and in the mitochondrial and cytosolic activities in the jaw muscle of C. granulata after 24 h of acclimation to a dilute media in summer did not require an increase in PEPCK gene expression. During hypoosmotic shock in winter, the gluconeogenenic capacity and the PEPCK activity are still remarkably low, and the PEPCK gene expression remains undetectable.The muscle gluconeogenesis seems to be one of the pathways implicated in the metabolic adjustment during hypo- and hyperosmotic shock in C. granulata. On the other hand, the present study highlights the importance of seasonal environmental differences in determining the development of metabolic patterns.

Strychar, K. B., M. Coates, et al. "Loss of Symbiodinium from bleached soft corals Sarcophyton ehrenbergi, Sinularia sp. and Xenia sp." Journal of Experimental Marine Biology and Ecology **In Press, Corrected Proof** http://www.sciencedirect.com/science/article/B6T8F-4FN7706-1/2/80c429bb1e2e4934cbd91f1b19a107a7

The deleterious effects of temperature-induced coral bleaching, a process by which corals lose their endosymbiotic algae (zooxanthellae; genus Symbiodinium) primarily at temperatures above mean yearly maximums, has not been well described for alcyonacean soft corals (Coelenterata, Octocorallia). The study of Symbiodinium cells lost from Sarcophyton ehrenbergi, Sinularia sp., and Xenia sp., which have not been compared in bleaching studies, indicate that the soft coral S.

ehrenbergi released the greatest number of symbiont cells, however, it was less susceptible to heat stress surviving temperatures of 34 [deg]C for >39 h. Sinularia sp. showed intermediate levels of bleaching tolerance to elevated temperatures, surviving prolonged exposures at 32 [deg]C, but dying within 24 h at 34 [deg]C. Xenia sp., however, was the most vulnerable to high heat stress maximally releasing Symbiodinium at temperatures [less-than or equal to]30 [deg]C. This evidence indicates that Xenia sp. is even more susceptible to elevated temperatures than Acropora spp., previously reported to be the most vulnerable coral species to elevated temperature-induced bleaching.Molecular analysis showed that the more resistant soft coral species (S. ehrenbergi) had the same type of Symbiodinium (clade C) as less resistant soft corals (Xenia sp.). In comparison to scleractinian corals collected from the same region that show similar bleaching resistance to high temperatures (e.g. Porities solida--more robust; Favites complanata--moderate resistance; Acropora hyacinthus--less robust), all scleractinian corals were symbiotic with Symbiodinium from clade C. A. hyacinthus, however, was found to possess multiple symbionts (clades B and C), and this represents a first report of Clade B in any Acropora species.

Udomkit, A., S. Chooluck, et al. (2000). "Molecular cloning of a cDNA encoding a member of CHH/MIH/GIH family from Penaeus monodon and analysis of its gene structure." Journal of Experimental Marine Biology and Ecology **244**(1): 145.

http://www.sciencedirect.com/science/article/B6T8F-3YGDCSP-8/2/0d8701129cd355e5932b01f996e10d50

We report the nucleotide and deduced amino acid sequences of Pem-CMG peptide, a member of crustacean CHH/MIH/GIH peptide family, in black tiger prawn (Penaeus monodon). The 5' and 3' fragments of Pem-CMG cDNA were cloned by the method of rapid amplification of cDNA ends (RACE). The two fragments constitute a combined cDNA length of 593 bp with a 77 bp overlapping region. Sequence analysis reveals the presence of a 384 bp open reading frame which was subsequently cloned. The open reading frame encodes a precursor peptide that is comprised of 128 amino acids, with a putative processing site, KR. The mature peptide consists of 74 amino acid residues, the sequence of which is significantly homologous to those of the CHH/MIH/GIH family known from other crustaceans. Analysis of a genomic fragment of Pem-CMG reveals a single intron of 314 bp interrupting the coding sequence for the mature peptide. The presence of only one intron in Pem-CMG gene suggests that this gene is structurally different from the previously reported MIH gene of Charybdis feriatus and CHH-like gene of Metapenaeus ensis which possess two introns in their coding sequences.

Journal of Fermentation and Bioengineering (4)

Chartrain, M., B. Jackey, et al. (1998). "Bioconversion of indene to cis (1S,2R) indandiol and trans (1R,2R) indandiol by Rhodococcus species." Journal of Fermentation and Bioengineering **86**(6): 550.

http://www.sciencedirect.com/science/article/B6T8G-3W4PNJF-5/2/719fa2896b60027a2ba4b081017bb402

cis (1S,2R) indandiol or trans (1R,2R) indandiol are both potential precursors to (-)-cis (1S,2R)-1-

aminoindan-2-ol, a key chiral synthon for Crixivan(R) (Indinavir), a leading HIV protease inhibitor. Enrichment and isolation studies yielded two Rhodococcus sp. strain B 264-1 (MB 5655) and strain I-24 (MA 7205) capable of biotransforming indene to cis (1S,2R) indandiol and trans (1R,2R) indandiol respectively. Isolate MB 5655 was found to have a toluene dioxygenase, while isolate MA 7205 was found to harbor both toluene and naphthalene dioxygenases as well as a naphthalene monooxygenase. When scaled up in a 14-l bioreactor, MB 5655 produced up to 2.0 g/l of cis (1S,2R) indandiol with an enantiometric excess greater than 99%. MA 7205 cultivated under similar conditions produced up to 1.4 g/l of trans (1R,2R) indandiol with an enantiomeric excess greater than 98%. Process development studies yielded titers greater that 4.0 g/l of cis indandiol for MB 5655. Due to their resistance to indene toxicity and easy cultivation in bioreactors, both Rhodococcus sp. strains appeared as good candidates for future strain engineering and process development work.

Intapruk, C., K. Yamamoto, et al. (1993). "Cloning of cDNAs encoding two peroxidases of Arabidopsis thaliana and their organ-specific expression." Journal of Fermentation and Bioengineering **75**(3): 166.

http://www.sciencedirect.com/science/article/B6T8G-4865H3Y-8R/2/f12392d089e0d349ff9da80d0a6ba66a

Two cDNAs, prxCa and prxEa, encoding peroxidase isozymes of Arabidopsis thaliana, ARP Ca and ARP Ea, respectively, were isolated by polymerase chain reaction using total RNA from stem and primers designed from genomic DNAs. The coding regions of the prxCa and prxEa cDNAs were identical to the putative exon regions in the genomic DNAs (intapruk, C. et al., Gene, 98, 237-241, 1991) and contained 1092 and 1078 bp, respectively. The deduced amino acid sequences of ARP Ca and ARP Ea suggested the presence of leader peptides of 31 and 29 amino acid residues at the N-termini, and 308 and 307 amino acid residues in mature proteins, respectively. The amino acid sequence of ARP was compared with those of other plant peroxidases: ARP showed highly conserved alignment with the horseradish peroxidase (HRP) family (group I), with 64 to 91% homology except for HRP n; lower similarity to rice, tobacco, cucumber, turnip, wheat and peanut peroxidases (group II) with 40 to 51% homology; and a very low level of homology of about 37% for potato and tomato peroxidases. The prxCa and prxEa were close to HRP neutral and basic isozyme genes, respectively, but the estimated isoelectric points of ARP Ca and ARP Ea showed values of 8.05 and 6.34, respectively. By Northern blot hybridization, the prxCa gene was shown to be expressed non-specific expression in many organs of A. thaliana, while the level of transcripts of the prxEa gene was found to be abundant in root but very low in leaf and stem.

Karita, S., T. Kimura, et al. (1997). "Purification of the Ruminococcus albus endoglucanase IV using a cellulose-binding domain as an affinity tag." Journal of Fermentation and Bioengineering **84**(4): 354.

http://www.sciencedirect.com/science/article/B6T8G-3RXXYBF-F/2/61e72c887f2cb34b633d625faa8181e1

The gene encoding the single cellulose-binding domain II (CBD II) of Clostridium stercorarium xylanase A was fused to the egIV gene encoding endoglucanase IV (EGIV) from Ruminococcus albus. The fusion protein (EGIV + CBDII) expressed in Escherichia coli can be readily purified from the cell-free extract of E. coli in a single step using the affinity of CBD to cellulose. The purified enzyme was cleaved into two moieties, i.e. the catalytic domain and CBD, at a specific site in the linker region by partial digestion with trypsin at 4[deg]C. This result indicates that this CBD belonging to family VI of CBD families can be used as an affinity tag for purification of the

recombinant protein.

Park, J. K., T. Okamoto, et al. (1997). "Molecular cloning, nucleotide sequencing, and regulation of the chiA gene encoding one of chitinases from Enterobacter sp. G-1." Journal of Fermentation and Bioengineering **84**(6): 493.

http://www.sciencedirect.com/science/article/B6T8G-3S6MP0K-2/2/c522e5956790b615d88340057e43b360

The chiA gene encoding a chitinase of 60-kDa has been cloned from Enterobacter sp. G-1 by PCR using synthetic oligonucleotides corresponding to the amino acid sequences of the purified enzyme and subsequently genomic library screening was performed. The products of the positive clones were found to degrade water-insoluble chitin. The primary structure of the chiA gene consisted of 1,686-bp encoding 562 amino acid residues. Comparison of the deduced amino acid sequence of the cloned chitinase gene product (chiA) with other chitinases revealed a high homology (95.7% identity) with chitinase A from Serratia marcescens QMB1466. The coding region of the chiA gene for higher expression in Escherichia coli was identified using deletion and sequence analysis. The expression of the chiA gene in Enterobacter sp. G-1 was controlled by presence of chitin, as determined by Northern blotting hybridization analysis. We found that the expression of the chiA gene in E. coli was controlled by an inverted repeat sequence located in the upstream region from a promoter region.

Journal of Hospital Infection (4)

Chiew, Y. F. and L. M. C. Hall (1998). "Comparison of three methods for the molecular typing of Singapore isolates of enterococci with high-level aminoglycoside resistances." Journal of Hospital Infection **38**(3): 223.

http://www.sciencedirect.com/science/article/B6WJP-4CDJ0YX-8/2/be18b13d8fc4a465721b243c12f7b9b4

Enterococci are frequently isolated as nosocomial pathogens and have often acquired intrinsic drug resistances. Molecular typing techniques have been developed to assist in epidemological and infection control measures. This study investigates enterococci with high-level aminoglycoside resistance (HLAR) from the National University Hospital (NUH) of Singapore, and evaluates and compares three methods for typing: restriction enzyme analysis by conventional gel electrophoresis [restriction fragment length polymorphism (RFLP)], pulsed-field gel electrophoresis (PFGE), and polymerase chain reaction (PCR) using random amplified polymorphic DNA (RAPD). Fifty-two isolates of Enterococcus faecalis and 13 isolates of Enterococcus faecium were used for the study. The numbers of patterns obtained for E. faecalis and E. faecium were 26 and 4, respectively by the RFLP method, and very similar discrimination was obtained by PFGE. RAPD PCR results were not reliably reproducible. A single pattern type by RFLP accounted for 16 of the E. faecalis isolates, suggesting hospital spread.

Loudon, K. W., A. P. Coke, et al. (1996). "Kitchens as a source of Aspergillus niger infection." Journal of

Hospital Infection **32**(3): 191.

http://www.sciencedirect.com/science/article/B6WJP-4C7633Y-1H/2/9fca7908a67e0478a30d86b22390fc03

In this study we investigated the epidemiology of a cluster of cutaneous infections owing to Aspergillus niger, which occurred in neutropenic patients in a bone marrow transplant unit. Heavy environmental contamination with the mould was found in the ward kitchen adjacent to the unit. The clinical and environmental isolates were typed by random amplification of polymorphic DNA (RAPD), which showed one of the patients was infected with the same strain as that isolated repeatedly from the kitchen area. In another case, contaminated stockinette material was implicated as the source of infection. Thorough cleaning of the ward kitchen resulted in no further cases on the unit. This highlights the fact that aspergilli may spread to patients by air, food or other vehicles, and underlines the importance of searching for a source and ensuring high levels of hospital hygiene are maintained.

Skibsted, U., D. L. Baggesen, et al. (1998). "Random amplification of polymorphic DNA (RAPD), pulsed-field gel electrophoresis (PFGE) and phage-typing in the analysis of a hospital outbreak of Salmonella Enteritidis." Journal of Hospital Infection **38**(3): 207.

http://www.sciencedirect.com/science/article/B6WJP-4CDJ0YX-6/2/20297cd3c4e6394eb02fa38c441c3d3e

Isolates of Salmonella Enteritidis from 81 patients from Herlev Hospital or from Copenhagen County were analysed by random amplification of polymorphic DNA (RAPD), pulsed-field gel electrophoresis (PFGE) and phage-typing. Fourteen polymorphic markers from five decamer primers unambiguously placed all isolates into six RAPD groups: 65 isolates of phage-type 6 (PFGE type I) were resolved into three RAPD groups constituting 86, 12, and 2%, respectively. A fourth RAPD group of 10 isolates was coincident with phage type 8 (PFGE type II) and two isolates, one phage-type 1, the other phage-type 4 (both PFGE type I) formed the fifth group. The sixth group of four isolates was not phage typeable and was PFGE type III. Forty outbreak-related isolates of phage-type 6 were resolved into three strains. No diversity of phage-type 6 was found among isolates unrelated to the outbreak. It is concluded that RAPD is useful as a tool in investigations of microbial outbreaks in its own right, or to supplement phage-typing and PFGE of Salmonella Enteritidis.

Xu, J., C. L. Smyth, et al. (2004). "Employment of 16 S rDNA gene sequencing techniques to identify culturable environmental eubacteria in a tertiary referral hospital." Journal of Hospital Infection **57**(1): 52.

http://www.sciencedirect.com/science/article/B6WJP-4C59SHV-2/2/369500e641c6fb788f1db3e26453da6e

Universal or 'broad-range' eubacterial polymerase chain reaction (PCR) was performed on 53 isolates from environmental water-associated sites in a haematology unit (N=22), and the outer surfaces of cleaning lotion containers sited throughout a tertiary referral hospital (N=31). 16 S rDNA PCR was performed using two sets of universal primers, including the novel reverse primer, XB4, to generate a composite amplicon of 1068 bp, which was sequenced to obtain each isolate's identity. Sequence analysis was able to identify 51 isolates. Most (75% from the haematology unit and 81% from cleaner containers) were Gram-positive. Nine different genera were identified from the haematology unit and 13 from the cleaning lotion containers. This study

provides the first reports of Terrabacter spp. and Brachybacterium paraconglomeratum isolated from a hospital environment. As unusual and difficult-to-identify environmental organisms are unlikely to be clinically significant, and molecular identification is costly and labour-intensive, we recommend that molecular methods are only used as an adjunct to first-line phenotypic identification schemes where a definitive identification is required. Where molecular identification methods are justified, partial 16 S rDNA PCR and sequencing employing the novel universal primer XB4, is a valuable and reliable technique.

Journal of Infection (6)

Al-Salloom, F. S., A. Al Mahmeed, et al. (2003). "Campylobacter-stimulated INT407 cells produce dissociated cytokine profiles." Journal of Infection **47**(3): 217.

http://www.sciencedirect.com/science/article/B6WJT-491RP1C-2/2/baa36fb3c0864f794a504ef4f9d227a3

Objectives. To study the action of factors produced by living Campylobacter jejuni (C. jejuni) against those present within sonicated and filtrated bacteria on induction of potential cytokines by the human intestinal cell line INT407.Methods. We used immunohistochemical technique modified to detect intracellular production of cytokines protein and RT-PCR to read RNA messages for evaluation of de novo cytokine synthesis.Results. The data herein display dissociation of cytokine profiles induced on by living C. jejuni. Exposure of INT407 cells to 106 live bacteria showed the highest numbers of cytokine producing cells of all examined cytokines. IFN-[gamma] was the highest induced cytokine followed by IL-10, TNF-[alpha] and lastly IL-4. Also, abrogation of induction of the proinflammatory cytokines IFN-[gamma] and TNF-[alpha] but not the antiinflammatory cytokines IL-4 and IL-10 by sonicated and filtrated bacteria was depicted. At the mRNA level, TNF-[alpha] signals were noted in accordance with its protein levels since increased TNF-[alpha] mRNA signals were registered only after stimulation with living bacteria. Very low or no induction of TNF-[alpha] was registered with non-stimulated cells.Conclusions. These results illustrate for the first time a role for factors from living bacteria in directing the immune response towards Th1 type. Characterization of such factors may be essential for future immunotherapeutic interventions during severe bacterial infections.

Ashshi, A. M., P. E. Klapper, et al. (2003). "Detection of human cytomegalovirus, human herpesvirus type 6 and human herpesvirus type 7 in urine specimens by multiplex PCR." Journal of Infection **47**(1): 59.

http://www.sciencedirect.com/science/article/B6WJT-48JSJH1-1/2/46b03e4f2b4b8c13cc17d86143e26ed7

Objectives. To develop a sensitive multiplex PCR to detect HCMV, HHV6 and HHV7, to test this PCR on urine specimens sent to the virus diagnostic laboratory and on stored urine samples from HIV-positive patients and their HIV-negative partners and to compare the sensitivity of the multiplex PCR with the diagnostic laboratory's routine service for the detection of HCMV.Study design. Primers specific for each of the three viruses were combined in a multiplex PCR that was then optimised for sensitivity. This PCR was applied prospectively to 413 unselected routine urine specimens over a 1 year period and retrospectively to 258 urine specimens from 63 HIV-positive

patients and 10 HIV-negative partners.Methods. In the prospective study, the multiplex PCR detected 40 specimens positive for HCMV alone, 10 for HHV6, 3 for HHV7 and 3 with a dual infection of HCMV and HHV6. The sensitivity for HCMV was 93.5% by multiplex PCR compared to 28.3% by culture. HHV6 DNA was detected in 6 neonates (2-21 days) and HHV7 DNA in 2 neonates (4 and 20 days). In the retrospective study of HIV patients, HCMV was the most commonly detected virus (55.6%) compared to HHV6 (7.9%) and HHV7 (4.8%).Conclusions. The multiplex PCR was significantly more sensitive than non-DNA based procedures for the detection of HCMV. Urine may be a useful non-invasive specimen for the detection of HHV6 and HHV7 and their presence in neonates suggest perinatal transmission or the possibility of in utero infection.

Bakhiet, M., F. S. Al-Salloom, et al. (2004). "Induction of [alpha] and [beta] chemokines by intestinal epithelial cells stimulated with Campylobacter jejuni." Journal of Infection **48**(3): 236.

http://www.sciencedirect.com/science/article/B6WJT-4BDM2K7-1/2/cc5d7040b208975927e4039ca0875a22

Objectives. To investigate the production of dynamic [alpha] and [beta] chemokines represented by interleukin-8 (IL-8) as [alpha] chemokine and CCL2 (monocyte-chemoattractant protein-1, CCR2 ligand), CCL4 (macrophage-inflammatory protein-1[beta], CCR5 ligand), CCL3 (macrophage-inflammatory protein-1[alpha], CCR1/5 ligand), (CCL5, regulated upon activation, normal T-cell expressed and secreted (RANTES, CCR5 ligand) as [beta] chemokines by the human intestinal cell line INT407 stimulated with factors produced by living Campylobacter jejuni (C. jejuni) and those present within sonicated and filtrated bacteria.Methods. We used immunohistochemical technique modified to detect intracellular production of cytokines protein and RT-PCR to read RNA messages for evaluation of de novo cytokine synthesis.Results. Living bacteria induced increased numbers of IL-8, CCL4 and CCL2 but not CCL3 or CCL5 producing cells. Low numbers of IL-8, CCL4 and CCL2 producing cells were detected with filtrated supernatant compared to living and sonicated bacteria. A non-significant low number of chemokine producing cells was noted when comparing numbers of chemokine producing cells stimulated with living C. jejuni to those stimulated with sonicated bacteria, indicating that the triggering factors involved in stimulation with living bacteria were still active after sonication, but they were largely lost upon filtration. The mRNA signals for IL-8 were noted in conformity with its protein levels as increased IL-8 mRNA signals were registered after stimulation with living and sonicated bacteria but not with filtrated supernatant.Conclusions. Preferential production of chemokines probably induced by membrane associate factors of C. jejuni acting on intestinal epithelial cells is presented. These chemokines are suggested to be part of an inflammatory network affecting cell types that contribute to initiation and/or resolution of the infection.

Boone, S. A. and C. P. Gerba "The occurrence of influenza A virus on household and day care center fomites." Journal of Infection **In Press, Corrected Proof**
http://www.sciencedirect.com/science/article/B6WJT-4DPYJMT-2/2/1dd74cc83157ae5332b07fc981d46d29

ObjectiveThe goal of this study was to evaluate the prevalence of influenza A virus on surfaces in day care and home settings to better assess the potential role of fomites in the transmission of influenza.MethodsDuring two and a half years, 218 fomites were tested from 14 different day care centers. Ten different fomites from bathrooms, kitchens and play areas were sampled. In addition, 92 fomites from eight different homes with children were tested over 6 months. Fourteen different household fomites from bathrooms, kitchens and living areas were sampled. Influenza A viral RNA was detected using reverse transcriptase-polymerase chain reaction.ResultsInfluenza was detected on 23% of day care fomites sampled during the fall and 53% of fomites sampled during the spring. Spring and fall sample data was determined to be statistically different at the

0.05 [alpha]-level by Chi-square analysis PP=0.00002. There was no statistical difference found between moist and dry fomites (Chi square P=0.13998). No influenza was detected on home fomites sampled during the summer. In contrast, influenza was detected on 59% of home fomites sampled during March.ConclusionsInfluenza A virus was detected on over 50% of the fomites tested in homes and day care centers during influenza season.

Xu, J., J. E. Moore, et al. (2003). "Identification of a novel [alpha]-proteobacterium causing bacteraemia in an immunocompetent patient." Journal of Infection **47**(2): 167.

http://www.sciencedirect.com/science/article/B6WJT-48S4KR9-1/2/a2e902ae243a0ac54f03fdfdf8e41d71

An 89-year male with pyrexia and suspected bacteremia was admitted to hospital, where a Gram-negative rod was identified from blood culture. The organism was difficult to identify phenotypically and the resulting sequencing of a 559 bp section of the 16S rRNA gene did not have a high homology score (>97.0%) with any deposited GenBank accession numbers and hence was not able to be assigned to a species within any genus. Given that the isolate was a member of the alpha subclass of the Proteobacteria but did not fall into any of the known genera with more than 93.7% homology (Brucella, Rhizobium, Ochrobactrum, Agrobacterium), we believe this isolate to represent a novel [alpha]-proteobacterium, which was the cause of bacteraemia in this patient.

Yee, E. K., L. J. Melkerson-Watson, et al. (2004). "Genomic analysis of penicillin-resistant Streptococcus pneumoniae in Southeastern Michigan." Journal of Infection **49**(2): 126.

http://www.sciencedirect.com/science/article/B6WJT-4C604VR-1/2/0be762e87f0f3e339a3778882e328e39

Objective. The emergence of multidrug resistance within Streptococcus pneumoniae population was analysed, correlating penicillin resistance Pen(R) with secondary antibiotic resistance, capsular serotype, and genetic diversity among isolates.Methods. DNA fingerprinting, following macro-restriction enzyme digestion and pulse field gel electrophoresis (PFGE), and restriction fragment analysis of the PBP 2b gene, following PCR amplification, were performed on the Pen(R) S. pneumoniae, among 377 clinical isolates obtained from the clinical microbiology laboratory (University of Michigan Medical Center).Results. Overall 35% of the isolates were Pen(R) of which 45% demonstrated high-level penicillin (Pen(R)-R, MIC>1). Respiratory isolates were more likely to be Pen(R) (ppConclusion. The emergence of multidrug resistance in the S. pneumoniae population in SE Michigan is not due to expansion of a single or limited number of resistant clones, is occurring most frequently in the paediatric population and is associated with a decreased susceptibility to penicillin.

Journal of Insect Physiology (5)

Albert, S. and J. Klaudiny (2004). "The MRJP/YELLOW protein family of Apis mellifera: Identification of new members in the EST library." Journal of Insect Physiology **50**(1): 51.

http://www.sciencedirect.com/science/article/B6T3F-4B1SDRY-1/2/4968d9a683b0f6abb17ecddfcf60b3d9

Major royal jelly proteins (named MRJP1-5) of honeybee (Apis mellifera), yellow proteins of Drosophila, together with putative proteins found in several bacteria, form a protein family termed the MRJP/yellow family. Members of the family exert diverse physiological functions and amongst eukaryotes appear to be restricted to the order Insecta. MRJPs constitute about 90% of total protein of royal jelly, which is secreted by nurse bees to feed the queen and growing larvae.We looked for mrjp and yellow homologues in a honeybee brain expressed sequence tags (EST) library. In addition to the five mrjp cDNAs previously characterized, we found three additional cDNAs encoding novel MRJPs and importantly, two cDNAs coding for orthologues of Drosophila yellow proteins. One yellow cDNA and all three cDNAs coding for the novel MRJPs were assembled completely, the sequence of the other yellow homologue was partially assembled.The data we present here supports the view that repeated duplications and functional divergence occurred during the evolution of MRJPs in honeybees, with even closely related MRJPs appearing to perform diverse physiological functions. Conversely, yellow protein orthologues appear to be conserved and thus candidates for maintaining the former function(s) of yellow proteins.

Goto, S. G. (2000). "Expression of Drosophila homologue of senescence marker protein-30 during cold acclimation." Journal of Insect Physiology **46**(7): 1111.

http://www.sciencedirect.com/science/article/B6T3F-408C8N2-5/2/98c1c4a70dddecc7b7f84b2d055e88f9

Gene expression during cold acclimation at a moderately low temperature (15[deg]C) was studied in Drosophila melanogaster using a subtraction technique. A gene homologous to senescence marker protein-30 (SMP30), which has a Ca2+-binding function, was up-regulated at the transcription level after acclimation to 15[deg]C. This gene (henceforth referred to as Dca) was also expressed at a higher level in individuals reared at 15[deg]C from the egg stage than in those reared at 25[deg]C. Moreover, DCA mRNA increased at the senescent stage in Drosophila, although SMP30 is reported to decrease at senescent stages in mammals. In situ hybridization to polytene chromosomes revealed that the Dca gene was located at 88D on chromosome 3R. The 5' flanking region of this gene had AP-1 (a transcription factor of SMP30) binding sites, stress response element and some other transcription factor binding sites. The function of DCA was discussed in relation to the possible regulation of cytosolic Ca2+ concentration.

Jamroz, R. C., F. D. Guerrero, et al. (2000). "Molecular and biochemical survey of acaricide resistance mechanisms in larvae from Mexican strains of the southern cattle tick, Boophilus microplus." Journal of Insect Physiology **46**(5): 685.

http://www.sciencedirect.com/science/article/B6T3F-3YWXJFY-9/2/03258d254390c4b93c36b2683c21eafa

We examined the larvae of several organophosphate and pyrethroid-resistant Mexican strains of Boophilus microplus using biochemical and molecular tests to investigate the mechanisms conferring acaricide resistance. The electrophoretic profiles of esterase activity in protein extracts from coumaphos and permethrin-resistant strains compared to the susceptible strain revealed distinct differences, which inhibitor studies attributed to carboxylesterases. Esterase hydrolysis assays showed significant enhancement of both total and permethrin hydrolysis in one pyrethroid-resistant strain, with no enhancement in two other strains with very high resistance to pyrethroids.

Sequence analysis of sodium channel mRNA fragments in all pyrethroid-resistant strains determined that they did not possess the classic kdr and super-kdr mutations known to confer pyrethroid resistance in several insect species. Using reverse transcriptase-polymerase chain reaction (RT-PCR) with degenerate primers designed from conserved regions of insect esterase amino acid sequences, a B. microplus larval cDNA fragment was isolated whose deduced amino acid sequence was significantly similar to esterases from a wide range of species. In Northern blot RNA analysis the cDNA hybridized to a 2.1 kb mRNA that was abundant in all resistant strains except one, in which a very low abundance could provide a marker for the mechanism conferring resistance in this strain.

Lewis, D. K., D. Spurgeon, et al. (2002). "A hexamerin protein, AgSP-1, is associated with diapause in the boll weevil." Journal of Insect Physiology **48**(9): 887.

http://www.sciencedirect.com/science/article/B6T3F-46SNK0B-7/2/bd8f7bd10a6266e8bbdaf9dbd22423b5

The objective of this research was to identify a reliable biochemical indicator for diapause (dormancy) in the boll weevil, Anthonomus grandis. Hemolymph polypeptides from reproductive and diapausing weevils were compared using denaturing sodium dodecyl sulpfate (SDS)-polyacrylamide gel electrophoresis (PAGE). A 77-kDa protein, which proved to be a hexamerin (AgSP-1), strongly correlated with morphological diapause characters in both male and female adult weevils. N-terminal sequence analysis identified the first 25 amino acids of the mature protein and was used to develop an antibody to AgSP-1. Anti-AgSP-1 reacted only with hemolymph from diapausing weevils of both sexes but not with hemolymph from reproductive weevils. Also, the yolk protein, vitellogenin (VG), inversely correlated with AgSP-1. When hemolymph VG was high, AgSP-1 was absent or barely perceptible.Juvenile hormone regulates VG synthesis in most insect species. Juvenile hormone is reported to stimulate reproductive maturation in the boll weevil (Physiological Entomology 22 (1997) 261) and to be absent during diapause (Physiological Entomology 22 (1997a) 269). Therefore, the juvenile hormone (JH) mimic, methoprene, was used to assess the role of JH activity in the boll weevil for terminating diapause, stimulating reproductive maturation and possibly influencing AgSP-1 titers. Application of methoprene was not effective in activating reproductive development. Hemolymph from methoprene-treated, females contained VG and AgSP-1 titers that were similar to acetone-treated and untreated control weevils.Using a genomic DNA library and 3' RACE, two clones were isolated that yielded the complete sequence of AgSP-1 as well as a portion of the 5' untranslated region. Northern blot analysis confirmed the presence of a 2.5 kB transcript for AgSP-1 in the fat body of diapausing weevils. AgSP-1 was also present in the fat body of reproductive weevils, but to a lesser extent. No sex-related differences in gene expression were observed; diapausing weevils of both sexes showed similar levels of AgSP-1 expression. An inverse correlation was observed between the VG transcript and AgSP-1 mRNA. VG was highly expressed in the fat body of reproductive females and only slightly expressed in tissue from diapausing females. Our data suggests that AgSP-1 is a diapause-specific protein in adult weevils and that JH, alone, is not effective in terminating diapause.

Nakabachi, A. and H. Ishikawa (1999). "Provision of riboflavin to the host aphid, Acyrthosiphon pisum, by endosymbiotic bacteria, Buchnera." Journal of Insect Physiology **45**(1): 1.

http://www.sciencedirect.com/science/article/B6T3F-3VKS2SP-1/2/955600051653b8c6360eaee42deb6832

Differential cDNA display and quantitative RT-PCR suggested that the riboflavin synthase complex of the aphid endosymbiont, Buchnera, is active only when the symbiotic system is

maintained and well organized in young hosts. Since this finding suggested the provision of riboflavin by Buchnera, we examined the effect of dietary riboflavin on the performance of symbiotic and aposymbiotic aphids using chemically-defined diets. Our results indicate: (1) dietary riboflavin is slightly detrimental to young, symbiotic aphids; (2) dietary riboflavin is essential to aposymbiotic aphids; (3) dietary riboflavin remarkably improves the performance of aposymbiotic aphids. These results strongly suggest that young, symbiotic aphids are provided with riboflavin by their endosymbionts, Buchnera.

Journal of Invertebrate Pathology (4)

Castrillo, L. A., J. D. Vandenberg, et al. (2003). "Strain-specific detection of introduced Beauveria bassiana in agricultural fields by use of sequence-characterized amplified region markers." Journal of Invertebrate Pathology **82**(2): 75.

http://www.sciencedirect.com/science/article/B6WJV-47VYRMD-2/2/bafbe1ad0fb428b62ee1878b121e06d2

Field studies on the efficacy and persistence of an introduced strain of Beauveria bassiana for insect control require detection assays to differentiate the non-native strain from indigenous populations. In this study we developed strain-specific molecular markers based on polymerase chain reaction amplification of sequence-characterized amplified regions (SCAR) in combination with dilution plating on semi-selective medium to detect and estimate density of propagules of a commercial strain of B. bassiana (strain GHA) in field samples. Using random amplified polymorphic DNA (RAPD) analysis, unique fragments that distinguished GHA from other strains of B. bassiana were obtained. Three amplicons, OPA-140.44, OPA-150.44, and OPB-90.67, generated with RAPD primers were cloned and sequenced and used as bases for designing SCAR primers OPA14 F/R445, OPA15 F/R441, and OPB9 F/R677, respectively. All three SCAR primers were highly sensitive, capable of detecting 100 pg B. bassiana GHA genomic DNA, and thus could be used to detect varying levels of the fungus in the field.

Fuxa, J. R., M. L. Milks, et al. (2005). "Interaction of an entomopathogen with an insect social form: an epizootic of Thelohania solenopsae (Microsporidia) in a population of the red imported fire ant, Solenopsis invicta." Journal of Invertebrate Pathology **88**(1): 79.

http://www.sciencedirect.com/science/article/B6WJV-4DS94TM-2/2/a5dcf8e07ccbd314422934e2d71d3898

This is the first report of Thelohania solenopsae infections in monogyne (single-queen) Solenopsis invicta colonies in the field. In a 0.2-ha plot near Baton Rouge, Louisiana, inter-colony prevalence was 63% infection in June, 1999, when the population was 100% monogyne. In February, 2000, 21% of 33 monogyne and 90% of 10 polygyne colonies were infected. By May, 2001, the polygyne colonies had disappeared and only one of 34 monogyne colonies was infected, the final detection of T. solenopsae in the plot. Colony size did not differ significantly among the four types (monogyne versus polygyne X infected versus uninfected).

Nielsen, C. B., D. Cooper, et al. (2002). "DNA polymerase gene sequences indicate western and forest tent caterpillar viruses form a new taxonomic group within baculoviruses." Journal of Invertebrate Pathology **81**(3): 131.

http://www.sciencedirect.com/science/article/B6WJV-47G3RXM-5/2/85e5f47319c2de0120a327d067ff6d0d

Vandergast, A. G. and G. K. Roderick (2003). "Mermithid parasitism of Hawaiian Tetragnatha spiders in a fragmented landscape." Journal of Invertebrate Pathology **84**(2): 128.

http://www.sciencedirect.com/science/article/B6WJV-49J8V7T-1/2/3fb5ce019e231e8e44b794d4da04c844

Hawaiian Tetragnatha spiders inhabiting small forest fragments on the Big Island of Hawaii are parasitized by mermithid nematodes. This is the first report of mermithid nematodes infecting spiders in Hawaii, and an initial attempt to characterize this host-parasite interaction. Because immature mermithids were not morphologically identifiable, a molecular identification was performed. A phylogenetic analysis based on 18S small ribosomal subunit nuclear gene sequences suggested that Hawaiian spider mermithids are more closely related to a mainland presumptive Aranimemis species that infects spiders, than to an insect-infecting mermithid collected on Oahu, HI, or to Mermis nigrescens, also a parasite of insects. Measured infection prevalence was low (ranging from 0 to 4%) but differed significantly among forest fragments. Infection prevalence was associated significantly with fragment area, but not with spider density nor spider species richness. Results suggest that mermithid populations are sensitive to habitat fragmentation, but that changes in infection prevalence do not appear to affect spider community structure.

Journal of Microbiological Methods (58)

Baric, S. and J. Dalla-Via (2004). "A new approach to apple proliferation detection: a highly sensitive real-time PCR assay." Journal of Microbiological Methods **57**(1): 135.

http://www.sciencedirect.com/science/article/B6T30-4BMK1J2-1/2/a6785d054a1cac445558c18f12262182

The present paper describes a new approach for diagnosis of apple proliferation (AP) phytoplasma in plant material using a multiplex real-time PCR assay simultaneously amplifying a fragment of the pathogen 16S rRNA gene and the host, Malus domestica, chloroplast gene coding for tRNA leucine. For the first time, such an approach, with an internal analytical control, is described in a diagnostic procedure for plant pathogenic phytoplasmas enabling distinction between uninfected plant material and false-negative results caused by PCR inhibition. Pathogen detection is based on the highly conserved 16S rRNA gene to ensure amplification of different AP phytoplasma strains. The newly designed primer/probe set allows specific detection of all examined AP strains, without amplifying other fruit tree phytoplasmas or more distantly related phytoplasma strains. Apart from its specificity, real-time PCR with serial dilutions of initial template DNA ranging over almost five orders of magnitude (undiluted to 80,000-fold diluted) demonstrated linear amplification over the whole range, while conventional PCR showed a

reliable detection only up to 500-fold or 10,000-fold dilutions, respectively. Compared to existing analytical diagnostic procedures for phytoplasmas, a rapid, highly specific and highly sensitive diagnostic method becomes now available.

Barlaan, E. A., M. Sugimori, et al. (2005). "Profiling and monitoring of microbial populations by denaturing high-performance liquid chromatography." Journal of Microbiological Methods **61**(3): 399.

http://www.sciencedirect.com/science/article/B6T30-4FH5K2W-2/2/d1763515fc5c067c10dc446f217b0607

We describe a new molecular technique for the analysis of microbial species and complex microbial populations based on the separation of PCR-amplified 16S rDNA fragments by denaturing high-performance liquid chromatography (DHPLC). Using marine bacterial samples, we determined the optimum conditions for the analysis of bacterial species and the examination of complex bacterial assemblages obtained from different environments. The incorporation of a 40-bp GC clamp into the amplification primer was essential to effectively discriminate genetic differences in DHPLC--primers with a 20-, 10-, or 0-bp GC clamp length were less efficient. A 64.5 [deg]C column temperature in DHPLC allowed optimal separation of species in a complex bacterial population. PCR-DHPLC analysis of bacterial assemblages demonstrated profiles with distinguishable peaks, which constituted the different populations and their degree of abundance. Fraction collection and DNA sequencing from profile peaks enabled bacterial identification. PCR-DHPLC analysis can also provide opportunities for describing bacterial communities, cloning bacteria, and monitoring bacterial populations in environments of interest.

Berg, E. S. and K. Skaug (2003). "Liposome encapsulation of the internal control for whole process quality assurance of nucleic acid amplification-based assays." Journal of Microbiological Methods **55**(1): 303.

http://www.sciencedirect.com/science/article/B6T30-492VY3R-4/2/57ca25a5f6f59fa5b6590691798be078

A system intended for whole process quality assurance of nucleic acid amplification assays was developed based on the use of liposomes as cell-mimicking vehicles for the internal control, allowing introduction of the internal control directly into the crude biological specimens. By the proof of principle testing, the Roche Cobas Amplicor(R) CT assay was chosen as model system and the Roche CT/NG Internal Control was thus loaded into the liposomes. The liposome/DNA particles were spiked into a Chlamydia trachomatis-positive urine specimen. A quantitative "in-house" duplex real-time C. trachomatis PCR assay showed that liposomes having Blue Dextran 2000 polysaccharide co-entrapped were the most suited particles as they were efficiently deposited by the centrifugation carried out according to the Roche urine specimen preparation procedure. Furthermore, it was demonstrated that the liposome/DNA particles might be used for whole process quality assurance of Amplicor(R) assay without major modifications of the assay protocol. An additional feature of the use of these liposomes was that the pellet became blue coloured and that might facilitate a thorough removal of the urine supernatant without increasing the risk of disturbance of the pellet. Principally, the liposome/internal control system is versatile and seems to be applicable for whole process quality control of amplification-based assays for detection of various pathogens.

Bertrand, I., C. Gantzer, et al. (2004). "Improved specificity for Giardia lamblia cyst quantification in

wastewater by development of a real-time PCR method." Journal of Microbiological Methods **57**(1): 41.

http://www.sciencedirect.com/science/article/B6T30-4B8BJ5M-1/2/f7ddb9cc37add799be085343f7166ef9

The protozoan parasite Giardia lamblia is the most common cause of waterborne disease outbreaks associated with drinking water in the United States. The conventional method used for the enumeration of Giardia cysts in water is based on immunofluorescence with monoclonal antibodies. It is tedious and time-consuming and has the major drawback to be non-specific for the only species infecting humans, G. lamblia. We have developed a real-time polymerase chain reaction (PCR) method using fluorescent TaqMan technology, which improved the specificity of G. lamblia cyst quantification compared to the immunofluorescence assay (IFA). However, this PCR was not totally specific for G. lamblia species and amplified Giardia ardeae target as well. This method showed a sensitivity of 0.45 cysts per reaction and an efficiency of 95% in purified suspensions. We have then applied this quantification method to raw wastewater, a medium containing numerous debris, particles and PCR inhibitors. The adaptation to these environmental samples was realized by a screening of three cyst purification methods and six DNA extraction protocols. Real-time quantification was accomplished by the simultaneous amplification of unknown samples and a tenfold serial dilution of purified G. lamblia cysts. For all samples, the concentrations observed with TaqMan PCR method were compared to the IFA values. Giardia spp. cysts were detected in all non-spiked raw wastewater samples with IFA procedure and the concentrations of Giardia spp. cysts used for the comparison between the two methods ranged between 3.3 x 102/l and 4.3 x 103/l. The highest TaqMan PCR/IFA ratios were observed when Percoll(R)/sucrose flotation was combined with DNA extraction protocol optimized for cyst wall lysis, impurities adsorption on a resin, and double step protein digestion and column purification. The concentrations observed with this TaqMan PCR method ranged from 2.5 x 102 to 2.4 x 103 G. lamblia cysts/l and only one sample resulted in a no amplification curve. Thus, we developed a TaqMan PCR method increasing the rapidity and specificity of G. lamblia cyst quantification. The combination of Percoll(R)/sucrose flotation and DNA extraction optimized protocol before TaqMan assay has provided a good indication of the G. lamblia contamination level in raw sewage samples.

Brancart, F., H. Rodriguez-Villalobos, et al. (2005). "Quantitative TaqMan PCR for detection of Pneumocystis jiroveci." Journal of Microbiological Methods **61**(3): 381.

http://www.sciencedirect.com/science/article/B6T30-4FG4V75-2/2/da8c604a81720555dd94149e0597ebd0

We developed a quantitative real-time PCR assay for detection and quantification of Pneumocystis jiroveci in bronchoalveolar lavage (BAL) specimens based on primers and probe targeting the gene encoding beta-tubulin. The assay was able to detect 50 DNA copies per ml of a standard plasmid containing the target sequence. The intra- and interassay coefficients of variation were 0.46%-4.27% and 0.05-2.00% over 5 log10 values. Fifty-seven controls of human, viruses, bacteria and fungi DNA samples were amplified and found negative. Fifty-three BAL samples sent to the laboratory for diagnosis of pneumocystosis were prospectively investigated by real-time PCR and direct microscopic examinations (DME) using Giemsa stain and direct immunofluorescence. All PCR negative samples were negative by microscopy. Among the 24 (45%) BAL found PCR positive, 8 were positive by microscopy (35%). The copy numbers of the target gene were between 4.4X103 and 2.8X106 per ml for the microscopically positive samples and between 8 and 9.2X103 per ml for the microscopically negative samples. In conclusion, we developed a rapid, sensitive and specific real time PCR for the diagnosis and quantification of Pneumocystis jiroveci in BAL samples.

Calvo, L. and L. J. Garcia-Gil (2004). "Use of amoB as a new molecular marker for ammonia-oxidizing bacteria." Journal of Microbiological Methods **57**(1): 69.

http://www.sciencedirect.com/science/article/B6T30-4BBHF0Y-1/2/7123f69d1b8b60f6d85c680eba21b437

Specific molecular determination and classification of ammonia-oxidizing bacteria have relied on the use of conventional markers such as 16S rDNA. However, this gene does not satisfactorily provide a wide vision of all phylogenetic lineages. Despite the initial expectations, the use of functional genes as for example amoA has only been useful to corroborate the established taxonomy. Ammonia-oxidizing bacteria constitute a physiological group that crosses over principal phylogenetic radiations. Therefore, it is necessary to look for novel functional markers, which are needed for both diversity and taxonomic studies.In this work, the available amoB sequences have been used to design a new degenerate set of primers flanking a ca. 500-bp region. Partial amoB gene sequences of up to 16 AOB strains (5 Nitrosomonas, 10 Nitrosospira, and 1 Nitrosococcus) belonging to both the [beta]- and the [gamma]-Proteobacteria have been obtained. Comparison of both DNA and deduced amino acid sequences results in three subgroups, two of them of the [beta]-Proteobacteria and a third one of the [gamma]-Proteobacteria displaying 75% and 35% homology in their deduced amino acid sequences, respectively. This gene has proven to be a suitable molecular marker to study AOB, as well as providing a new insight into the classification of this group.

Chung, E., J. E. Aldom, et al. (1999). "PCR-based quantitation of Cryptosporidium parvum in municipal water samples." Journal of Microbiological Methods **38**(1-2): 119.

http://www.sciencedirect.com/science/article/B6T30-3XF07V4-F/2/41704d1beb3d48109efaee04a68ae063

A PCR method for the quantitation of Cryptosporidium parvum oocysts in municipal drinking water samples was investigated. Quantitative PCR uses an internal standard (IS) template with unknown target numbers to compare to standards of known concentrations in a standard curve. The IS template was amplified using the same primers used to amplify a portion of a 358 bp gene fragment that encodes a repetitive oocyst wall protein in C. parvum. Municipal water samples spiked with known numbers of C. parvum oocysts were tested by quantitative PCR using the IS and the Digene SHARP Signal(TM) System Assay for PCR product detection. The absorbance readings for target DNA and IS templates versus the number of molecules of the target DNA were plotted to generate standard curves for estimating oocyst numbers. The method allowed the quantitation of oocysts from log 3 to log 5 spiked into municipal water samples.

Chung, E., J. E. Aldom, et al. (1998). "Detection of Cryptosporidium parvum oocysts in municipal water samples by the polymerase chain reaction." Journal of Microbiological Methods **33**(2): 171.

http://www.sciencedirect.com/science/article/B6T30-3T87G0D-9/2/968d5f4392fd402812481d4f5793d1e8

The enteric protozoan, Cryptosporidium parvum, the causative agent for cryptosporidiosis, has been isolated from drinking water, fecal samples from humans and animals, and environmental samples such as sediment and soil. The currently available water sampling methods for detection of this parasite are labor-intensive and the efficiency of oocyst recovery is poor. A recent

improved method utilizing membrane filtration and dissolution followed by polymerase chain reaction (PCR) amplification, and confirmatory nested PCR was evaluated for the sensitive and specific detection of C. parvum oocysts. Detection of PCR products by the ELISA-based Digene SHARP Signal(TM) System Assay was assessed for sensitivity. Seventy-two municipal water samples ranging in volume from 230 to 1,000 l from southwestern Ontario, Canada were spiked with varying concentrations of formalin-killed C. parvum oocysts for use in this study. Oocyst recovery on the filters was determined by the Merifluor immunofluoresence assay for Cryptosporidium/Giardia. Oocyst detection using the PCR assay showed an 84.7% correlation with immunofluoresence assay (IFA) results. During optimization studies, the correlation between PCR and IFA reached 98%. The sensitivity of a primary PCR assay ranged from 1 to 10 oocysts per reaction, which was equivalent to 102 to 103 oocysts per 100 l municipal water. The PCR assay also showed potential for application to untreated water samples and naturally contaminated municipal water from a recent Cryptosporidium outbreak. Further application of nested PCR may improve overall sensitivity and specificity for detecting C. parvum in municipal water samples since combined primary and nested PCR results showed 97.2% correlation with IFA. The Digene SHARP Signal(TM) System assay offers a sensitive and specific alternative for detection of C. parvum amplification products.

Cohen, S. D. (2005). "A protocol for direct sequencing of multiple gene specific PCR products from Discula umbrinella, a fungal endophyte, utilizing bufferless precast electrophoresis." Journal of Microbiological Methods **61**(1): 131.

http://www.sciencedirect.com/science/article/B6T30-4DXSRKG-2/2/7c4b85d8c3c4f40a32672a8787e2e013

A protocol for direct sequencing of polymerase chain reaction (PCR) products from mycelia of Discula umbrinella, a fungal endophyte, using bufferless electrophoresis is described. This improved method allows researchers to conduct high-capacity screening of multiple gene regions for fungal endophytes applicable to microbial ecology and population genetic studies.

Costa, C., D. Vidaud, et al. (2001). "Development of two real-time quantitative TaqMan PCR assays to detect circulating Aspergillus fumigatus DNA in serum." Journal of Microbiological Methods **44**(3): 263.

http://www.sciencedirect.com/science/article/B6T30-42G0M1Y-9/2/c15ca754866fc01aba2bbf370417b738

Several PCR assays have been developed for detecting Aspergillus fumigatus DNA in blood of patients with invasive aspergillosis. However, the best blood fraction to be assayed has not been defined and the multicopy genes used as the DNA targets for amplification not characterized. Firstly, we developed a real-time PCR assays based on the TaqMan technology targeted to a single copy gene. To compare serum, white cell pellet, and plasma for effectiveness as blood assay fractions, we spiked whole blood with A. fumigatus DNA and processed these fractions similarly. The difference between white cell pellet and serum was not significant. In contrast, the yield from plasma was 10 times lower than from serum. Then, we compared serum processed immediately or after 24 h at room temperature and observed a lower yield after 24 h. Secondly, a real-time PCR assay targeted to a mitochondrial gene was also developed. The copy number was estimated between 9 and 10 mitochondrial genes per single copy gene. Therefore, we recommend serum, stored and frozen as soon as possible, to be used for detecting circulating A. fumigatus DNA for diagnosis. Moreover, the mitochondrial multicopy gene was characterized in order to compare results from different patients.

Dawson, S. L., J. C. Fry, et al. (2002). "A comparative evaluation of five typing techniques for determining the diversity of fluorescent pseudomonads." Journal of Microbiological Methods **50**(1): 9.

http://www.sciencedirect.com/science/article/B6T30-44XTRVC-1/2/51db3d950497df1ad7c30c3e716311f8

Five typing methods were evaluated, utilising 63 strains of fluorescent pseudomonads, to assess their usefulness as tools to study the bacterial diversity within this complex group. The methods used were Biolog metabolic profiling, restriction fragment length polymorphism ribotyping, PCR ribotyping, and repetitive element sequence-based PCR (rep-PCR) utilising BOX and enterobacterial repetitive intergenic consensus (ERIC) primers. Cluster analysis of the results clearly demonstrated the considerable homogeneity of Pseudomonas aeruginosa isolates and, conversely, the heterogeneity within the other species, in particular P. putida and P. fluorescens, which need further taxonomic investigation. Biolog metabolic profiling enabled the best differentiation among the species. Rep-PCR proved to be highly discriminatory, more so than the other DNA fingerprinting techniques, demonstrating its suitability for the analysis of highly clonal isolates. RFLP ribotyping, PCR ribotyping, and rep-PCR produced specific clusters of P. aeruginosa isolates, which corresponded to their origins of isolation, hence we recommend these methods for intraspecific typing of bacteria.

de Nijs, M., L. Nabben, et al. (1996). "Isolation of Fusarium DNA for molecular analysis with and without mechanical cell disruption." Journal of Microbiological Methods **27**(1): 13.

http://www.sciencedirect.com/science/article/B6T30-3W25BXD-2/2/b8f8f320be1289f89bd54fc65b0c148d

When isolating DNA from fungal cells, the first step in many methods is disruption of cell walls to release the cell contents into the extraction buffer. Often, cell wall disruption is achieved by grinding of the mycelium under liquid nitrogen. There is a large variety in DNA yields and cross contamination can occur due to the cumbersomeness of this method. To shorten and facilitate the DNA isolation procedure, Fusarium cell walls were disrupted by a Polytron homogenizer. The use of the homogenizer for extended periods of time, yielded increasing amounts of DNA but, at the same time, caused increased shearing of DNA. The shearing, however, had no effect on RAPD patterns. In the course of the experiments, it was found that the DNA extraction buffer was capable of releasing substantial quantities of DNA from the Fusarium mycelium (range 0.5-1.6 mg g-1 mycelium), without a separate step for mechanical disruption of cell walls. Both methods described here for isolation of Fusarium DNA, with and without the use of a Polytron homogenizer, substantially reduce the risks of contamination and the risks related to the use of liquid nitrogen. When large quantities of DNA are required, mechanical disruption of the cells using a Polytron homogenizer is suitable. Direct addition of mycelium to the extraction buffer, without a separate step for mechanical cell disruption, is favourable when relatively small amounts of DNA are needed for analysis.

Del Prete, R., M. Quaranta, et al. (1998). "Detection of Mycobacterium paratuberculosis in stool samples of patients with inflammatory bowel disease by IS900-based PCR and colorimetric detection of amplified DNA." Journal of Microbiological Methods **33**(2): 105.

http://www.sciencedirect.com/science/article/B6T30-3T87G0D-1/2/0ad211aec2a643c4dd4a157410bfe63d

The hypothesis that Crohn's disease (CD) and ulcerative colitis (UC) may result from mycobacterial infection has been proposed. Among the atypical mycobacteria, Mycobacterium paratuberculosis has been often involved in the pathogenesis of CD and UC. The polymerase chain reaction (PCR) with a single primer pair from the nucleotide sequence of the 'Insertion Sequence' IS900 of M. paratuberculosis followed by a non-isotopic ELISA-like detection method of amplification products for the specific detection of M. paratuberculosis in human feces was developed. Fifteen (46.8%) of the 32 stool samples from patients with histologically confirmed CD, and nine (33.3%) of the 27 stool samples from patients with UC had a 229-bp fragment of M. paratuberculosis DNA detected by ethidium bromide agarose gel electrophoresis. Of the 41 stool samples used as negative control, 30 were from healthy subjects, nine from patients with other non-specific gastrointestinal diseases, and two from patients with colon cancer. Only one of these samples, namely from one of the patients with colon cancer, was positive by PCR. With regard to cultural technique, eight stool samples from patients with CD, five samples from patients with UC and one sample from a patient with colon cancer allowed the mycobacterial growth. The amplified PCR products were identified by using a colorimetric detection procedure designed DNA Enzyme ImmunoAssay (DEIA), based on the hybridization of the denatured DNA with a non-radioactively labelled inter-primer specific oligonucleotide probe. Severe precautions were taken to exclude either the possible contamination among the samples or false-positive results. Our findings confirm other works in which M. paratuberculosis has been considered the putative etiologic agent responsible for CD and UC. In addition, the newly developed PCR-DEIA technique, revealing a higher sensitivity than cultural technique and being much more rapid, represents a useful tool for both epidemiological and therapeutic purposes.

Deng, M.-Q. and D. O. Cliver (1999). "Rapid DNA extraction methods and new primers for randomly amplified polymorphic DNA analysis of Giardia duodenalis." Journal of Microbiological Methods **37**(2): 193.

http://www.sciencedirect.com/science/article/B6T30-3WV9H3M-B/2/81345858129633d4242000eda892e30b

A randomly amplified polymorphic DNA (RAPD) procedure using simple genomic DNA preparation methods and newly designed primers was optimized for analyzing Giardia duodenalis strains. Genomic DNA was extracted from in vitro cultivated trophozoites by five freezing-thawing cycles or by sonic treatment. Compared to a conventional method involving proteinase K digestion and phenol extraction, both freezing-thawing and sonication were equally efficient, yet with the advantage of being much less time- and labor-intensive. Five of the 10 tested RAPD primers produced reproducible polymorphisms among five human origin G. duodenalis strains, and grouping of these strains based on RAPD profiles was in agreement among these primers. The consistent classification of two standard laboratory reference strains, Portland-1 and WB, in the same group confirmed previous results using other fingerprinting methods, indicating that the reported simple DNA extraction methods and the selected primers are useful in RAPD for molecular characterization of G. duodenalis strains.

Deng, S.-J., R. J. Forster, et al. (1993). "Simulataneous amplification and sequencing of genomic DNA (SAS): sequencing of 16S rRNA genes using total genomic DNA from Butyrivibrio fibrisolvens, and detection and genotyping of nonculturable mycoplasma-like organisms directly from total DNA isolated from infected plants." Journal of Microbiological Methods **17**(2): 103.

http://www.sciencedirect.com/science/article/B6T30-476MNGF-G/2/fae7aa8b7b02bc06cd6b8909c7090657

A protocol for simultaneous amplification and sequencing of genomic DNA (SAS) was developed

which combines Sanger's dideoxy chain-terminating sequencing method and Mullis' polymerase chain reaction (PCR) technique into a single step procedure. Sequencing ladders were generated in SAS by the addition of ddNTPs into a PCR amplification mixture. The technique allowed the determination of 16S rRNA gene sequence for the identification of the rumen bacterium Butyrivibrio fibrisolvens directly from genomic DNA, without prior amplification by cloning or PCR. It also allowed the detection and genotyping of plant pathogenic mycoplasma-like organisms (MLOs) from a complex total DNA mixture prepared from infected plants without prior amplification.

Deng, S. and C. Hiruki (1991). "Amplification of 16S rRNA genes from culturable and nonculturable Mollicutes." Journal of Microbiological Methods **14**(1): 53.

http://www.sciencedirect.com/science/article/B6T30-47DKKG6-7/2/ceda9969737d45d41ada0d6ecde9de8c

Five polymerase chain reaction (PCR) primer pairs were synthesized on the basis of the aligned 16S-like rRNA sequences of eukaryotes or 16S rRNA sequences of eubacteria, Mollicutes, and intracellular organelles. These PCR primer pairs had high sequence homology to the conserved 16S rRNA genes of various culturable and nonculturable Mollicutes, but less sequence homology to the eukaryotic nuclear 16S-like rRNA or 16S rRNA genes of intracellular organelles. Full-length 16S rRNA genes and partial-length 16S rRNA genes of evolutionarily variable regions were successfully amplified when DNA preparations from culturable Mollicutes such as Mycoplasma flocculare and three Spiroplasma strains and nonculturable Mollicutes associated with various plant diseases were used as PCR templates. Amplifications were not detected when Escherichia coli genomic DNA and DNA preparations from healthy plants were used under high stringency annealing conditions in thermocycling. The results suggest the possibility that 16S rRNA genes of culturable and nonculturable Mollicutes can be amplified for detection and for a phylogenetic study using crude Mollicutes DNA preparations under appropriately controlled thermocycling conditions.

Duarte, G. F., A. S. Rosado, et al. (1998). "Extraction of ribosomal RNA and genomic DNA from soil for studying the diversity of the indigenous bacterial community." Journal of Microbiological Methods **32**(1): 21.

http://www.sciencedirect.com/science/article/B6T30-3SBNK2T-3/2/477a00f8da918e58c1424bf44dff5401

A method for the indirect (cell extraction followed by nucleic acid extraction) isolation of bacterial ribosomal RNA (rRNA) and genomic DNA from soil was developed. The protocol allowed for the rapid parallel extraction of genomic DNA as well as small and large ribosomal subunit RNA from four soils of different texture. DNA and rRNA yields from these soils were 15-30 and 0.25-1.0 [mu]g g-1 soil, respectively. Following different purification steps, the rRNA as well as genomic DNA extracts obtained were sufficiently pure for either reverse transcription and polymerase chain reaction (PCR) amplification, or direct PCR amplification. Using a set of universal bacterial primers based on conserved regions of the 16S rRNA sequence, both approaches yielded mixed target molecules for subsequent denaturing gradient gel electrophoresis fingerprinting of soil microbial diversity. The amplified rRNA-based bacterial diversity assessment was compared with diversity assessments based on amplified DNA in one selected soil. Results showed similarities as well as differences between the profiles generated on the basis of rRNA and those based on genomic DNA, which suggested that the bacterial communities defined on the basis of their genomic DNA contained variable amounts of rRNA.

Fach, P., S. Perelle, et al. (2003). "Comparison of different PCR tests for detecting Shiga toxin-producing Escherichia coli O157 and development of an ELISA-PCR assay for specific identification of the bacteria." Journal of Microbiological Methods **55**(2): 383.

http://www.sciencedirect.com/science/article/B6T30-4991VY1-4/2/7c4812942463184cd20e1576851a846e

In an attempt to develop a standard for ELISA-PCR detection of Shiga toxin producing Escherichia coli (STEC) O157, six published PCR tests were tested in a comparative study on a panel of 277 bacterial strains isolated from foods, animals and humans. These tests were based on the detection of the genes rfbE [J. Clin. Microbiol. 36 (1998) 1801] and rfbB [Appl. Environ. Microbiol. 65 (1999) 2954], the 3' end of the eae gene [Epidemiol. Infect. 112 (1994) 449], the region immediately flanking the 5' end of the eae gene [Int. J. Food. Microbiol. 32 (1996) 103], the flicH7 gene [J. Clin. Microbiol. 35 (1997) 656], or a part of the recently described 2634-bp Small Inserted Locus (SILO157 locus) of STEC O157 [J. Appl. Microbiol. 93 (2002) 250]. Unlike the other PCR assays, those amplifying the rfb sequences were unable to distinguish toxigenic from nontoxigenic O157. These assays were relatively specific to STEC O157, giving essentially a cross reaction with clonally related E. coli O55 and to a lesser extent with E. coli O145, O125, O126. They also detected the Shiga toxin (stx)-negative derivatives of STEC O157. Based on these results, an ELISA-PCR assay consisting of the solution hybridization of amplicons with two probes that ensured the specificity of the amplification was developed. The ELISA-PCR assay, which used an internal control (IC) of inhibition, was able to detect 1 to 10 copies of STEC O157 in the PCR tube. Adaptation of PCR into ELISA-PCR assay format facilitates specific and sensitive detection of PCR amplification products and constitutes a method of choice for screening STEC O157.

Flint, J. F. and E. R. Angert (2005). "Development of a strain-specific assay for detection of viable Lactobacillus sp. HOFG1 after application to cattle feed." Journal of Microbiological Methods **61**(2): 235.

http://www.sciencedirect.com/science/article/B6T30-4F65K92-2/2/26a4ad413e384265fa875c0dd9c04c6a

A strain-specific assay was developed for the detection of viable Lactobacillus on cattle feed. The DNA sequences of the 16S rRNA gene and four different 16S/23S rRNA intergenic spacer regions (ISR) from Lactobacillus sp. HOFG1 were determined. Based on these sequences, a strain-specific primer was designed for the amplification of one of the ISRs. When combined with a Lactobacillus group primer, the polymerase chain reaction (PCR) assay detected only Lactobacillus sp. HOFG1 and not other closely related L. animalis or L. murinus strains. The feed assay uses a combination of enrichment culturing and PCR to detect and enumerate viable Lactobacillus sp. HOFG1 after its application onto cattle feed. The high degree of primer specificity and use of selective culturing allows for the detection of viable Lactobacillus which is useful in tracking bacteria applied to complex feed mixtures that contain a high background of endogenous bacteria.

Fontaine, M. and E. Guillot (2003). "An immunomagnetic separation-real-time PCR method for quantification of Cryptosporidium parvum in water samples." Journal of Microbiological Methods **54**(1): 29.

http://www.sciencedirect.com/science/article/B6T30-480CRT1-3/2/0f14bafbd0900f11d289aff2ea694326

The protozoan parasite Cryptosporidium parvum is known to occur widely in both raw and drinking water and is the cause of waterborne outbreaks of gastroenteritis throughout the world. The routinely used method for the detection of Cryptosporidium oocysts in water is based on an immunofluorescence assay (IFA). It is both time-consuming and nonspecific for the human pathogenic species C. parvum. We have developed a TaqMan polymerase chain reaction (PCR) test that accurately quantifies C. parvum oocysts in treated and untreated water samples. The protocol consisted of the following successive steps: Envirochek(R) capsule filtration, immunomagnetic separation (IMS), thermal lysis followed by DNA purification using Nanosep(R) centrifugal devices and, finally, real-time PCR using fluorescent TaqMan technology. Quantification was accomplished by comparing the fluorescence signals obtained from test samples with those from standard dilutions of C. parvum oocysts. This IMS-real-time PCR assay permits rapid and reliable quantification over six orders of magnitude, with a detection limit of five oocysts for purified oocyst solutions and eight oocysts for spiked water samples. Replicate samples of spiked tap water and Seine River water samples (with approximately 78 and 775 oocysts) were tested. C. parvum oocyst recoveries, which ranged from 47.4% to 99% and from 39.1% to 68.3%, respectively, were significantly higher and less variable than those reported using the traditional US Environmental Protection Agency (USEPA) method 1622. This new molecular method offers a rapid, sensitive and specific alternative for C. parvum oocyst quantification in water.

Gelsomino, A., A. C. Keijzer-Wolters, et al. (1999). "Assessment of bacterial community structure in soil by polymerase chain reaction and denaturing gradient gel electrophoresis." Journal of Microbiological Methods **38**(1-2): 1.

http://www.sciencedirect.com/science/article/B6T30-3XF07V4-1/2/04c7dc9092859b72798dedc8bf36f8bd

Bacterial community structure was studied in a Flevo silt loam (FSL) soil microplot, as well as in 15 other soils, by using DNA extraction followed by molecular fingerprinting. Total community DNA was extracted and purified by a direct method, which yielded amplifiable DNA of high molecular weight for all soils. A variable region of the 16S rRNA gene was then amplified by PCR with bacterial primers, resulting in a mixture of amplicons separable via denaturing gradient gel electrophoresis (DGGE). The DGGE profiles of FSL soil were indicative of dominant soil bacterial types, as evidenced by assessing the amplification of Enterobacter cloacae and Arthrobacter sp. targets in a soil DNA background. These targets produced barely detectable bands when present in soil DNA at roughly 5 x 106 genome equivalents per g dry soil, and strong bands at 27-fold higher levels. The PCR-DGGE analysis of the FSL soil was highly reproducible. Furthermore, different single versus composite topsoil samples yielded similar DGGE profiles with respect to major bands. In addition, samples taken along vertical soil cores (0-45 cm depth) revealed relative stability of the DGGE profiles. The profiles produced with DNA obtained from different aggregate size fractions of this soil were also similar with respect to the main bands. Moreover, FSL topsoil samples taken over a 1-year period (fallow soil) yielded stable profiles. These data suggested that the soil bacterial communities thus determined were dominated by a limited number of stable and ubiquitous types. The 16 soils, representing varying types and geographical locations, were assessed for differences in their bacterial DGGE profiles. There were striking differences between the profiles obtained for these soils. Evidence was found for the hypothesis that similar soil types tend to contain similar structures of the dominating bacterial types as revealed by the DGGE profiles.

Haugland, R. A., N. Brinkman, et al. (2002). "Evaluation of rapid DNA extraction methods for the quantitative detection of fungi using real-time PCR analysis." Journal of Microbiological Methods **50**(3): 319.

http://www.sciencedirect.com/science/article/B6T30-45J9474-1/2/1cd9cf90bb9b3bfb631ff0fc926dbb3a

Three comparatively rapid methods for the extraction of DNA from fungal conidia and yeast cells in environmental (air, water and dust) samples were evaluated for use in real-time PCR (TaqMan(TM)) analyses. A simple bead milling method was developed to provide sensitive, accurate and precise quantification of target organisms in air and water (tap and surface) samples. However, quantitative analysis of dust samples required further purification of the extracted DNA by a streamlined silica adsorption procedure.

Hookey, J. V., V. Edwards, et al. (1999). "Use of fluorescent amplified fragment length polymorphism (fAFLP) to characterise methicillin-resistant Staphylococcus aureus." Journal of Microbiological Methods **37**(1): 7.

http://www.sciencedirect.com/science/article/B6T30-3WMJT3S-2/2/fedf387ef13aeeb21544343bdcad82bb

The new PCR-based genotyping technique, fluorescent amplified fragment length polymorphism (fAFLP), was compared for discriminatory power and reproducibility with standard phenotypic methods, a coagulase gene (coa) restriction fragment length polymorphism (RFLP) method and pulsed-field gel electrophoresis (PFGE), in typing 34 isolates and four reference strains of methicillin-resistant Staphylococcus aureus (MRSA). The fAFLP showed from 40 to 75 fragments, 50 to 450 base pairs (bp) in size. Based on replicate studies, the isolates were judged indistinguishable when their fAFLP pattern was >93.7% similar. Only two of the isolates were indistinguishable by this criterion. Thirty-one MRSA fell into four major fAFLP groups (1, 2, 3 and 4) at the level of >79.9% similarity. Three other isolates and an EMRSA-16 strain fell outside these major groups. Within both fAFLP groups 1 and 2, two subgroups, A and B, could be identified at ~82.0% similarity. While most isolates within group 1 could also be separated by their phenotypic and coagulase gene (coa) RFLP pattern, all the isolates within fAFLP groups 2A and 2B were identical on the basis of these characters. The MRSA within fAFLP groups 3 and 4 were heterogeneous by their phenotypic characteristics and coa gene RFLP patterns. fAFLP was reproducible and distinguished between MRSA isolates that appeared identical by other methods. It is likely to contribute to the epidemiological analysis of outbreaks of MRSA infection.

Jenkins, M. C., J. Trout, et al. (2000). "Estimating viability of Cryptosporidium parvum oocysts using reverse transcriptase-polymerase chain reaction (RT-PCR) directed at mRNA encoding amyloglucosidase." Journal of Microbiological Methods **43**(2): 97.

http://www.sciencedirect.com/science/article/B6T30-41S524P-6/2/2cd6835cf07553e5bda3655d4703a6df

The purpose of the present study was to determine if reverse transcriptase-polymerase chain reaction (RT-PCR) directed at mRNA encoding the enzyme amyloglucosidase (CPAG) could serve as a indicator for C. parvum oocyst viability. Oocysts were stored for 1-11 months in the refrigerator and at monthly intervals extracted for total RNA for RT-PCR analysis. An aliquot of these C. parvum oocysts was inoculated into neonatal mice which were necropsied 4 days later for ileal tissue that was analyzed by semi-quantitative PCR to determine the level of parasite

replication. The CPAG RT-PCR assay detected RNA from as few as 103 C. parvum oocysts. An effect of storage time on both RT-PCR signal and mouse infectivity was observed. RNA from oocysts stored for 1-7 months, unlike oocysts stored for 9 or 11 months, contained CPAG mRNA that was detectable by RT-PCR. A gradual decrease in the RT-PCR signal intensity was observed between 5 and 7 months storage. The intensity of RT-PCR product from oocysts and the signal from semi-quantitative PCR of ileal tissue DNA from mice infected with these same aged oocysts were comparable. The RT-PCR assay of CPAG mRNA in cultured cells infected with viable C. parvum oocysts first detected expression at 12 h with highest expression levels observed at 48 h post-infection. These results indicate that CPAG RT-PCR may be useful for differentiating viable from non-viable C. parvum oocysts and for studying the expression of the gene for amyloglucosidase in vitro.

Joshi, B. and S. K. Walia (1996). "Detection of metapyrocatechase homologous genes in petroleum hydrocarbon contaminated groundwater by polymerase chain reaction." Journal of Microbiological Methods **27**(2-3): 121.

http://www.sciencedirect.com/science/article/B6T30-3W31DM7-3/2/7116b951cd7e2445ef59423154c7537d

PCR assay was developed and used for rapid detection of metapyrocatechase (MPC) homologous gene sequences among hydrocarbon-degrading bacterial populations present in contaminated environments. The primers for the PCR assay were selected from the DNA sequence of MPC gene that has been cloned in Escherichia coli pAW313 after aligning with other published sequences. The primary primers, PF313 and PR313 were located 882 bp apart and were able to amplify the conserved region of the MPC homologous gene sequences under standardized PCR conditions. The nested primers, NF313 and NR313 amplified a confirmatory internal fragment of 506 bp from within the 882 bp region of MPC gene. Specific amplification of the unique 506 bp nested fragment was also obtained using DNA extracted from nine naturally occurring hydrocarbon degrading bacteria while, no amplification was observed when the DNAs extracted from 18 unrelated bacterial strains were used as template. The specificity and identity of the amplified 506 bp nested DNA fragment from hydrocarbon-degrading bacterial strains was confirmed by restriction digestion with EcoRI and by southern hybridization using digoxigenin-labeled internal probe. The MPC homologous gene sequences were also amplified when DNA directly extracted from petroleum hydrocarbon contaminated groundwater samples was used as template. Humic substances present in the groundwater samples did not inhibit the amplification reaction when the DNA extracted directly from groundwater was used for the PCR assay without further purification.

Jutras, E. M., R. M. Miller, et al. (1995). "Optimization of arbitrarily primed PCR for the identification of bacterial isolates." Journal of Microbiological Methods **24**(1): 55.

http://www.sciencedirect.com/science/article/B6T30-3W0P5P4-M/2/6e732a0a586cc44e9d91f506eed178e7

Arbitrarily primed polymerase chain reaction (AP-PCR) has been used extensively for genetic mapping, and the identification of bacterial isolates. To ensure that the results will be reproducible and due to true genetic variations, the AP-PCR reaction conditions must be optimized. In this study, three cultured bacterial isolates were screened with 100 arbitrary primers. Of these, five were chosen for the optimization study. The parameters optimized included: the operating conditions of the thermal cycler, the agarose gel concentration, the annealing temperature, and the concentrations of Taq polymerase enzyme, magnesium chloride, primer, and template. The final optimized PCR reaction conditions were 1 x buffer (3.5 mM MgCl2, 10 mM Tris-HCl, 50 mM

KCl and 0.1 mg ml-1 gelatin), 200 [mu]M dNTP, 0.4 [mu]M primer, 2.5 U AmpliTaq(R) (Perkin-Elmer Cetus) polymerase enzyme, and 5 [mu]l of template (at least 106 lysed bacterial cells). The Perkin-Elmer Gene-Amp(TM) 9600 PCR System was used with the following cycling conditions; a 94[deg]C 15 s denaturation step, a 45[deg]C 15 s annealing step, and 72[deg]C 30 s extension step for a total of 35 cycles. Reproducible, unique fingerprints were generated for the three isolates using each of the five arbitrary primers.

Kehrmeyer, S. R., B. M. Applegate, et al. (1996). "Combined lipid/DNA extraction method for environmental samples." Journal of Microbiological Methods **25**(2): 153.

http://www.sciencedirect.com/science/article/B6T30-3VYTYB9-5/2/c10a1629e8b35a9232c50bba68a175a9

Previously, separate methods have been developed for the extraction and purification of lipids and DNA from soils and sediments. This paper describes a new method for the isolation of both lipids and DNA from the same environmental sample. This combined method is based on the Bligh and Dyer lipid extraction technique. Upon phase separation, lipids partition into the organic phase and DNA partitions into the aqueous phase. DNA extraction and recovery from the solid phase is necessary under certain conditions. Preliminary experiments performed with 32P-labeled DNA in the absence of soil showed that greater than 98% of the total DNA was present in the aqueous phase after the modified Bligh and Dyer extraction. Analysis of the DNA by polyacrylamide gel electrophoresis and autoradiography demonstrated that no degradation of DNA occurred during the lipid extraction procedure. Lipid extraction of lyophilized cells showed that DNA was released from Pseudomonas putida and Bacillus subtilis cells corresponding to 26+/-5 and 14+/-4% of the theoretical DNA yield, respectively. The combined lipid/DNA extraction method was applied to both lyophilized cells and wet cells added to soil. Analysis by DNA:DNA hybridization showed that approx. 40-50% of the DNA from cells added to soil was recovered after lipid extraction relative to samples treated only with conventional DNA extraction. Estimation of cell number per gram soil based on either lipid or DNA analysis showed good agreement with actual numbers added based on plate counts of the inocula. DNA extracts from samples which had been lipid-extracted also had lower amounts of humic material. Although some DNA was not recovered after lipid extraction, that which was recovered was of sufficiently high quality for hybridization analysis. This method shows utility for the co-recovery of both lipids and DNA from a single sample; this is particularly useful when a small sample size is all that is available or procurable.

Lee, C.-Y., G. Panicker, et al. (2003). "Detection of pathogenic bacteria in shellfish using multiplex PCR followed by CovaLink(TM) NH microwell plate sandwich hybridization." Journal of Microbiological Methods **53**(2): 199.

http://www.sciencedirect.com/science/article/B6T30-4817HYH-5/2/a8eb6f3e66dbc524f2289b7398c7dfe7

Outbreak of diseases associated with consumption of raw shellfish especially oysters is a major concern to the seafood industry and public health agencies. A multiplex PCR amplification of targeted gene segments followed by DNA-DNA sandwich hybridization was optimized to detect the etiologic agents. First, a multiplex PCR amplification of hns, spvB, vvh, ctx and tl was developed enabling simultaneous detection of total Salmonella enterica serotype Typhimurium, Vibrio vulnificus, Vibrio cholerae and Vibrio parahaemolyticus from both pure cultures and seeded oysters. Amplicons were then subjected to a colorimetric CovaLink(TM) NH microwell plate sandwich hybridization using phophorylated and biotinlylated oligonucleotide probes, the nucleotide sequences of which were located internal to the amplified DNA. The results from the

hybridization with the multiplexed PCR amplified DNA exhibited a high signal/noise ratio ranging between 14.1 and 43.2 measured at 405 nm wavelength. The sensitivity of detection for each pathogen was 102 cells/g of oyster tissue homogenate. The results from this study showed that the combination of the multiplex PCR with a colorimetric microwell plate sandwich hybridization assay permits a specific, sensitive, and reproducible system for the detection of the microbial pathogens in shellfish, thereby improving the microbiological safety of shellfish to consumers.

Lehner, A., S. Loncarevic, et al. (1999). "A rapid differentiation of Listeria monocytogenes by use of PCR-SSCP in the listeriolysin O (hlyA) locus." Journal of Microbiological Methods **34**(3): 165.

http://www.sciencedirect.com/science/article/B6T30-3VCK5Y0-1/2/54a78d871d9924b1e73f082ec208f0e9

Single strand conformation polymorphism of PCR-amplified fragments (PCR-SSCP) was employed to develop a typing protocol for Listeria monocytogenes. Twelve sets of PCR primers were designed to amplify fragments within the coding and non-coding region of hlyA locus. In parallel, PFGE analysis of ApaI and AscI digested L. monocytogenes DNA was performed to determine the number of different genotypes and distribution of strains within serovar-subgroups. SSCP analysis of PCR generated amplicon K9 derived from the non-coding region of the hlyA gene revealed reproducible and highly polymorphic patterns whereas other amplicons showed either monomorphic or 2 to 6 different patterns. Combining the results of all 12 primer pairs, 25 genotypes were observed in 39 strains representing seven serovars. Results were confirmed by PFGE typing, only two differences in the contribution to subgroups in serovar 3b strains were observed. The data substantiate that the PCR-SSCP analysis is a reliable and highly discriminating method for characterizing L. monocytogenes strains on the molecular level.

Lunge, V. R., B. J. Miller, et al. (2002). "Factors affecting the performance of 5' nuclease PCR assays for Listeria monocytogenes detection." Journal of Microbiological Methods **51**(3): 361.

http://www.sciencedirect.com/science/article/B6T30-462BNY0-1/2/7b93cd32641d12045a545ebb21fe851e

The design and operating parameters affecting the performance of 5' nuclease PCR (TaqMan) assays for the detection of Listeria monocytogenes was investigated. A system previously developed and based on the hlyA gene was used as a model [Appl. Environ. Microbiol. 61 (1995) 3724]. A series of fluorogenic probes labeled with a reporter and a quencher dye was synthesized to explore the effect of probe position and sequence content on the efficiency of probe hydrolysis. In addition, a series of PCR primer pairs that altered the distance between the upstream primer and the interceding probe was examined. The effects of various assay parameters were evaluated by measuring the ratio of the fluorescence intensity of the reporter dye over the quencher dye ([Delta]RQ). For a given probe sequence, the [Delta]RQ was typically lower if the 5' terminus was a G residue. Decreasing the probe concentration increased the [Delta]RQ, although this was at the expense of reproducibility in the assay readout. The distance between the upstream primer and the interceding probe has a significant effect on probe hydrolysis. Reducing the primer-probe distance from, for example, 127 to 4 nt increased the [Delta]RQ from 2.87 to 5.00. These general rules were used to develop a 5' nuclease PCR (TaqMan) assay with enhanced signal output, providing higher and more reproducible [Delta]RQ values for L. monocytogenes detection.

Mikula, M., A. Dzwonek, et al. (2003). "Quantitative detection for low levels of Helicobacter pylori infection in experimentally infected mice by real-time PCR." Journal of Microbiological Methods **55**(2): 351.

http://www.sciencedirect.com/science/article/B6T30-492VY3R-3/2/35105370a05d3aa61b480c236cb5dc4b

Accurate diagnosis of Helicobacter pylori infection is important in both clinical practice and clinical research. Molecular methods are highly specific and sensitive, and various PCR-based tests have been developed to detect H. pylori in gastric biopsy specimens. We optimized a sensitive and specific quantitative SYBR Green I real-time PCR assay for detection of H. pylori based on amplification of the fragment of a 26-kDa Helicobacter species-specific antigen gene that allows for detection of 5 bacterial cells per PCR sample. Under the assay conditions, SYBR Green I real-time PCR is highly reproducible with a precise log-linear relation in the range of six orders of magnitude of bacterial DNA concentrations. For accurate comparison of H. pylori infection in different tissue samples, the amount of total host DNA in each sample is normalized by TaqMan real-time PCR of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) pseudogenes. The developed method was validated in prophilactically immunized and experimentally infected mice and revealed a level of H. pylori gastric colonisation that was below the limit of detection for a rapid urease test. This new method established for a quantitative analysis of H. pylori in the host's stomach may be useful in experimental studies evaluating new anti-H. pylori drugs and vaccines.

Millar, B. C., X. Jiru, et al. (2000). "A simple and sensitive method to extract bacterial, yeast and fungal DNA from blood culture material." Journal of Microbiological Methods **42**(2): 139.

http://www.sciencedirect.com/science/article/B6T30-419JGT1-4/2/835e4a88ce004302c3d0580c1d0b5d94

This study investigated the various commercially available kits and 'in-house' methods to extract DNA from Gram-negative and Gram-positive bacteria, yeast and fungal agents in commonly employed blood culture material. The main methods investigated were as follows; Qiagen QIAmp Blood kit, Roche high PCR template preparation kit, Puregene DNA extraction kit, boiling, glass beads/sonication and wash/alkali/heat lysis. The results indicated that a simple wash/alkali/heat lysis method was the most sensitive, reproducible, simple and cost-effective extraction method. This was the only method which removed any PCR inhibitors and inherent DNA which existed in virgin BacT/Alert aerobic, anaerobic and paediatric blood culture material. Contaminating microbial DNA from Lactococcus lactis or Bacillus coagulans was identified in all batches of BacT/Alert(R) FAN(R) aerobic blood culture material examined.

Mills, D. K., K. Fitzgerald, et al. (2003). "A comparison of DNA profiling techniques for monitoring nutrient impact on microbial community composition during bioremediation of petroleum-contaminated soils." Journal of Microbiological Methods **54**(1): 57.

http://www.sciencedirect.com/science/article/B6T30-481N1WB-1/2/536cfbc557d19d3004d6fa4f23111924

Amplicon length heterogeneity PCR (LH-PCR) and terminal restriction fragment length polymorphisms (TRFLP) were used to monitor the impact that nutrient amendments had on microbial community dynamics and structural diversity during bioremediation of petroleum-contaminated soils. Slurried soils contaminated with petroleum hydrocarbons were treated in airlift bench-scale bioreactors and were either amended with optimal inorganic nutrients or left unamended. Direct DNA extraction and PCR amplification of whole eubacterial community DNA

were performed with universal primers that bracketed the first two or three hypervariable regions of the 16S rDNA gene sequences. The LH-PCR method profiled a more diverse microbial community than did the TRFLP method. The LH-PCR method also tracked differences between the communities due to nutrient amendments. An in silico database search for bacterial genera with amplicon lengths represented in the community fingerprints was performed. It was possible to qualitatively identify different groups in the microbial community based on the amplicon length variations. A similar "virtual" search was performed for the TRFLP fragments using the web-based TAP-TRFLP program. Cloning and sequencing of the PCR products confirmed the in silico database matches. The application of the LH-PCR method as a monitoring tool for bioremediation could greatly enhance and extend the current understanding of the microbial community dynamics during the biodegradation of environmental contaminants.

Mohammadi, T., H. W. Reesink, et al. (2005). "Removal of contaminating DNA from commercial nucleic acid extraction kit reagents." Journal of Microbiological Methods **61**(2): 285.

http://www.sciencedirect.com/science/article/B6T30-4F1H03C-1/2/b726dfa0245a19b905ec66cb92e86ab6

Due to contamination of DNA extraction reagents, false-positive results can occur when applying broad-range real-time PCR based on bacterial 16S rDNA. Filtration of the nucleic acid extraction kit reagents with GenElute Maxiprep binding columns was effective in removing this reagent-derived contaminating DNA while the sensitivity of the assay was maintained.

Moore, J. E., J. Xu, et al. (2002). "Improved molecular detection ofBurkholderia cepacia genomovar III and Burkholderia multivorans directly from sputum of patients with cystic fibrosis." Journal of Microbiological Methods **49**(2): 183.

http://www.sciencedirect.com/science/article/B6T30-44JD8RX-2/2/2d7c59041e7636c41abfb8009b984046

Optimum detection of the Burkholderia cepacia complex (BCC) from sputum of patients with cystic fibrosis (CF) is essential in preventing patient-to-patient transmission of this organism. The aim of this study was to develop an improved PCR assay with reference to sensitivity for the direct detection of BCC organisms from CF sputum employing the recA locus. The sensitivity results of three recA PCR assays were compared using various combinations of previously published primers. These included (i) a single-round approach using the primer set BCR1/BCR2, yielding a 1036-bp product, (ii) a single-round approach using the primer set BCR1/Mr, yielding a 465-bp product, and (iii) a semi-nested PCR (SN-PCR) approach using the primer set BCR1/BCR2 followed by BCR1/Mr. The sensitivity of these assays were determined by spiking B. cepacia-free sputum with known numbers of four strains of BCC, namely, genomovar II [B. multivorans] (C1576), genomovar IIIa (C5424, C6433) and genomovar IIIb (C1394). Following optimization, the chosen assay was performed on 14 patients. Employment of the single-round assay with BCR1/BCR2 was the least sensitive with a detection threshold of 107 cfu/g sputum for GIIIa and GIIIb, and 108 cfu/g sputum for GII. Sensitivity was improved by targeting the smaller amplification region of the recA locus (465 bp) employing the BCR1/Mr primer pair, in combination with a single-round approach, whereby the detection threshold was improved by 1 log for each genomovar. Employment of the semi-nested assay demonstrated optimum sensitivity, whereby the detection threshold increased to 101 and 102 cfu/g sputum for genomovar IIIa/IIIb and genomovar II, respectively. Subsequent genomovar characterisation can be performed by sequencing of the PCR amplicon without the need for culture which may be beneficial in patients in the initial stages of colonisation or who are transiently colonised and who may be culture-negative for BCC.

Oh, E.-J., S. Lee, et al. (2003). "Prevalence of metallo-[beta]-lactamase among Pseudomonas aeruginosa and Acinetobacter baumannii in a Korean University hospital and comparison of screening methods for detecting metallo-[beta]-lactamase." Journal of Microbiological Methods **54**(3): 411.

http://www.sciencedirect.com/science/article/B6T30-48F5SRF-1/2/3fb8613c54e76696ed856eb8080b283c

To identify the metallo-[beta]-lactamases (MBLs) prevalent in Korea, a total of 130 clinical isolates of Pseudomonas aeruginosa and Acinetobacter baumannii (99 P. aeruginosa and 31 A. baumannii) with a reduced susceptibility to imipenem (IPM) and/or ceftazidime (CAZ) was subjected to PCR analyses with primers specific to blaIMP-1, blaVIM-1, and blaVIM-2. In addition, inhibitor-potentiated disk diffusion methods (IPD) using two kinds of substrate-inhibitor combinations (ceftazidime-2-mercaptopropionic acid (2MPA) and imipenem-EDTA) were investigated. Thirty-three isolates (29 P. aeruginosa and 4 A. baumannii) carried blaVIM-2 and two P. aeruginosa isolates harbored blaIMP-1. The enterobacterial repetitive intergenic consensus PCR (ERIC-PCR) pattern revealed that many of the VIM-2-producing P. aeruginosa isolates were clonally related, whereas the A. baumannii isolates were diverse. The inhibitor-potentiated disk diffusion test using imipenem-EDTA was highly sensitive and specific for detecting the VIM-2 producer. These results suggest that VIM-2 is an important MBL in P. aeruginosa and A. baumannii in the Korean hospital of this study and that the IMP-1-producing P. aeruginosa has also emerged. Screening for MBLs and strict infection control for these isolates will contribute to prevent further spread of resistance.

Patel, B. K. R., D. K. Banerjee, et al. (1991). "Extraction and characterization of mRNA from mycobacteria: implication for virulence gene identification." Journal of Microbiological Methods **13**(2): 99.

http://www.sciencedirect.com/science/article/B6T30-47DD949-2/2/1e3aec5e8392c62321b184db9436601c

A method for the extraction of intact total RNA from mycobacteria (Mycobacterium bovis BCG and M. leprae) has been developed. The presence of specific mRNA transcripts within this population has been confirmed by the following evidence: hybridization of Northern blots with gene-specific probes revealed discrete size classes of transcript; increases in mRNA levels for the 71kDa heat-shock protein were seen after heat shock and this was abolished with rifampicin; cDNA specific for hsp-71kDa mRNA could be synthesized from total RNA and characterized after PCR amplification. The ability to analyse mRNA by this method has implications for the study of gene expression and the molecular mechanisms of pathogenicity in mycobacteria.

Privitera, O., F. Sisto, et al. (1999). "Reverse transcription polymerase chain reaction method for the detection of glycopeptide resistance in enterococci." Journal of Microbiological Methods **35**(2): 95.

http://www.sciencedirect.com/science/article/B6T30-3VYY2GN-1/2/210f97a1c61126e265affce206894d17

In this work we have developed reverse transcription polymerase chain reaction (RT-PCR) methods for detecting specific mRNA from enterococci, particularly vanA and vanB genes, responsible for glycopeptide resistance in this genus. mRNA from the two genes was detected

immediately after RNA extraction of a midlog phase culture, determined by growth rate analysis. Because of the short half-life associated with many bacterial RNA species (1.5-2 min), time is an important factor in obtaining RNA of good yield and high purity. Our results showed that: (i) the transcription of mRNA to vanA ligase in enterococci showing Van A phenotype happens only after induction with both vancomycin and teicoplanin; (ii) the transcription of mRNA related to vanB ligase happens only in the presence of vancomycin and (iii) there was no transcription of mRNA in the two strains positive to vanA gene after PCR experiments.RT-PCR methodology can have numerous applications in microbiology for studying gene expression in isolated bacteria and also in nonculturable cells in environmental samples, for studies of mechanisms and/or as an indicator of viability in bacterial communities.

Purohit, H. J., A. Kapley, et al. (2003). "A novel approach for extraction of PCR-compatible DNA from activated sludge samples collected from different biological effluent treatment plants." Journal of Microbiological Methods **52**(3): 315.

http://www.sciencedirect.com/science/article/B6T30-4700SPG-3/2/dda1d58c616b80a63f44fcc766b497db

This paper describes a method that facilitates the extraction of PCR-compatible DNA from different activated sludge samples. The approach involves a novel preprocessing step in DNA extraction, which removes potential PCR inhibitors. The sludge was washed with different ratios of acetone and petroleum ether after pretreatment with 0.01% Tween-20 at 50 [deg]C. It was observed that an initial washing step with 50 mM Tris-HCl, pH 9.0, before the detergent-solvent step, improved the quality of the extracted DNA. The extraction protocol resulted in amplifiable amounts of DNA when 10 mg of a sludge sample was used, even in the presence of phenol as a sludge contaminant. The usefulness of the extracted template was demonstrated by carrying out different PCR reactions. The random amplified polymorphic DNA (RAPD) patterns demonstrated the diversity of sludge samples.

Rivas, R., E. Velazquez, et al. (2004). "Identification of microorganisms by PCR amplification and sequencing of a universal amplified ribosomal region present in both prokaryotes and eukaryotes." Journal of Microbiological Methods **56**(3): 413.

http://www.sciencedirect.com/science/article/B6T30-4B66CCS-1/2/af91e0f3240d728a1ae5b5ce236095be

The small ribosomal subunit contains 16S rRNA in prokaryotes and 18S rRNA in eukaryotes. Even though it has been known that some small ribosomal sequences are conserved in 16S rRNA and 18S rRNA molecules, they have been used separately for taxonomic and phylogenetic studies. Here, we report the existence of two highly conserved ribosomal sequences in all organisms that allow the amplification of a zone containing approximately 495 bp in prokaryotes and 508 bp in eukaryotes which we have named the "Universal Amplified Ribosomal Region" (UARR). Amplification and sequencing of this zone is possible using the same two universal primers (U1F and U1R) designed on the basis of two highly conserved ribosomal sequences. The UARR encompasses the V6, V7 and V8 domains from SSU rRNA in both prokaryotes and eukaryotes. The internal sequence of this zone in prokaryotes and eukaryotes is variable and the differences become less marked on descent from phyla to species. Nevertheless, UARR sequence allows species from the same genus to be differentiated. Thus, by UARR sequence analysis the construction of universal phylogenetic trees is possible, including species of prokaryotic and eukaryotic microorganisms together. Single isolates of prokaryotic and eukaryotic microorganisms from different sources can be identified by amplification and sequencing of UARR using the same pair of primers and the same PCR and sequencing conditions.

Rodrigues, J. L. M., M. R. Aiello, et al. (2002). "Use of both 16S rRNA and engineered functional genes with real-time PCR to quantify an engineered, PCB-degrading Rhodococcus in soil." Journal of Microbiological Methods **51**(2): 181.

http://www.sciencedirect.com/science/article/B6T30-45R7VGX-3/2/002745a42a69eab4833ca17d3f28acca

A real-time PCR (RTm-PCR) assay using fluorescently labeled oligonucleotides (TaqMan probes) was used to detect and quantify the recombinant Rhodococcus sp. strain RHA1(fcb) in soil. One primer and probe set targeted a hypervariable region of the 16S rRNA gene unique to strain RHA1(fcb) and its phylogenetic relatives, and the other set targeted the recombinant 4-chlorobenzoate (4-CBA) degradation operon (fcb) and was strain-specific. The method had a 6-log dynamic range of detection (102-107 cells ml-1) for both probes when DNA from pure cultures was used. Although the method was less sensitive in soil, the estimated number of cells in soil by real-time PCR corresponded to the measured number of RHA1(fcb) cells determined by colony-forming units.

Saint, C. P. and L. Ho (1999). "A PCR test for the identification and discrimination of Legionella longbeachae serogroups 1 and 2." Journal of Microbiological Methods **37**(3): 245.

http://www.sciencedirect.com/science/article/B6T30-3X3BN58-5/2/c96e142431917fed56d3a9604c19a12f

A PCR test has been developed for the specific identification of Legionella longbeachae. The test targeted sequence unique to both L. longbeachae serogroups 1 and 2 within the mip gene and permitted both species and serogroup identification. The test was trialed on a range of closely related species and 20 clinical isolates originating from Australia, the USA and Israel. Results were consistent with previous identification analyses. From 20 water samples known to contain Legionella spp. one sample yielded isolates which consistently tested positive by L. longbeachae serogroup 1 PCR. DNA sequencing of the PCR product, 5S rRNA gene sequence and hybridisation analysis with a specific oligonucleotide probe definitively identified one isolate as L. longbeachae serogroup 1. PCR testing was demonstrated as a superior method of identification to traditional seroagglutination reactions, which were ambiguous and could explain the previous failure to identify the presence of this microorganism in water.

Saris, P. E. J., L. G. Paulin, et al. (1990). "Direct amplication of DNA from colonies of Bacillus subtilis and Escherichia coli by the polymerase chain reaction." Journal of Microbiological Methods **11**(2): 121.

http://www.sciencedirect.com/science/article/B6T30-476TXNN-DP/2/d9f83825bf237474df9ef17b4d2b8ee6

The polymerase chain reaction (PCR) catalyzed by Taq DNA polymerase has been used to amplify genomic and plasmid DNA sequences. In all reported cases, the template has been extensively purified. We show here that genomic and plasmid DNA sequences can be amplified by the PCR directly from single colonies of both Escherichia coli and Bacillus subtilis without any purification of the template. This technique makes the use of the PCR even more applicable in the automation processes, screening and diagnosis.

Savill, M. G., S. R. Murray, et al. (2001). "Application of polymerase chain reaction (PCR) and TaqMan(TM) PCR techniques to the detection and identification of Rhodococcus coprophilus in faecal samples." Journal of Microbiological Methods **47**(3): 355.

http://www.sciencedirect.com/science/article/B6T30-44DY2J7-C/2/dbc5cd1cda020ab6f8064e70dc612efc

Rhodococcus coprophilus, a natural inhabitant of herbivore faeces, has been suggested as a good indicator of animal (as opposed to human) faecal contamination of aquatic environments. However, conventional detection methods limit its use for this as they require up to 21 days to obtain a result. In this paper an optimised method for extracting R. coprophilus DNA from faecal samples is described. PCR and 5'-nuclease (TaqMan(TM)) PCR methods were developed to allow the detection and enumeration of R. coprophilus in faecal samples within 2-3 days. Both PCR methods targeted the 16S rRNA gene, producing an amplicon of 443 bp which was specific for R. coprophilus. Sixty cells were required to produce an amplification product by conventional PCR, while as little as one cell was required for the TaqMan(TM) PCR method. The latter approach gave a linear quantitative response over at least four log units with both bacterial cells and DNA. Successful amplification by PCR was achieved using DNA extracted from cow, sheep, horse and deer faeces but was negative for samples from humans, pig, possum, duck and rabbit. These PCR methods enhance the feasibility of using R. coprophilus to distinguish faecal pollution of farmed herbivores from human pollution.

Shelburne, C. E., A. Prabhu, et al. (2000). "Quantitation of Bacteroides forsythus in subgingival plaque: Comparison of immunoassay and quantitative polymerase chain reaction." Journal of Microbiological Methods **39**(2): 97.

http://www.sciencedirect.com/science/article/B6T30-3XSJG15-1/2/df569bcd451d8d6335033effd589c289

Our objective was to compare three methods (enzyme-linked immunosorbent assay [ELISA], endpoint and quantitative polymerase chain reaction [E-PCR and Q-PCR]) for detection and quantitation of Bacteroides forsythus in 56 plaque samples from seven subjects with progressive periodontal disease. Samples collected in buffer were pelleted and resuspended in 500 [mu]l of water. Fifty [mu]l aliquots were removed for an ELISA performed on bacteria or plaque immobilized on 96-well plates and probed with B. forsythus specific antibody. An occurrence of 3.7+/-0.6.104 or more bacteria were detected by ELISA in pure culture; 26 of 54 plaque samples were positive, two samples could not be analyzed. Samples for PCR were autoclaved for 10 min prior to use. The detection level of E-PCR using primers specific for B. forsythus 16S rRNA was 200 cells and 42 out of 56 samples were positive based on ethidium bromide stained agarose gels. Q-PCR using the same primers combined with a nested fluorescent oligonucleotide probe detected 10+/-0.32 bacteria in pure culture; 43 of 56 plaque samples were positive. The ELISA and Q-PCR obtained identical results with 36 of the 54 samples assayed; there were one false positive and 17 false negative ELISA results using Q-PCR as standard. The positive proportions of plaque samples were almost the same for E-PCR and Q-PCR. We conclude that the PCR methods are more appropriate for a multicenter study because of greater sensitivity and convenience of sample transportation from clinics to a central laboratory.

Sicinschi, L. A., P. Correa, et al. (2003). "A positive assay for identification of cagA negative strains of Helicobacter pylori." Journal of Microbiological Methods **55**(3): 625.

http://www.sciencedirect.com/science/article/B6T30-49NVM34-1/2/3fc4aa77eb5432f370dc5be6af80feb5

A new PCR protocol was developed for the positive identification of cagA negative Helicobacter pylori strains. Amplification of a portion of the genome across the insertion point of the cag pathogenicity island (the ES--"empty site") generated a 106-bp fragment, which produces a positive signal for cagA negative strains. Combined with the results of the cagA assay, the signals for ES allowed the complete characterization of the patients' cagA status. DNA sequencing analysis confirmed the identity of the ES fragment. The new protocol and cagA assay were applied to 22 DNA preparations isolated from stools from H. pylori infected adult patients and to 21 DNA preparations isolated from stools from H. pylori infected children. The same analysis was also performed on nine colonies of H. pylori derived from gastric biopsies of nine of the adult patients. The total number of cagA positive cases from adult patients was 14 or 63.6% (11 mono- and 3 mixed) and of the cagA negative cases (or ES positive) was 9 or 40.9% (6 mono- and 3 mixed). Of the 21 stool DNA samples from children, 6 (28.6%) were cagA positive, 12 (57.1%) were cagA negative and 3 (14.3%) were positive for cagA and for the ES simultaneously. The proportions of mixed cagA positive and cagA negative H. pylori infections were almost equal in adults and children (13.6% and 14.3%, respectively). No reaction products of the proper fragment sizes for cagA or the empty site (ES) were obtained from any of the stool DNA samples of 10 H. pylori uninfected subjects (100% specificity). This noninvasive assay discriminates consistently cagA negative cases from cagA positive strains and from amplification failures. It can be a useful tool for clinical and epidemiological studies of H. pylori infection.

Stich, R. W., B. Byrum, et al. (2004). "Evaluation of an automated system for non-radiometric detection of Mycobacterium avium paratuberculosis in bovine feces." Journal of Microbiological Methods **56**(2): 267.

http://www.sciencedirect.com/science/article/B6T30-4B4Y2NX-1/2/3ae187e44367568a75ae8163445be7ab

Cultivation of Mycobacterium avium subsp. paratuberculosis (M. paratuberculosis) from feces remains the most reliable method to detect infected animals. The purpose of this study was to evaluate a broth-based automated system used for cultivation of mycobacteria such as M. tuberculosis from human hosts, for the detection of M. paratuberculosis in bovine feces. Bovine feces was spiked with tenfold serial dilutions of M. paratuberculosis (5 x 105 to 5 x 10-1 organisms), then processed with a double-centrifugation technique that included disinfection prior to inoculation into broth tubes. The same pathogen dilution series was also inoculated directly into broth and broth with uninfected processed feces. All of the system signal-positive bottles were identified within 30 days, with the highest concentration of M. paratuberculosis detected by the system in as few as 8 days. The presence of the pathogen was confirmed with acid-fast staining and an IS900-based PCR assay when growth of M. paratuberculosis was indicated by the system. However, some of the signal-negative cultures inoculated with the equivalent of 0.5 organisms tested PCR-positive 56 days post-inoculation, indicating that longer culture periods may lead to detection of small quantities of the organisms. Additionally, it was indicated that the processing step had a detrimental effect on detection of the organism. Comparison of the broth- and Herrold's egg yolk medium (HEYM) solid media-based culture methods with defined check test specimens corroborated the experimental evaluation of this system, indicating that broth-based detection could provide a more rapid assay for M. paratuberculosis. These results suggest that this automated system could be used to detect this organism in bovine feces, but that new approaches to processing the feces for culture should be explored.

Suh, S.-J., L. A. Silo-Suh, et al. (2004). "Development of tools for the genetic manipulation of

Pseudomonas aeruginosa." Journal of Microbiological Methods **58**(2): 203.

http://www.sciencedirect.com/science/article/B6T30-4C9HRN1-3/2/4900538879bc31ab43a854e6353f9642

To facilitate study of the opportunistic bacterial pathogen Pseudomonas aeruginosa, several genetic tools were developed. These tools include a series of cassettes carrying (a) the minimal sequence for the origin of transfer (oriT) of RP4 plasmid for introducing plasmid into P. aeruginosa via conjugation, (b) a minimal sequence for P. aeruginosa replicon (stabilizing fragment or SF) for maintenance of plasmids in P. aeruginosa, and (c) the transcriptionally non-polar tetracycline resistance gene (TcR) for insertional mutagenesis. Additional genetic constructs include (d) two conjugative and suicide lacZ reporter fusion plasmids for studying gene expression at the transcriptional or translational level, (e) a gentamicin resistant promoter-probing mini-Tn5 lacZ, and (f) a tightly regulated T7 promoter/repressor system to control gene expression in P. aeruginosa.

Te'o, V. S. J., P. L. Bergquist, et al. (2002). "Biolistic transformation of Trichoderma reesei using the Bio-Rad seven barrels Hepta Adaptor system." Journal of Microbiological Methods **51**(3): 393.

http://www.sciencedirect.com/science/article/B6T30-46B789B-2/2/227ae8ac096a449f04acde94435c19ee

Effective biolistic transformation of intact conidia from the filamentous fungus Trichoderma reesei was achieved using the Bio-Rad Hepta Adaptor system with seven barrels for particle launch. Transformation frequencies of up to 39 colonies per [mu]g of circular DNA and 37 colonies per [mu]g of linear DNA were obtained at an optimal target distance of 3 cm and a helium pressure of 1350 psi. These values are about 3.5- to 6-fold higher than transformant yields reported earlier for T. reesei using the hygromycin phosphotransferase (hph) gene conferring resistance to the antibiotic hygromycin B as a selectable marker in combination with the PDS-1000/He single barrel system. High mitotic stability of the transformants (98-100%) was demonstrated. The Hepta Adaptor device allowing bombardment of seven lots of conidia in a single plate offers clear advantage in terms of transformant numbers over the single barrel system where target cells are restricted to the center of the plate.

Tjhie, H. T. J., R. Roosendaal, et al. (1993). "Detection of Chlamydia pneumoniae using a general Chlamydia polymerase chain reaction with species differentiation after hybridisation." Journal of Microbiological Methods **18**(2): 137.

http://www.sciencedirect.com/science/article/B6T30-476TX8F-8X/2/3f13a7a2d0224f7a8cbee80add31fc49

A general polymerase chain reaction (PCR) for the detection of Chlamydia trachomatis, C. psittaci and C. pneumoniae was developed. By computer assisted sequence analysis two sets of sense primers CM1 and CM2 and one set of antisense primers CM3, each consisting of a mixture of two primers, were selected from conserved regions on the gene coding for the major outer membrane protein (MOMP-gene). Internal species specific oligoprobes were selected for the differentiation of the three species. The general PCR using primer combinations CM1/CM3 and CM2/CM3 generated a DNA fragment of approximately 360 and 320 bp respectively with 15 serovars of C. trachomatis, 6 strains of C. pneumoniae and 2 strains of C. psittaci. All amplified fragments hybridised with the general Chlamydia probe. The species specific probes detected only the Chlamydia strains of the corresponding species. Although both combinations gave rise to

an identical specificity in the PCR, the CM2/CM3-PCR was 10-100 times more sensitive. The sensitivity of the CM2/CM3-PCR was one inclusion forming unit (IFU) or ten copies of isolated MOMP-DNA. Application of this general Chlamydia PCR on a throat sample weakly positive in culture for C. pneumoniae (one IFU) scored also positive after hybridisation with the general probe and the C. pneumoniae probe. The results indicate that the general Chlamydia PCR can be used for the sensitive detection of all Chlamydia species and could prove useful in the detection of Chlamydia pneumoniae in clinical specimens.

Urzi, C., F. De Leo, et al. (1999). "Intra-specific diversity of Aureobasidium pullulans strains isolated from rocks and other habitats assessed by physiological methods and by random amplified polymorphic DNA (RAPD)." Journal of Microbiological Methods **36**(1-2): 95.

http://www.sciencedirect.com/science/article/B6T30-3WBWTMS-C/2/da7f0e7eab0ac895762c131466b12924

Intra-specific diversity of Aureobasidium pullulans strains isolated from environmental sources and from stones was studied by assessment of morphological, biochemical and physiological characters as well as random amplified polymorphic DNA (RAPD) using microsatellite or minisatellite DNA primers (GTG)5, (GACA)4, M13.The results showed that both classical and molecular techniques evidenced a phenotypic and genetic diversity of analysed A. pullulans strains.A different behaviour was observed in reference to the growth responses with -glucosamine, citrate, galactitol and with different salt concentrations and range of growth temperature. Molecular analysis partially confirmed the data obtained with biochemical and physiological tests, additionally showing common fragments in all strains, to be used for a possible application as 'in situ' probes and for a rapid identification of A. pullulans strains.

van der Schee, C., H. J. F. Sluiters, et al. (2001). "Host and pathogen interaction during vaginal infection by Trichomonas vaginalis and Mycoplasma hominis or Ureaplasma urealyticum." Journal of Microbiological Methods **45**(1): 61.

http://www.sciencedirect.com/science/article/B6T30-42R0S9B-7/2/a0b5c8a401c81f7b00be2b504de6ecd1

Vaginal infections by Trichomonas vaginalis and Mycoplasma hominis have been shown to be associated. Since M. hominis and Ureaplasma urealyticum are similar pathogens, both belonging to the class of the mycoplasmata, we describe here a molecular study into the interdependence of U. urealyticum and T. vaginalis during infection. Susceptibility towards infection by U. urealyticum depends on genetic polymorphism in the interleukin-1 receptor antagonist (IL-1RA) gene. Now, we defined the relation between IL-1RA genotypes and infection by M. hominis and T. vaginalis. Finally, we also developed a restriction fragment length polymorphism (RFLP) tool for mapping variation in the T. vaginalis AP33 adhesin in order to define putative associations between parasite subtype and mycoplasmata or host. Studies using crudepellets from T. vaginalis culture broth clearly confirm the association between T. vaginalis and M. hominis infection. The association between IL-1RA genotype 2,2 and lack of U. urealyticum infection is corroborated as well. U. urealyticum infection and infection by T. vaginalis are independent. Furthermore, T. vaginalis and M. hominis infection are not depending on IL-1RA genotypes. Interestingly, one of the three AP33 RFLP types identified appeared to be associated with the absence of U. urealyticum infection. In conclusion, the complex interaction between bacterial and parasitic pathogens and the infected host is determined by genetic characteristics of host and microorganisms involved.

Varma, M., J. D. Hester, et al. (2003). "Detection of Cyclospora cayetanensis using a quantitative real-time PCR assay." Journal of Microbiological Methods **53**(1): 27.

http://www.sciencedirect.com/science/article/B6T30-47G445R-1/2/7d6df9100bfb7161af9d3c597f30610a

Cyclospora cayetanensis, a coccidian parasite, with a fecal-oral life cycle, has become recognized worldwide as an emerging human pathogen. Clinical manifestations include prolonged gastroenteritis. While most cases of infection with C. cayetanensis in the United States have been associated with foodborne transmission, waterborne transmission has also been implicated. We report on the development and application of a real-time, quantitative polymerase chain reaction assay for the detection of C. cayetanensis oocysts, which is the first reported use of this technique for this organism. Both a species-specific primer set and dual fluorescent-labeled C. cayetanensis hybridization probe were designed using the inherent genetic uniqueness of the 18S ribosomal gene sequence of C. cayetanensis. The real-time polymerase chain reaction assay has been optimized to specifically detect the DNA from as few as 1 oocyst of C. cayetanensis per 5 [mu]l reaction volume.

Vescio, P. A. and S. A. Nierzwicki-Bauer (1995). "Extraction and purification of PCR amplifiable DNA from lacustrine subsurface sediments." Journal of Microbiological Methods **21**(3): 225.

http://www.sciencedirect.com/science/article/B6T30-3YCM197-1/2/c412a38ec48de3e774ec98e39626ead6

An extraction procedure which recovers high quality DNA from microbial communities in lacustrine type sediments has been developed. This method employs direct lysis of cells in an agarose-sediment mixture, electroelution of community DNA, followed by ammonium acetate precipitation for further purification. The extracted community DNA was found to be suitable for PCR amplification with 16S rRNA gene-specific primers when T4 gene 32 protein was present in amplification reactions.

Vickery, M. C. L., A. L. Smith, et al. (1998). "Optimization of the arbitrarily-primed polymerase chain reaction (AP-PCR) for intra-species differentiation of Vibrio vulnificus." Journal of Microbiological Methods **33**(2): 181.

http://www.sciencedirect.com/science/article/B6T30-3T87G0D-B/2/34d0b99b1341f20fc22ce73419eb8876

Optimal parameters were established for obtaining unique and reproducible DNA fingerprints of selected clinical and environmental isolates of Vibrio vulnificus by the arbitrarily primed polymerase chain reaction (AP-PCR). Genomic DNA from selected strains was subjected to AP-PCR amplification using a single, arbitrarily selected oligonucleotide primer, R-PSE420. Amplified DNA was analyzed by agarose gel electrophoresis and ProRFLP(R) computer software. Reproducibility of the fingerprint was dependent upon the concentrations of the purified genomic DNA, MgCl2 and oligonucleotide primer, and on PCR cycling parameters. Using 1 [mu]g of purified genomic DNA, 2.5 mM MgCl2, 1.04 [mu]M of R-PSE420 oligonucleotide primer and thermal cycling protocols with stepwise increases in the annealing temperatures, DNA fingerprints which were reproducible and free of primer artifacts were generated. By following the optimized AP-PCR amplification protocol, unique DNA fingerprint profiles for each V. vulnificus strain tested

were produced. These AP-PCR generated unique DNA fingerprint profiles can be used in the identification, and investigation of the distribution and diversity of various strains of V. vulnificus in bivalve shellfish and their surrounding waters.

Watanabe, K., Y. Kodama, et al. (2001). "Design and evaluation of PCR primers to amplify bacterial 16S ribosomal DNA fragments used for community fingerprinting." Journal of Microbiological Methods **44**(3): 253.

http://www.sciencedirect.com/science/article/B6T30-42G0M1Y-8/2/577eab17de3b1b3eccfade31668f1f4a

Denaturing gradient gel electrophoresis of PCR-amplified 16S ribosomal DNA (rDNA) fragments has frequently been applied to the fingerprinting of natural bacterial populations (PCR/DGGE). In this study, sequences of bacterial universal primers frequently used in PCR/DGGE were compared with 16S rDNA sequences that represent recently proposed divisions in the domain Bacteria. We found mismatches in 16S rDNA sequences from some groups of bacteria. Inosine residues were then introduced into the bacterial universal primers to reduce amplification biases caused by these mismatches. Using the improved primers, phylotypes affiliated with Verrucomicrobia and candidate division OP11, were detected in DGGE fingerprints of groundwater populations, which have not been detected by PCR/DGGE with conventional universal primers.

Wilson, V. L., B. C. Tatford, et al. (1999). "Species-specific detection of hydrocarbon-utilizing bacteria." Journal of Microbiological Methods **39**(1): 59.

http://www.sciencedirect.com/science/article/B6T30-3XR2S4Y-7/2/ae9829c87053e2383ef66e6af7c628ec

Rapid detection and quantitative assessment of specific microbial species in environmental samples is desirable for monitoring changes in ecosystems and for tracking natural or introduced microbial species during bioremediation of contaminated sites. In the interests of developing rapid tests for hydrocarbon-degrading bacteria, species-specific PCR primer sets have been developed for Pseudomonas aeruginosa, Stentrophomonas (Xanthomonas) maltophilia, and Serratia marsescens. Highly variable regions of the 16S rRNA gene were used to design these primer sets. The amplification products of these primer sets have been verified and validated with hemi-nested PCR and with ligase chain reaction (LCR) techniques, and have been applied to the analyses of environmental water samples. These species-specific primer sets were also chosen to amplify in conjunction with a universal set of PCR primers chosen from highly conserved neighboring sequences in the same gene. These multiplex or competitive PCR procedures enable testing with an internal marker and/or the quantitative estimation of the relative proportion of the microbial community that any one of these species occupies. In addition, this universal PCR primer set amplified the same size amplicon from a wide spectrum of procaryotic and eucaryotic organisms and may have potential in earth biota analyses.

Wu, S.-J., A. Chan, et al. (2004). "Detection of PCR amplicons from bacterial pathogens using microsphere agglutination." Journal of Microbiological Methods **56**(3): 395.

http://www.sciencedirect.com/science/article/B6T30-4B7N8Y9-2/2/209f4a33e6817adc1dd8030055a6ac99

For rapid and inexpensive detection of polymerase chain reaction (PCR) amplicons, a novel microsphere agglutination assay has been developed. PCR is carried out using biotinylated forward and reverse primers, and the amplified DNA fragments are able to agglutinate streptavidin-coated microspheres (5.7 [mu]m in diameter). Purification of PCR amplicons is unnecessary when initial primer concentrations are 250 nM. Agglutination can be identified visually within 2 min without any additional equipment or reagents. Using listeriolysin (lisA)-specific biotinylated primers, we have successfully detected and identified Listeria monocytogenes lisA+ cells among Salmonella typhimurium, Staphylococcus aureus, Campylobacter jejuni and Escherichia coli O157:H7 cells. The simplicity of this protocol considerably reduces the time and cost of diagnostic PCR experiments. This procedure is potentially useful for various studies and field applications.

Journal of Molecular Biology (58)

Abts, H. F., T. Welss, et al. (1999). "Cloning and characterization of hurpin (protease inhibitor 13): a new skin-specific, UV-repressible serine proteinase inhibitor of the ovalbumin serpin family." Journal of Molecular Biology **293**(1): 29.

http://www.sciencedirect.com/science/article/B6WK7-45R8710-5F/2/880e1abeaa46674c2c137960639d31e0

Epidermal keratinocytes are the primary target of the midrange ultraviolet part (UVB, 280-320 nm) of terrestrial sunlight. Analysis of the resulting UV response at the transcriptional level by differential display PCR identified a formerly unrecognized large group of repressed genes. Among those UV-repressible genes, a novel serine proteinase inhibitor (serpin) termed hurpin (HaCaT UV-repressible serpin) has been identified. The isolated full-length cDNAs harbour a 1176 bp open reading frame encoding a potential protein with 391 amino acid residues and a predicted molecular mass of ~44 kDa. The novel serpin has nearly 59% amino acid identity with the squamous cell carcinoma antigen 1 (SCCA1) and squamous cell carcinoma antigen 2 (SCCA2). In addition, it displays all of the structural features unique to the ovalbumin family of serpins (ov-serpins). The amino acid sequence of the hinge region in the reactive site loop suggests that hurpin has the potential for protease inhibition. The putative reactive center P1-P1' residues were identified as Thr356-Ser357 by alignment with other ov-serpins. The physiological target protease is unknown and the in vitro translated hurpin does not form SDS-stable complexes with a variety of known serine proteases. Expression of hurpin is restricted to epidermal cells where two distinct transcripts of 3.0 and 3.4 kb are detectable. Furthermore, expression of hurpin appears to be related to the activation or proliferation state of keratinocytes, since hurpin transcripts are more abundant in immortalized keratinocytes (HaCaT) and in cultured normal human keratinocytes, compared to the expression in normal skin. Moreover, in psoriasis, a skin disease characterized by hyperproliferation of keratinocytes and responsive to therapeutic UV irradiation, overexpression of hurpin is noted in psoriatic skin lesions compared to non-lesional skin.

Alcocer, M. J. C., G. J. Murtagh, et al. (2002). "The Disulphide Mapping, Folding and Characterisation of Recombinant Ber e 1, an Allergenic Protein, and SFA8, Two Sulphur-rich 2 S Plant Albumins." Journal of Molecular Biology **324**(1): 165.

http://www.sciencedirect.com/science/article/B6WK7-4741XW5-G/2/d53a775b7a1905fcaced07704454e5e8

We have cloned and expressed genes encoding the allergenic brazil nut 2 S albumin (Ber e 1) and the sunflower albumin 8 (SFA8) in the methylotrophic yeast Pichia pastoris. We show that both proteins were secreted at high levels and that the purified proteins were properly folded. We also showed that Ber e 1 is glycosylated during secretion and that the glycan does not interfere with the folding or immunoreactivity. The disulphide map of the Ber e 1 protein was experimentally established and is in agreement with the conserved disulphide structure of other members of the 2 S albumin family. A model three-dimensional structure of the allergen was generated. During the expression studies and through mutation we have also shown that alteration of the sequences around the Kex2 endoproteolytic processing site in the expressed fusion protein can compromise the secretion by targeting part of the protein for possible degradation. The secreted production of these properly folded sulphur-rich plant albumins presents an opportunity to delineate the attributes that make an allergen and to facilitate the diagnosis and therapy of type I allergy.

Alcocer, M. J. C., G. J. Murtagh, et al. (2004). "The Major Human Structural IgE Epitope of the Brazil Nut Allergen Ber e 1: A Chimaeric and Protein Microarray Approach." Journal of Molecular Biology **343**(3): 759.

http://www.sciencedirect.com/science/article/B6WK7-4D97J05-3/2/632e9eee6148082b3241fdf64685e2b6

A protein microarray system containing different dilutions of 77 related and non-related proteins was used to show that IgE from subjects allergic to Brazil nut specifically recognise the seed 2S albumin protein (Ber e 1). Further, correctly folded chimaeric 2S albumin proteins containing structural epitope replacement were constructed and directed to the secretion pathway of the methylotropic yeast Pichia pastoris. Through the use of a chimaeric protein microarray system together with sera from a panel of 18 well-characterised Brazil nut allergic subjects, a structural IgE epitope of Ber e 1 was mapped to a helix-loop-helix region. The same structural region has been previously reported as the immunodominant region in related food allergens by different techniques. In conclusion, the combination of chimaeric proteins and protein microarrays will greatly facilitate the screening of a large number of individuals for a particular structural epitope and help to further our understanding of how proteins are recognised by the adaptive immune system.

Arezi, B., C. J. Hansen, et al. (2002). "Efficient and High Fidelity Incorporation of Dye-terminators by a Novel Archaeal DNA Polymerase Mutant." Journal of Molecular Biology **322**(4): 719.

http://www.sciencedirect.com/science/article/B6WK7-46SNK20-B/2/02a12014d1bdc45fa21eea48e540845f

We examined the molecular basis of ddNTP selectivity in archaeal family B DNA polymerases by randomly mutagenizing the gene encoding Thermococcus sp. JDF-3 DNA polymerase and screening mutant libraries for improved ddNTP incorporation. We identified two mutations, P410L and A485T, that improved ddNTP uptake, suggesting the contribution of P410 and A485 to ddNTP/dNTP selectivity in archaeal DNA polymerases. The importance of A485 was identified previously in mutagenesis studies employing Pfu (A486) and Vent (A488) DNA polymerases, while the contribution of P410 to ddNTP/dNTP selectivity has not been reported. We demonstrate that a combination of mutations (P410L/A485T) has an additive effect in improving ddNTP

incorporation by a total of 250-fold. To assess the usefulness of the JDF-3 P410L/A485T in fluorescent-sequencing applications, we compared the archaeal mutant to Taq F667Y with respect to fidelity and kinetic parameters for DNA and dye-ddNTPs. Although the Taq F667Y and JDF-3 P410L/A485T mutants exhibit similar Km and Vmax values for dye-ddNTPs in single-base extension assays, the archaeal mutant exhibits higher fidelity due to a reduced tendency to form certain (ddG:dT, ddT:dC) mispairs. DNA polymerases exhibiting higher insertion fidelity are expected to provide greater accuracy in SNP frequency determinations by single-base extension and in multiplex minisequencing assays.

Arias, A., C. M. Ruiz-Jarabo, et al. (2004). "Fitness Increase of Memory Genomes in a Viral Quasispecies." Journal of Molecular Biology **339**(2): 405.

http://www.sciencedirect.com/science/article/B6WK7-4C476TX-F/2/10bbef7d177d43ea4b0764c6e8617aff

Viral quasispecies may contain a subset of minority genomes that reflect those genomic sequences that were dominant at an early phase of quasispecies evolution. Such minority genomes are referred to as memory in viral quasispecies. A memory marker previously characterized in foot-and-mouth disease virus (FMDV) is an internal oligoadenylate tract of variable length that became dominant upon serial plaque-to-plaque transfers of FMDV clones. During large population passages, genomes with internal oligoadenylate were outcompeted by wild-type revertants but remained in the mutant spectra as memory genomes. Here, we report a quantification of relative fitness of several FMDV clones, harboring internal oligoadenylate tracts of different length, and that were retrieved at early or late times (passage number) after implementation of memory. The results show that for any given length range of the oligoadenylate, maintenance in memory resulted in an increase in relative fitness, comparable to the increase undergone by the entire population. The fitness increase is in agreement with the Red Queen hypothesis, and implies a replicative memory mechanism. Thus, permanence of memory genomes may be a source of high fitness variants despite their initial low fitness, and despite having remained hidden in mutant spectra. This reinforces the interest of diagnosing minority genomes during chronic human and animal viral infections.

Azriel-Rosenfeld, R., M. Valensi, et al. (2004). "A Human Synthetic Combinatorial Library of Arrayable Single-chain Antibodies based on Shuffling in Vivo Formed CDRs into General Framework Regions." Journal of Molecular Biology **335**(1): 177.

http://www.sciencedirect.com/science/article/B6WK7-4B41W2K-K/2/9ec7756ed0a5a9f73a396a42d5e64846

We describe a novel approach for high-throughput screening of recombinant antibodies, based on their immobilization on solid cellulose-based supports. We constructed a large human synthetic single-chain Fv antibody library where in vivo formed complementarity determining regions were shuffled combinatorially onto germline-derived human variable-region frameworks. The arraying of library-derived scFvs was facilitated by our unique display/expression system, where scFvs are expressed as fusion proteins with a cellulose-binding domain (CBD). Escherichia coli cells expressing library-derived scFv-CBDs are grown on a porous master filter on top of a second cellulose-based filter that captures the antibodies secreted by the bacteria. The cellulose filter is probed with labeled antigen allowing the identification of specific binders and the recovery of the original bacterial clones from the master filter. These filters may be simultaneously probed with a number of antigens allowing the isolation of a number of binding specificities and the validation of specificity of binders. We screened the library against a number of cancer-related peptides, proteins, and peptide-protein complexes and yielded antibody

fragments exhibiting dissociation constants in the low nanomolar range. We expect our new antibody phage library to become a valuable source of antibodies to many different targets, and to play a vital role in facilitating high-throughput target discovery and validation in the area of functional cancer genomics.

Banci, L., I. Bertini, et al. (2002). "Solution structure of the N-terminal domain of a potential copper-translocating P-type ATPase from Bacillus subtilis in the apo and Cu(I) loaded states." Journal of Molecular Biology **317**(3): 415.

http://www.sciencedirect.com/science/article/B6WK7-45KNCPH-63/2/3849a38a788e3f187a8bc64f6c5f8dfb

A putative partner of the already characterized CopZ from Bacillus subtilis was found, both proteins being encoded by genes located in the same operon. This new protein is highly homologous to eukaryotic and prokaryotic P-type ATPases such as CopA, Ccc2 and Menkes proteins. The N-terminal region of this protein contains two soluble domains constituted by amino acid residues 1 to 72 and 73 to 147, respectively, which were expressed both separately and together. In both cases only the 73-147 domain is folded and is stable both in the copper(I)-free and in the copper(I)-bound forms. The folded and unfolded state is monitored through the chemical shift dispersion of 15N-HSQC spectra. In the absence of any structural characterization of CopA-type proteins, we determined the structure of the 73-147 domain in the 1-151 construct in the apo state through 1H, 15N and 13C NMR spectroscopies. The structure of the Cu(I)-loaded 73-147 domain has been also determined in the construct 73-151. About 1300 meaningful NOEs and 90 dihedral angles were used to obtain structures at high resolution both for the Cu(I)-bound and the Cu(I)-free states (backbone RMSD to the mean 0.35(+/-0.06) A and 0.39(+/-0.07) A, respectively). The structural assessment shows that the structures are accurate. The protein has the typical [beta][alpha][beta][beta][alpha][beta] folding with a cysteine in the C-terminal part of helix [alpha]1 and the other cysteine in loop 1. The structures are similar to other proteins involved in copper homeostasis. Particularly, between BsCopA and BsCopZ, only the charges located around loop 1 are reversed for BsCopA and BsCopZ, thus suggesting that the two proteins could interact one with the other. The variability in conformation displayed by the N-terminal cysteine of the CXXC motif in a number of structures of copper transporting proteins suggests that this may be the cysteine which binds first to the copper(I) carried by the partner protein.

Beaulieu, M., E. Levesque, et al. (1998). "Isolation and characterization of a simian UDP-glucuronosyltransferase UGT2B18 active on 3-hydroxyandrogens." Journal of Molecular Biology **275**(5): 785.

http://www.sciencedirect.com/science/article/B6WK7-45P0FFJ-16/2/5d40ecd20f0f573d3ccd6a0052917334

A monkey cDNA, UGT2B18, encoding a UDP-glucuronosyltransferase (UGT) active on 3-hydroxyandrogens, has been isolated and characterized. Previous results suggested that the monkey represents the most appropriate animal model for studying the physiologic relevance of steroid UGTs. UGT2B18 was isolated from a cynomolgus monkey prostate cDNA library using human UGT2B7, UGT2B10 and UGT2B15 cDNA as probes. The cDNA is 1748 bp in length and contains an open reading frame of 1587 bp encoding a protein of 529 residues. The UGT2B18 cDNA clone was transfected into HK293 cells and a stable cell line expressing UGT2B18 protein was established. Western blot analysis of the UGT2B18-HK293 cell line using a human UGT2B17 polyclonal antibody (EL-93) revealed high expression of a 53 kDa UGT2B protein. The transferase activity of UGT2B18 was tested with over 60 compounds and was demonstrated to

be principally active on C19 steroids having an hydroxyl group at position 3[alpha] of the steroid molecule. UGT2B18 was also active on planar phenols and bile acids. Kinetic analysis revealed that UGT2B18 glucuronidates 3-hydroxyandrogens with high velocity and affinity. Using cell homogenates, Kmvalues of 5.1, 7.8 and 23 [mu]M for androsterone (ADT), etiocholanolone and androstane-3[alpha], 17[beta] diol (3[alpha]-diol) were obtained, respectively. Specific RT-PCR analysis demonstrated the expression of UGT2B18 transcripts in several tissues including liver, prostate, kidney, testis, adrenal, bile duct, bladder, colon, small intestine, cerebellum and pancreas suggesting a contribution of this isoenzyme to the high plasma levels of glucuronidated ADT and 3[alpha]-diol found in the cynomolgus monkey.

Bespalov, I. A., J. P. Bond, et al. (1999). "Fabs specific for 8-oxoguanine: control of DNA binding." Journal of Molecular Biology **293**(5): 1085.

http://www.sciencedirect.com/science/article/B6WK7-45R86XX-48/2/76416f3f9882a378313e99e35ff3fb0f

Free radicals produce a broad spectrum of DNA base modifications including 7,8-dihydro-8-oxoguanine (8-oxoG). Since free radicals have been implicated in many pathologies and in aging, 8-oxoG has become a benchmark for factors that influence free radical production. Fab g37 is a monoclonal antibody that was isolated by phage display in an effort to create a reagent for detecting 8-oxoG in DNA. Although this antibody exhibited a high degree of specificity for the 8-oxoG base, it did not appear to recognize 8-oxoG when present in DNA. Fab g37 was modified using HCDR1 and HCDR2 segment shuffling and light chain shuffling. Fab 166 and Fab 366 which bound to 8-oxoG in single-stranded DNA were isolated. Fab 166 binds more selectively to single-stranded oligonucleotides containing 8-oxoG versus control oligonucleotides than does Fab 366 which binds DNA with reduced dependency on 8-oxoG. Numerous other clones were also isolated and characterized that contained a spectrum of specificities for 8-oxoG and for DNA. Analysis of the primary sequences of these clones and comparison with their binding properties suggested the importance of different complementarity determining regions and residues in determining the observed binding phenotypes. Subsequent chain shuffling experiments demonstrated that mutation of SerH53 to ArgH53 in the Fab g37 heavy chain slightly decreased the Fab's affinity for 8-oxoG but significantly improved its binding to DNA in an 8-oxoG-dependent manner. The light chain shuffling experiments also demonstrated that numerous promiscuous light chains could enhance DNA binding when paired with either the Fab g37 or Fab 166 heavy chains; however, only the Fab 166 light chain did so in an additive manner when combined with the Fab 166 heavy chain that contains ArgH53. A three-point model for Fab 166 binding to oligonucleotides containing 8-oxoG is proposed. We describe a successful attempt to generate a desired antibody specificity, which was not present in the animal's original immune response.

Boraston, A. B., V. Notenboom, et al. (2003). "Structure and Ligand Binding of Carbohydrate-binding Module CsCBM6-3 Reveals Similarities with Fucose-specific Lectins and "Galactose-binding" Domains." Journal of Molecular Biology **327**(3): 659.

http://www.sciencedirect.com/science/article/B6WK7-482XFVD-5/2/897503ff87c4b42229aa2c322d98ff0d

Carbohydrate-binding polypeptides, including carbohydrate-binding modules (CBMs) from polysaccharidases, and lectins, are widespread in nature. Whilst CBMs are classically considered distinct from lectins, in that they are found appended to polysaccharide-degrading enzymes, this distinction is blurring. The crystal structure of CsCBM6-3, a "sequence-family 6" CBM in a xylanase from Clostridium stercorarium, at 2.3 A reveals a similar, all [beta]-sheet fold to that from MvX56, a module found in a family 33 glycoside hydrolase sialidase from Micromonospora

viridifaciens, and the lectin AAA from Anguilla anguilla. Sequence analysis leads to the classification of MvX56 and AAA into a family distinct from that containing CsCBM6-3. Whilst these polypeptides are similar in structure they have quite different carbohydrate-binding specificities. AAA is known to bind fucose; CsCBM6-3 binds cellulose, xylan and other [beta]-glucans. Here we demonstrate that MvX56 binds galactose, lactose and sialic acid. Crystal structures of CsCBM6-3 in complex with xylotriose, cellobiose, and laminaribiose, 2.0 A, 1.35 A, and 1.0 A resolution, respectively, reveal that the binding site of CsCBM6-3 resides on the same polypeptide face as for MvX56 and AAA. Subtle differences in the ligand-binding surface give rise to the different specificities and biological activities, further blurring the distinction between classical lectins and CBMs.

Borovjagin, A. V. and S. A. Gerbi (1999). "U3 small nucleolar RNA is essential for cleavage at sites 1, 2 and 3 in pre-rRNA and determines which rRNA processing pathway is taken in Xenopus oocytes." Journal of Molecular Biology **286**(5): 1347.

http://www.sciencedirect.com/science/article/B6WK7-45SJFV2-9/2/48e6481d3169189696347e57d8c856ea

A molecular dissection of U3 small nucleolar RNA (snoRNA) was performed in vivo inXenopus oocytes and the effects on rRNA processing were analyzed. Oocyte injection of antisense oligonucleotides against parts of U3 snoRNA resulted in specific fragmentation of U3 by endogenous RNase H. Fragmentation of U3 domain II correlated with a decrease in 20 S pre-rRNA and a concomitant increase in 36 S pre-rRNA, indicating reduced cleavage at site 3. Conversely, fragmentation of U3 domain I completely blocked 18 S rRNA formation, increased the 20 S rRNA precursor, and decreased 36 S pre-rRNA, indicating inhibition of cleavage at sites 1+2. rRNA processing defects at sites 1+2 or 3 after destruction of intact endogenous U3 snoRNA were rescued by injection of in vitro transcripts of U3 snoRNA or certain U3 fragments. Thus, cleavage at sites 1+2 and 3 is U3 snoRNA dependent. Moreover, U3 snoRNA has two functional modules: domain I for sites 1+2 cleavage and domain II for site 3 cleavage. The data suggest that whichever of these U3 domains acts first determines which rRNA processing pathway will be taken: cleavage first at site 3 of pre-rRNA leads to pathway A, whereas cleavage first at sites 1+2 leads to pathway B for rRNA processing. Predictions of this model were validated by rescue of site 3 cleavage by injection of just domain II after U3 depletion. Rescue of sites 1+2 cleavage required covalent continuity of domain I with the hinge region and non-covalent association with domain II. We could experimentally shift which rRNA processing pathway was taken by injecting fragments of U3 to compete with endogenous U3 snoRNA.

Boyer, P. L., J. Lisziewicz, et al. (1999). "Analysis of amino insertion mutations in the fingers subdomain of HIV-1 reverse transcriptase." Journal of Molecular Biology **286**(4): 995.

http://www.sciencedirect.com/science/article/B6WK7-45V8044-N/2/3657469e1b17c220bb3a53bd907bc0c2

In response to dideoxy inosine/hydroxyurea dual therapy, HIV-1 (human immunodeficiency virus type-1) variants were isolated that had a small amino acid insertion and flanking amino acid substitutions in the fingers subdomain of HIV-1. We have analyzed the reverse transcriptase variants for their effects on HIV-1 reverse transcriptase activity. The data suggests that the inserted amino acid residues are responsible for low-level resistance to the nucleoside analog ddITP, while the role of the flanking amino acid substitutions is to compensate for the deleterious effects of the insertion.

Campbell, M. J. and R. W. Davis (1999). "Toxic mutations in the recA gene of E. coli prevent proper chromosome segregation." Journal of Molecular Biology **286**(2): 417.

http://www.sciencedirect.com/science/article/B6WK7-45R87NG-JV/2/8a8beba9c7aea9b988034362f1fcd217

The recA gene of Escherichia coli is the prototype of therecA/RAD51/DMC1/uvsX gene family of strand transferases involved in genetic recombination. In order to find mutations in the recA gene important in catalytic turnover, a genetic screen was conducted for dominant lethal mutants. Eight single amino acid substitution mutants were found to prevent proper chromosome segregation and to kill cells in the presence or absence of an inducible SOS system. All mutants catalyzed some level of recombination and constitutively stimulated LexA cleavage. The mutations occur at the monomer-monomer interface of the RecA polymer or at residues important in ATP hydrolysis, implicating these residues in catalytic turnover. Based on an analysis of the E96D mutant, a model is presented in which slow RecA-DNA dissociation prevents chromosome segregation, engendering lexA -independent, lethal filamentation of cells.

Ciotta, C., S. Ceccotti, et al. (1998). "Increased somatic recombination in methylation tolerant human cells with defective DNA mismatch repair." Journal of Molecular Biology **276**(4): 705.

http://www.sciencedirect.com/science/article/B6WK7-45S49X9-J6/2/85a32dbecaadcc712d65ce5ef28d39fa

We have studied whether spontaneous intrachromosomal recombination is altered in methylation tolerant human cells with a defect in mismatch repair. Somatic recombination was analysed in HeLaMR cells containing the vector pTPSN, which carries two copies of the gene for hygromycin resistance. The hygromycin genes are both inactivated by an inserted HindIII linker but hygromycin-resistant clones can arise by recombination. The spontaneous rate of recombination in a clone of HeLaMR cells containing a single integrated copy of pTPSN (HeLaG1) was 3.1 x 10-6/cell per generation. Two methylation tolerant variants from HeLaG1 cells (clone 12 and clone 15) were isolated by exposure to MNNG. Clone 12 cells exhibited a 16-fold increase in spontaneous mutation rate at the HPRT gene and extensive microsatellite instability at both mono- and dinucleotide repeats. Microsatellite instability limited to mononucleotide repeats was found in clone 15, whereas the mutation rate at HPRT was not significantly affected. A mismatch binding defect in extracts of clone 15 could be complemented by exogenous GTBP but not by purified hMSH2 protein. These data suggest that clone 15 is defective in GTBP. Extracts of clone 12 were unable to correct a single C:T mispair and complementation by extracts of human colorectal carcinoma cells with known deficiencies in mismatch repair indicated a defect in hMutL[alpha]. Western blotting with antibodies against different human mismatch repair proteins showed that clone 12 cells did not express hPMS2 protein, but expression of hMLH1, hMSH2 and GTBP appeared normal. The spontaneous recombination rate of clone 12 was 19-fold higher than the parental HeLaG1 cells, whereas no increase was observed in clone 15. Analysis of individual recombinants showed that hygromycin resistance arose exclusively by gene conversion. Our data indicate that mismatch correction regulates somatic recombination in human cells.

DePriest, P. T. and M. D. Been (1992). "Numerous group I introns with variable distributions in the ribosomal DNA of a lichen fungus." Journal of Molecular Biology **228**(2): 315.

http://www.sciencedirect.com/science/article/B6WK7-4DN3YPC-5Y/2/7029c742118694426ba65cc5a08b84db

The length of the small subunit ribosomal DNA (SSU rDNA) differs significantly among individuals from natural populations of the ascomycetous lichen complex Cladonia chlorophaea. The sequence of the 3' region of the SSU rDNA from two individuals, chosen to represent the shortest and longest sequences, revealed multiple insertions within a region that otherwise aligned with a 520-nucleotide sequence of the SSU rDNA in Saccharomyces cerevisiae. The high degree of variability in SSU rDNA size can be accounted for by different numbers of insertions; one individual had two group I introns and the second had five introns, two of which were clearly related to introns at identical positions in the other individual. Yet, introns in different positions, whether within an individual or between individuals, were not similar in sequence. The distribution of introns at three of the positions is consistent with either intron loss or acquisition, and clearly indicates the dynamic variability in this region of the nuclear genome. All seven insertions, which ranged in size from 210 to 228 nucleotides, had the conserved sequence and secondary structural elements of group I introns. The variation in distribution and sequence of group I introns within a short highly conserved region of rDNA presents a unique opportunity for examining the molecular evolution and mobility of group I introns within a systematics framework.

Dixon, A. K., T.-M. Tait, et al. (1997). "Expression of the dystrophin-related protein 2 (Drp2) transcript in the mouse." Journal of Molecular Biology **270**(4): 551.

http://www.sciencedirect.com/science/article/B6WK7-45N4XBS-6G/2/ca202705cd673cc0ebbfebfa341d8486

We have recently characterised a new member of the dystrophin gene family, DRP2, and its murine counterpart, Drp2, which encode dystrophin-related protein 2 (DRP2). DRP2 is predicted to resemble certain short C-terminal isoforms of dystrophin and dystrophin-related protein 1 (DRP1 or utrophin). We describe here a comprehensive survey of Drp2 expression in the mouse by RT-PCR, and compare the expression profile of Drp2 with that of the related genes Dmd, Drp1 and Dag1 that encode all the known isoforms of dystrophin, DRP1/utrophin and a component of the dystrophin-associated protein complex, dystroglycan, respectively. Drp2 was shown to be expressed throughout the central nervous system (CNS) and in several peripheral tissues including the eye, kidney, teeth, oesophagus, colon, epididymis and ovary. The expression of Drp2 in the CNS was then further defined by in situ hybridization. Overall, the pattern of Drp2 expression corresponds to a subset of the brain regions known to express Dag1, and shows substantial overlap with regions that express various isoforms of dystrophin (particularly in the cerebral cortex, hippocampus and cerebellum). These data define the distribution of Drp2 expression in the mouse, and raise the possibility that in the CNS it may be an important component in neuronal dystrophin-associated complexes.

Doherty, A. J. and D. B. Wigley (1999). "Functional domains of an ATP-dependent DNA ligase." Journal of Molecular Biology **285**(1): 63.

http://www.sciencedirect.com/science/article/B6WK7-45KNCTG-7R/2/910e0ea900197e3c1b8ee2939cff45c4

The crystal structure of an ATP-dependent DNA ligase from bacteriophage T7 revealed that the protein comprised two structural domains. In order to investigate the biochemical activities of these domains, we have overexpressed them separately and purified them to homogeneity. The larger N-terminal domain retains adenylation and ligase activities, though both at a reduced level.

The adenylation activity of the large domain is stimulated by the presence of the smaller domain, suggesting that a conformational change is required for adenylation in the full length protein. The DNA binding properties of the two fragments have also been studied. The larger domain is able to band shift both single and double-stranded DNA, while the smaller fragment is only able to bind to double-stranded DNA. These data suggest that the specificity of DNA ligases for nick sites in DNA is produced by a combination of these different DNA binding activities in the intact enzyme.

Dorgai, L., S. Sloan, et al. (1998). "Recognition of core binding sites by bacteriophage integrases." Journal of Molecular Biology **277**(5): 1059.

http://www.sciencedirect.com/science/article/B6WK7-45S49S7-G0/2/36df8d2e13ff4b21b9253613da307caf

Bacteriophage integrases promote recombination between DNA molecules that carry attachment sites. They are members of a large and widely distributed family of site-specific recombinases with diverse biological roles. The integrases of phages [lambda] and HK022 are closely related members of this family, but neither protein efficiently recombines the attachment sites of the other phage. The nucleotides responsible for this specificity difference are located close to the points of recombinational strand exchange, within an integrase binding motif called the extended core binding site. There are four imperfectly repeated copies of this motif in each set of phage attachment sites, but only two, B' and C, contain major specificity determinants. When these specificity determinants were replaced by the corresponding nucleotides from a site with the alternative specificity, the resulting mutant was recombined by both integrases. Thus, the determinants act by impeding recombination promoted by the non-cognate integrase. We found that identical nucleotide substitutions within different core site copies had different effects on recombination, suggesting that integrase does not recognize each of the extended core binding sites in the same way. Finally, substitution at several positions in [lambda] integrase with the corresponding HK022-specific amino acids prevents recombination of [lambda] attachment sites, and this defect can be suppressed in an allele-specific manner by appropriate substitutions of HK022-specific nucleotides in the extended core binding sites.

Duffy, C. and M. Feiss (2002). "The large subunit of bacteriophage [lambda]'s terminase plays a role in DNA translocation and packaging termination." Journal of Molecular Biology **316**(3): 547.

http://www.sciencedirect.com/science/article/B6WK7-458W9VP-D/2/c583ca0c55329744b2df0a79ae0001ac

The DNA packaging enzyme of bacteriophage [lambda], terminase, is a heteromultimer composed of a small subunit, gpNu1, and a large subunit, gpA, products of the Nu1 and A genes, respectively. The role of terminase in the initial stages of packaging involving the site-specific binding and cutting of the DNA has been well characterized. While it is believed that terminase plays an active role in later post-cleavage stages of packaging, such as the translocation of DNA into the head shell, this has not been demonstrated. Accordingly, we undertook a generalized mutagenesis of [lambda]'s A gene and found ten lethal mutations, nine of which cause post-cleavage packaging defects. All were located in the amino-terminal two-thirds of gpA, separate from the carboxy-terminal region where mutations affecting the protein's endonuclease activity have been found. The mutants fall into five groups according to their packaging phenotypes: (1) two mutants package part of the [lambda] chromosome, (2) one mutant packages the entire chromosome, but very slowly compared to wild-type, (3) two mutants do not package any DNA, (4) four mutants, though inviable, package the entire [lambda] chromosome, and (5) one mutant may be defective in both early and late stages of DNA packaging. These results indicate that gpA is actively involved in late stages of packaging, including DNA translocation, and that this enzyme

contains separate functional domains for its early and late packaging activities.

Dworkin, J., G. Jovanovic, et al. (1997). "Role of upstream activation sequences and integration host factor in transcriptional activation by the constitutively active prokaryotic enhancer-binding protein PspF." Journal of Molecular Biology **273**(2): 377.

http://www.sciencedirect.com/science/article/B6WK7-45RFFH6-16/2/a4cb585b955f22a23968cea21a488bea

PspF, the transcriptional activator of the pspA operon of Escherichia coli, which belongs to the enhancer binding protein (EBP) family of [sigma]54 activator proteins, is constitutively active in an in vitro transcription assay. PspF protein, together with RNA polymerase holoenzyme containing [sigma]54, is required for in vitro transcription from the pspA promoter. EBP proteins are typically subject to regulation either by post-translational modification or interaction of a specific ligand with an N-terminal regulatory domain. However, unlike other members of the EBP family, PspF lacks this domain. pspA is positively regulated by IHF in vitro, and this regulation is dependent on the topology of the DNA; a linear template is much more dependent on IHF than a supercoiled template. EBP binding to upstream activating sequences (UAS) in their target promoters is mediated by the C-terminal domain which contains a helix-turn-helix DNA-binding motif. A mutant PspF protein lacking the C-terminal DNA-binding domain is active in vitro, although at much higher concentrations than the wild-type protein. In vitro transcription from pspA templates missing one or both of the UAS sites is reduced relative to wild-type templates, but is still appreciable; however, IHF acts as a negative regulator of pspA transcription on these mutant templates. Thus, PspF bound to non-specific sequences upstream of the pspA promoter can activate pspA transcription, but this activation is inhibited by IHF. These data, taken together, support the model that a precise promoter geometry is necessary for IHF to positively regulate transcription and that IHF may act to prevent activation from inappropriately spaced upstream sites.

Fang, L. J., M. J. Simard, et al. (2001). "A novel mutation in the neurofibromatosis type 1 (NF1) gene promotes skipping of two exons by preventing exon definition." Journal of Molecular Biology **307**(5): 1261.

http://www.sciencedirect.com/science/article/B6WK7-457D1BS-68/2/1923a3e5a127832aa52314bce6705ccd

Using a protein truncation assay, we have identified a new mutation in the neurofibromatosis type 1 (NF1) gene that causes a severe defect in NF1 pre-mRNA splicing. The mutation, which consists of a G to A transition at position +1 of the 5' splice site of exon 12a, is associated with the loss of both exons 11 and 12a in the NF1 mRNA. Through the use of in vivo and in vitro splicing assays, we show that the mutation inactivates the 5' splice site of exon 12a, and prevents the definition of exon 12a, a process that is normally required to stimulate the weak 3' splice site of exon 12a. Because the 5' splice site mutation weakens the interaction of splicing factors with the 3' splice site of exon 12a, we propose that exon 11/exon 12a splicing is also compromised, leading to the exclusion of both exons 11 and 12a. Our results provide in vivo support for the importance of the exon definition model during NF1 splicing, and suggest that the NF1 region containing exons 11 and 12a plays an important role in the activity of neurofibromin.

Hoheisel, J. D., G. G. Lennon, et al. (1991). "Use of high coverage reference libraries of Drosophila

melanogaster for relational data analysis: A step towards mapping and sequencing of the genome." Journal of Molecular Biology **220**(4): 903.

http://www.sciencedirect.com/science/article/B6WK7-4FNGB0M-6V/2/d9609456aa362ba5e318e319df6e04a7

Three differently made, primary Drosophila cosmid libraries of 16-fold genome coverage have been generated. Also, a jumping library has been created by a new method that takes advantage of methylation differences between genomic DNA and vector. Thirdly, two cDNA libraries have been picked. All these libraries have been arrayed on high-density in situ filters, each containing 9216 clones. As a reference system, such filters are distributed and identified clones are provided. Single-copy probes have identified on average 1.4 cosmids per genome equivalent. Together with cytogenetically mapped yeast artificial chromosomes, the libraries are also being used for physically mapping the genome, mainly by oligonucleotide fingerprinting and pool hybridizations. cDNA clones are further examined by a partial sequencing analysis by oligomer hybridization.

Ito, E., G.-x. Xie, et al. (2000). "A core-promoter region functions bi-directionally for human opioid-receptor-like gene ORL1 and its 5'-adjacent gene GAIP." Journal of Molecular Biology **304**(3): 259.

http://www.sciencedirect.com/science/article/B6WK7-45F50T3-1K/2/7e8e20dbb7391e9424deea655117e386

We report the genomic structure and functional activities of the promoter regions of the human opioid-receptor-like gene ORL1 and its 5'-adjacent gene GAIP (G alpha interacting protein). The transcription and alternative splicing of human ORL1 are controlled by two alternate promoters, located approximately 10 kb apart. The two promoter regions lack a TATA-box and are GC rich. Promoter 1A initiates, from a single transcription start point (TSP), two transcripts: one consisting of exons 1A, 1B, 2, etc., the other without exon 1B. A potential ATG codon upstream of the initiation codon of ORL1 starts a new open-reading frame encoding a theoretical polypeptide of 205 amino acid residues. The promoter 1B transcribes, from multiple TSPs, only one mRNA starting with exon 1B. Two different repeat sequence polymorphisms are found in the ORL1 promoter regions. Luciferase reporter gene assays with promoter regions and a series of deletion mutants have mapped the core-promoter 1A and 1B within two short fragments. DNA sequencing and a database search reveal that the human GAIP gene is located upstream of ORL1 and is oriented in the opposite direction. The transcription and alternative splicing of GAIP are also under the control of alternate promoters. The first exons of ORL1 and GAIP are separated by only 83 bp. This 83 bp fragment, together with short surrounding sequences from both first exons, functions bi-directionally as a core-promoter for both genes. The transcription and alternative splicing of human ORL1 and GAIP are cell-type specific. While GAIP is expressed in both NT2 precursor cells and differentiated NT2 neuronal cells, ORL1 is only expressed in differentiated NT2 neurons. Since ORL1 is a G protein-coupled receptor and GAIP interacts with G protein [alpha] subunits, their physical linkage in the genome and co-operative transcriptional regulation may play a significant role in ORL1 receptor signal transduction.

Keeling, P. J. and B. S. Leander (2003). "Characterisation of a Non-canonical Genetic Code in the Oxymonad Streblomastix strix." Journal of Molecular Biology **326**(5): 1337.

http://www.sciencedirect.com/science/article/B6WK7-47XNNH2-6/2/60128e61f4e98446a343e6be02658e1e

The genetic code is one of the most highly conserved characters in living organisms. Only a small number of genomes have evolved slight variations on the code, and these non-canonical codes are instrumental in understanding the selective pressures maintaining the code. Here, we describe a new case of a non-canonical genetic code from the oxymonad flagellate Streblomastix strix. We have sequenced four protein-coding genes from S. strix and found that the canonical stop codons TAA and TAG encode the amino acid glutamine. These codons are retained in S. strix mRNAs, and the legitimate termination codons of all genes examined were found to be TGA, supporting the prediction that this should be the only true stop codon in this genome. Only four other lineages of eukaryotes are known to have evolved non-canonical nuclear genetic codes, and our phylogenetic analyses of [alpha]-tubulin, [beta]-tubulin, elongation factor-1 [alpha] (EF-1 alpha), heat-shock protein 90 (HSP90), and small subunit rRNA all confirm that the variant code in S. strix evolved independently of any other known variant. The independent origin of each of these codes is particularly interesting because the code found in S. strix, where TAA and TAG encode glutamine, has evolved in three of the four other nuclear lineages with variant codes, but this code has never evolved in a prokaryote or a prokaryote-derived organelle. The distribution of non-canonical codes is probably the result of a combination of differences in translation termination, tRNAs, and tRNA synthetases, such that the eukaryotic machinery preferentially allows changes involving TAA and TAG.

Koch, H.-G., C. Winterstein, et al. (2000). "Roles of the ccoGHIS gene products in the biogenesis of the cbb3-type cytochrome c oxidase." Journal of Molecular Biology **297**(1): 49.

http://www.sciencedirect.com/science/article/B6WK7-460PM8T-5/2/659d51207e683eef8e05574978d1bf0f

In many bacteria the ccoGHIS cluster, located immediately downstream of the structural genes (ccoNOQP) of cytochrome cbb3 oxidase, is required for the biogenesis of this enzyme. Genetic analysis of ccoGHIS in Rhodobacter capsulatus demonstrated that ccoG, ccoH, ccoI and ccoS are expressed independently of each other, and do not form a simple operon. Absence of CcoG, which has putative (4Fe-4S) cluster binding motifs, does not significantly affect cytochrome cbb3 oxidase activity. However, CcoH and CcoI are required for normal steady-state amounts of the enzyme. CcoI is highly homologous to ATP-dependent metal ion transporters, and appears to be involved in the acquisition of copper for cytochrome cbb3 oxidase, since a CcoI-minus phenotype could be mimicked by copper ion starvation of a wild-type strain. Remarkably, the small protein CcoS, with a putative single transmembrane span, is essential for the incorporation of the redox-active prosthetic groups (heme b, heme b3 and Cu) into the cytochrome cbb3 oxidase. Thus, the ccoGHIS products are involved in several steps during the maturation of the cytochrome cbb3 oxidase.

Kolmerer, B., J. Clayton, et al. (2000). "Sequence and expression of the kettin gene in Drosophila melanogaster and Caenorhabditis elegans." Journal of Molecular Biology **296**(2): 435.

http://www.sciencedirect.com/science/article/B6WK7-45F4TTJ-Y/2/617f4577f976f4d0bef6ca6d9b2ce245

Kettin is a large modular protein associated with thin filaments in the Z-disc region of insect muscles. The sequence of a 21.3 kb contig of the Drosophila gene has been determined. The corresponding protein sequence has 35 immunoglobulin-like (Ig) domains which are separated by shorter linker sequences, except near the N and C termini of the molecule where linker sequences are short or missing. This confirms a model in which each Ig domain binds to an actin protomer. The Drosophila kettin gene is at 62C 1-3 on the third chromosome. Two P-element insertions, l(3)j1D7 and l(3)rL182 are in the kettin gene, and complementation tests showed that

existing l(3)dre8 mutations are in the same gene. The RNA was detected in wild-type Drosophila embryos at stage 11, first in the gut invagination region of the mesoderm, and by stage 13 in both visceral and somatic mesoderm. Somatic mesoderm expression became segmental at stage 13. RNA expression was greatly reduced in embryos of P-element homozygotes but normal in heterozygotes. The structure of the flight muscle in all the heterozygous mutants was normal, including the myofibril-cuticle connections, and they were able to fly. Kettin sequence homologous to the Drosophila protein, was identified in the Caenorhabditis elegans genome database. The RNA was detected in pharyngeal, body wall and anal depressor muscles of larvae and adult worms, as well as in the male gonad. Antibody to insect kettin labelled the pharyngeal, body wall, anal depressor and proximal gonadal muscles in adult worms. Body wall muscles were labelled in an obliquely striated pattern consistent with the Z-disc localisation in insect muscle. The relationship of kettin to D-titin, which has been assigned to the same chromosomal locus in Drosophila, is discussed.

Krebitz, M., B. Wagner, et al. (2003). "Plant-based Heterologous Expression of Mal d 2, a Thaumatin-like Protein and Allergen of Apple (Malus domestica), and its Characterization as an Antifungal Protein." Journal of Molecular Biology **329**(4): 721.

http://www.sciencedirect.com/science/article/B6WK7-48PDMV4-D/2/d4ceb9f3402e5e5991e8bffc46fd81a0

Mal d 2 is a thaumatin-like protein and important allergen of apple fruits that is associated with IgE-mediated symptoms in apple allergic individuals. We obtained a full-length cDNA clone of Mal d 2 from RNA isolated from ripe apple (Malus domestica cv. Golden Delicious). The cDNA's open reading frame encodes a protein of 246 amino acid residues including a signal peptide of 24 residues and two putative glycosylation sites. The deduced amino acid sequence of the mature Mal d 2 protein results in a predicted molecular mass of 23,210.9 Da and a calculated pI of 4.55. Sequence comparisons and molecular modeling place Mal d 2 among those pathogenesis-related thaumatin-like proteins that contain a conserved acidic cleft. In order to ensure the correct formation of the protein's eight conserved disulfide bridges we expressed Mal d 2 in Nicotiana benthamiana plants by the use of a tobacco mosaic viral vector. Transfected N. benthamiana plants accumulated Mal d 2 to levels of at least 2% of total soluble protein. MALDI-TOF mass spectrometric analyses of the recombinant Mal d 2 and its proteolytic fragments showed that the apple-specific leader peptide was correctly cleaved off by the host plant and that the mature recombinant protein was intact and not glycosylated. Purified recombinant Mal d 2 displayed the ability to bind IgE from apple-allergic individuals equivalent to natural Mal d 2. In addition, the recombinant thaumatin-like Mal d 2 exhibited antifungal activity against Fusarium oxysporum and Penicillium expansum, implying a function in plant defense against fungal pathogens.

Lang, R., A. Kocourek, et al. (2001). "Substrate specificity determinants of human macrophage elastase (MMP-12) based on the 1.1 A crystal structure." Journal of Molecular Biology **312**(4): 731.

http://www.sciencedirect.com/science/article/B6WK7-457CY1P-6G/2/3cd94550943ef082bd061fd756454439

The macrophage elastase enzyme (MMP-12) expressed mainly in alveolar macrophages has been identified in the mouse lung as the main destructive agent associated with cigarette smoking, which gives rise to emphysema, both directly via elastin degradation and indirectly by disturbing the proteinase/antiproteinase balance via inactivation of the [alpha]1-proteinase inhibitor ([alpha]1-PI), the antagonist of the leukocyte elastase. The catalytic domain of human recombinant MMP-12 has been crystallized in complex with the broad-specificity inhibitor batimastat (BB-94). The crystal structure analysis of this complex, determined using X-ray data to

1.1 A and refined to an R-value of 0.165, reveals an overall fold similar to that of other MMPs. However, the S-shaped double loop connecting strands III and IV is fixed closer to the [beta]-sheet and projects its His172 side-chain further into the rather hydrophobic active-site cleft, defining the S3 and the S1-pockets and separating them from each other to a larger extent than is observed in other MMPs. The S2-site is planar, while the characteristic S1'-subsite is a continuous tube rather than a pocket, in which the MMP-12-specific Thr215 replaces a Val residue otherwise highly conserved in almost all other MMPs. This alteration might allow MMP-12 to accept P1' Arg residues, making it unique among MMPs. The active-site cleft of MMP-12 is well equipped to bind and efficiently cleave the AlaMetPhe-LeuGluAla sequence in the reactive-site loop of [alpha]1-PI, as occurs experimentally. Similarities in contouring and particularly a common surface hydrophobicity both inside and distant from the active-site cleft explain why MMP-12 shares many substrates with matrilysin (MMP-7). The MMP-12 structure is an excellent template for the structure-based design of specific inhibitors for emphysema therapy and for the construction of mutants to clarify the role of this MMP.

Lee, K., S. Varma, et al. (1997). "In vivo determination of RNA Structure-Function relationships: analysis of the 790 loop in ribosomal RNA." Journal of Molecular Biology **269**(5): 732.

http://www.sciencedirect.com/science/article/B6WK7-45RFFN2-2T/2/1fd530b69ee194eae6bda86293f8aa49

The 790 loop is a conserved hairpin located between positions 786 and 796 of Escherichia coli 16 S rRNA that is required for ribosome function. Using a novel genetic approach, all positions in the loop were simultaneously mutated and functional mutant sequences were selected in vivo. This "instant evolution" experiment revealed that approximately 190 of the 262,144 possible mutant sequences were functional. Analysis of functional mutant sequences allowed discrimination between nucleotides directly involved in protein synthesis and those involved primarily in loop structure. Among the functional mutant sequences, positions 789 and 791 were invariant and extensive covariation was observed among the nucleotides at the base of the loop at positions 787, 788, 794 and 795. NMR and thermodynamic analyses of model 790 hairpinsin vitro revealed weak pairing interactions between positions 787 and 795 and between positions 788 and 794 consistent with the in vivo mutational analysis. Functional analysis of site-directed mutants containing all possible nucleotide combinations at positions 787 and 795 in vivo showed that stable base-pairs at these positions prevent subunit association.

Leonhartsberger, S., A. Huber, et al. (2001). "The hydH/G genes from Escherichia coli code for a zinc and lead responsive two-component regulatory system." Journal of Molecular Biology **307**(1): 93.

http://www.sciencedirect.com/science/article/B6WK7-457D49J-B/2/f5510eedb32e8585aa7c93d665dacb2a

The hydH/G genes from Escherichia coli code for a two-component regulatory system that has been implicated in the regulation of hydrogenase 3 formation. In a detailed study of the function of HydH/G employing hycA'-'lacZ reporter gene fusions, it was shown that HydH/G indeed led to a stimulation of activation of the hycA promoter responsible for hydrogenase 3 synthesis but only when hydG is overexpressed from a plasmid in a strain lacking FhlA. Since the stimulation was not observed with an fdhF'-'lacZ fusion, and since it was independent from a functional hydH gene product, it must be considered as unspecific cross-talk. An extensive search for the actual physiological signal of HydH/G showed that the system responds to high concentrations of zinc or lead in the medium. Expression of zraP, a gene inversely oriented to hydH/G whose product seems to be involved in acquisition of tolerance to high Zn2+ concentrations, is stimulated by high Zn2+ and Pb2+ concentrations and this stimulation requires both HydH and HydG. Purified

HydG in the presence of phosphoryl donors binds to a region within the zraP-hydHG intergenic region that is characterised by two inverted repeats separated by a 14 bp spacer. Putative -12/-24 [sigma]54-dependent promoter motifs are present upstream of both the zraP and the hydHG transcriptional units; in accordance, transcription of zraP is strictly dependent on the presence of a functional rpoN gene. The expression of hydH/G is autoregulated: high Zn2+ and Pb2+ concentrations lead to a significant increase of the HydG protein content which took place only in a hydH+ genetic background. Since HydH binds to membranes tightly, it is assumed that the HydH/G system senses high periplasmic Zn2+ and Pb2+ concentrations and contributes to metal tolerance by activating the expression of zraP. The redesignation of hydH/G as zraS/R is suggested.

Lin, Y. and S. D. Jayasena (1997). "Inhibition of multiple thermostable DNA polymerases by a heterodimeric aptamer." Journal of Molecular Biology **271**(1): 100.

http://www.sciencedirect.com/science/article/B6WK7-45NJPJ0-4S/2/4e95e3f9ef55ff65634ffd87cbb718da

Single-stranded DNA aptamers that recognize DNA polymerase from Thermus acquaticus (Taq pol) with high affinity have been described recently. These aptamers have been shown to efficiently inhibit the polymerase activity of Taq pol and are useful in enhancing the amplification efficiency of low copy number targets by the polymerase chain reaction (PCR). Aptamers selected to bind to Taq pol fell into two different sequence families and inhibited several DNA polymerases isolated from the Thermus species, including that from Thermus thermophilus (Tth pol). Aptamers from one sequence family inhibited the Stoffel fragment of Taq pol efficiently, whereas those from the other family did not. Truncated aptamers derived from two parent ligands from both families were combined to form a heterodimeric aptamer that effectively inhibited all three polymerases and were shown to be useful in detecting a low copy number target by PCR amplification. These data demonstrate that the combination of aptamers with different properties into a single molecule broadens their spectrum of utility.

Linse, S., M. Voorhies, et al. (2000). "An EF-hand phage display study of calmodulin subdomain pairing." Journal of Molecular Biology **296**(2): 473.

http://www.sciencedirect.com/science/article/B6WK7-45F4TTJ-12/2/3ee0242bf4e5fde046518362347ebbb2

The interaction between the two EF-hands, EF3 and EF4, in the C-terminal domain of vertebrate calmodulin is addressed using an EF-hand phage display library. Significant specificity is observed in the presence of Ca2+, as EF3-EF4 heterodimers are favored over EF3-EF3 and EF4-EF4 homodimers. Primarily EF4-type (and not EF3-type) amino acids are selected when an EF3 peptide is used as the target and vice versa. The results show that this specificity is promoted by several factors. There are three positions, corresponding to Phe89, Ala102, and Leu105, that are strongly selected as EF3-type hydrophobic residues with an EF4 target. When EF3 is the target peptide, EF4-type residues, Ile125, Tyr138 and Phe141, are selected. Remarkably, this subset consists of the same three residue positions in EF3 or EF4 and seems to be involved in specifying the heterodimer preference in both cases. In addition, electrostatic repulsion between the acidic monomers in an EF4 homodimer may further influence the preferred stability of heterodimers. This hypothesis is based on the observation that positively charged residues are strongly selected at four positions when EF4 is the target. A survey of EF-hand pairs suggests that charge separation is a common way to achieve efficient attraction of Ca2+ without causing electrostatic repulsion between the subdomains. No significant specificity of binding is observed in the ion free state or in the presence of magnesium as no sequence is preferentially

selected. The residues at the interface between the two EF-hands are thus highly optimized for the Ca2+ bound state. At some residue positions, EF3-type amino acids are chosen with EF3-target in the presence of Ca2+. These residues are not involved in the preference for heterodimer over homodimer formation, but represent key positions to mutate in the intact domain to stabilize its Ca2+-bound state.

Loakes, D., F. Hill, et al. (1997). "Stability and structure of DNA oligonucleotides containing non-specific base analogues." Journal of Molecular Biology **270**(3): 426.

http://www.sciencedirect.com/science/article/B6WK7-45RFFJF-26/2/cbfbc4d243a76435d98752a0f9df0553

The nature of DNA containing the deoxyribosyl derivative of 5-nitroindole has been investigated. 5-Nitroindole has been shown to give good stability when present in duplexes opposite natural bases, with only slightly reduced melting temperatures. However, enhanced stability occurs when it is incorporated as an additional bulged base in duplexes. It also markedly enhances the stability of duplexes when it is present as a pendant base at either the 5' or 3'-ends of the two strands. The stabilisation is presumed to be due to enhanced stacking interactions for the nitroindole base. Oligomers containing a number of consecutive 5-nitroindole residues form stable, stacked secondary structures. An oligomer containing 21 such substitutions is presumed to exist as a hairpin structure. This was further investigated by circular dichroism melting experiments, which demonstrated that the single-stranded oligomer contains significant secondary structure in the region of the 5-nitroindole tract, which appears to contain a double-stranded stem. X-ray analysis of 5-nitroindole deoxyriboside provides some indication of how the mode of stacking observed in crystals of the nucleoside may also be responsible for stabilising secondary structures of oligonucleotides.

Lyu, Y. L., C.-T. Lin, et al. (1999). "Inversion/dimerization of plasmids mediated by inverted repeats." Journal of Molecular Biology **285**(4): 1485.

http://www.sciencedirect.com/science/article/B6WK7-45R8860-SH/2/3aefd67c88250b2a8378f39b2e2fbe1f

In contrast with earlier studies on the lambda and Escherichia coli genomes, recombination between inverted repeats on plasmids is highly efficient and shown to be recA-independent. In addition, the recombination product is exclusively a head-to-head inverted dimer. Here, we show that this recombination/rearrangement event can occur on different plasmid replicons and is not specific to the particular sequence within the inverted repeats. Transcription readthrough into the inverted repeats has little effect on this event. Genetic analysis has also indicated that most known recombination enzymes are not involved in this process. Specifically, single or double mutants defective in Holliday junction resolution systems (RuvABC and/or RecG/RusA) do not abolish this recombination/rearrangement event. This result does not support the previous models (i.e. the reciprocal-strand-switching and the cruciform-dumbbell models) in which intermediates containing Holliday junctions are proposed. Further analysis has demonstrated that the recombination/rearrangement frequency is dramatically (over 1000-fold) reduced if mismatches (2.8%) are present within the inverted repeats. Mutations in dam, mutH and mutL genes partially or completely restored the recombination/rearrangement frequency to the level exhibited by the perfect inverted repeats, suggesting the formation of heteroduplexes during recombination/rearrangement. Sequencing analysis of the recombination/rearrangement products have indicated that the majority of the products do not involve crossing-over. We discuss a possible mechanism in which blockage of the lagging strand polymerase by a hairpin triggers recombination/rearrangement mediated by inverted repeats.

Mahadeva, H., M. P. Starkey, et al. (1998). "A simple and efficient method for the isolation of differentially expressed genes." Journal of Molecular Biology **284**(5): 1391.

http://www.sciencedirect.com/science/article/B6WK7-45S48YR-1V/2/7526ea925f560ac44cdf4747640613e2

A simple and reproducible general approach for the isolation of differentially expressed genes is described. Digestion of cDNAs with a class IIs restriction endonuclease produces fragments with every combination of possible bases in the cohesive ends. Under stringent conditions, the specific ligation of adaptors with perfectly complementary overhangs partitions the cDNA fragments into non-overlapping subpopulations. Internal cDNA restriction fragments are exponentially amplified by adaptor primer PCR and visualised by non-denaturing polyacrylamide gel electrophoresis. The power of the technology was demonstrated using a rat model of pressure-induced left-ventricular hypertrophy (LVH). A set of 29 fragments, derived from a sample (6 %) of the possible adaptor pool combinations, displayed apparent differential expression. The differential expression of 19 (66 %) were confirmed by Northern blot analysis. Sequence analysis identified both genes known to be upregulated in LVH, and novel genes. The fidelity of adaptor ligation was demonstrated by the isolation of known gene fragments by appropriate adaptor combinations. The spiking of mRNA populations with known amounts of a synthetic mRNA demonstrated a current sensitivity equivalent to the detection of transcripts expressed at the level of as little as 1 in 10,000 molecules.

McGowan, S., I. S. Lucet, et al. (2002). "The FxRxHrS Motif: A Conserved Region Essential for DNA Binding of the VirR Response Regulator from Clostridium perfringens." Journal of Molecular Biology **322**(5): 997.

http://www.sciencedirect.com/science/article/B6WK7-46W1BV8-B/2/66cf9c38e97d5bb63acafc51b2124fd9

The VirSR two-component signal transduction pathway regulates virulence and toxin production in Clostridium perfringens, the causative agent of gas gangrene. The response regulator, VirR, binds to repeat sequences located upstream of the promoter and is directly responsible for the transcriptional activation of pfoA, the structural gene for the cholesterol-dependent cytolysin, perfringolysin O. Comparative sequence analysis of the 236 amino acid residue VirR protein revealed a two-domain structure: a typical N-terminal response regulator domain and an uncharacterised C-terminal domain. Database searching revealed that over 40 other proteins, many of which appeared to be response regulators or transcriptional activators, had homology with the VirR C-terminal domain (VirRc). Multiple sequence alignment of this VirRc family revealed a highly conserved region that was designated the FxRxHrS motif. By deletion analysis this motif was shown to be essential for the functional integrity of the VirR protein. Alanine scanning mutagenesis and subsequent phenotypic analysis indicated that conserved residues located within the motif were required for activity. These residues extended from L179 to N194. More detailed site-directed mutagenesis showed that amino acid residues R186, H188 and S190 were essential for activity since even conservative substitutions in these positions resulted in non-functional proteins. Three of the mutant proteins, R186K, S190A and S190C, were purified and shown by in vitro gel shift analysis to be unable to bind to the specific target DNA with the same efficiency as the wild-type protein. These data reveal for the first time that VirRc functions as a DNA binding domain in which the highly conserved FxRxHrS motif has a functional role. These studies have important implications for this new family of transcriptional factors since they imply that the conserved FxRxHrS motif may be involved in DNA binding in all of these proteins, irrespective of their biological role.

Meiss, G., F.-U. Gast, et al. (1999). "The DNA/RNA non-specific Serratia nuclease prefers double-stranded A-form nucleic acids as substrates." Journal of Molecular Biology **288**(3): 377.

http://www.sciencedirect.com/science/article/B6WK7-45R87KC-HD/2/546878ea8ef388a8a730c7231813b666

A steady-state kinetic analysis of the cleavage of the oligonucleotides d(CGCTTTTTTGC) (d(y)), d(GCAAAAAAGCG) (d(r)), r(CGCUUUUUUGC) (r(y)) and r(GCAAAAAAGCG) (r(r)) in single and double-stranded form by the extracellular Serratia marcescens endonuclease, in conjunction with structural data from a circular dichroism spectroscopic analysis of these substrates, suggests that oligonucleotides adopting the A-conformation are preferred over those adopting the B-conformation as substrates. Relative catalytic efficiencies (kcat/KM) for the cleavage of the homo- and heteroduplexes follow the order r(r).r(y) (1.0) > r(r).d(y) (0.9) > d(r).r(y) (0.7) > d(r).d(y) (0.3). The purine-rich single-stranded oligonucleotides r(r) and d(r), are cleaved more efficiently than the pyrimidine-rich oligonucleotides, r(y) and d(y), presumably because they adopt helical structures with pronounced base stacking. Except for the double-stranded oligodeoxynucleotide substrate, the individual strands are cleaved more efficiently when incorporated into a duplex, than in a single-stranded form. Cleavage experiments with various polynucleotides, including a viroid RNA and a specifically designed 167 bp DNA, confirm that double-stranded A-form nucleic acids are preferentially attacked by Serratia nuclease. In an attempt to analyze the basis of these preferences, we have mutated the amino acid residues Tyr76 and Trp123 of Serratia nuclease. These residues are located close to the active site and are conserved in all members of the Serratia nuclease family, suggesting that they could be involved in substrate binding, e.g. by stacking interactions with the bases, which could lead to the cleavage preferences observed. However, only effects on the activity, but no change of the sequence or substrate preferences, were detected upon substitution of these amino acid residues, ruling out any involvement of these residues in the A-form preference of Serratia nuclease.

Miki, T., J. Ae Park, et al. (1992). "Control of segregation of chromosomal DNA by sex factor F in Escherichia coli: Mutants of DNA gyrase subunit A suppress letD (ccdB) product growth inhibition." Journal of Molecular Biology **225**(1): 39.

http://www.sciencedirect.com/science/article/B6WK7-4DMP5N2-11/2/6577b2f5d02bb448011b690790e676c4

The letA (ccdA) and letD (ccdB) genes, located just outside the sequence essential for replication of the F plasmid, apparently contribute to stable maintenance of the plasmid. The letD gene product acts to inhibit partitioning of chromosomal DNA and cell division of the host bacteria, whereas the letA gene product acts to suppress the activity of the letD gene product. To identify the target of the letD gene product, temperature-sensitive growth-defective mutants were screened from bacterial mutants that had escaped the letD product growth inhibition that occurs in hosts carrying an FletA mutant. Of nine mutants analysed, three mutants were shown, by phage P1-mediated transduction and complementation analysis, to have mutations in the gyrA gene and the other six in the groE genes. The nucleotide sequence revealed that one of the gyrA mutants has a base change from G to A at position 641 (resulting in an amino acid change from Gly to Glu at position 214) of the gyrA gene. The mutant GyrA proteins produced by these gyrA(ts) mutants were trans-dominant over wild-type GyrA protein for letD tolerance. The wild-type GyrA protein, produced in excess amounts by means of a multicopy plasmid, overcame growth inhibition of the letD gene product. These observations strongly suggest that the A subunit of DNA gyrase is the target of the LetD protein.

Morosyuk, S. V., K. Lee, et al. (2000). "Structure and function of the conserved 690 hairpin in Escherichia coli 16 s ribosomal RNA: analysis of the stem nucleotides." Journal of Molecular Biology **300**(1): 113.

http://www.sciencedirect.com/science/article/B6WK7-45F51FD-DK/2/78734795ad7b131ed80c2917a316d7e2

Nucleotides 680 to 710 of Escherichia coli 16 S rRNA form a distinct structural domain required for ribosome function. The goal of this study was to determine the functional significance of pairing interactions in the 690 region. Two different secondary structures were proposed for this hairpin, based on phylogenetic and chemical modification studies. To study the effect of pairing interactions in the 690 hairpin on ribosome function and to determine which of the proposed secondary structures is biologically significant, we performed an instant-evolution experiment in which the nine nucleotides that form the proposed base-pairs and dangling ends of the 690 stem were randomly mutated, and functional mutant combinations were selected. A total of 96 unique functional mutants were isolated, assayed in vivo, and sequenced. Analysis of these data revealed extensive base-pairing and stacking interactions among the mutated nucleotides. Formation of either a Watson-Crick base-pair or G.U pair between positions 688 and 699 is absolutely required for ribosome function. We also performed NMR studies of a 31-nucleotide RNA which indicate the formation of a functionally important base-pair between nucleotides 688 and 699. Formation of a second base-pair between positions 689 and 698, however, is not essential for ribosome function, but the level of ribosome function correlates with the predicted thermodynamic stability of the nucleotide pairs in these positions. The universally conserved positions G690 and U697 are generally portrayed as forming a G.U mismatch. Our data show co-variation between these positions, but do not support the hypothesis that the G690:U697 pair forms a wobble structure. NMR studies of model 14-nt and 31-nt RNAs support these findings and show that G690 and U697 are involved in unusual stacking interactions but do not form a wobble pair. Preliminary NMR structural analysis reveals that the loop portion of the 690 hairpin folds into a highly structured and novel conformation.

Quevillon, S., J.-C. Robinson, et al. (1999). "Macromolecular assemblage of aminoacyl-tRNA synthetases: identification of protein-protein interactions and characterization of a core protein." Journal of Molecular Biology **285**(1): 183.

http://www.sciencedirect.com/science/article/B6WK7-45KNCTG-83/2/c0921c6e2162de009502f3187e5497b6

In eukaryotes, from fly to human, nine aminoacyl-tRNA synthetases contribute a multienzyme complex of defined and conserved structural organization. This ubiquitous multiprotein assemblage comprises a unique bifunctional aminoacyl-tRNA synthetase, glutamyl-prolyl-tRNA synthetase, as well as the monospecific isoleucyl, leucyl, glutaminyl, methionyl, lysyl, arginyl, and aspartyl-tRNA synthetases. Three auxiliary proteins of apparent molecular masses of 18, 38 and 43 kDa are invariably associated with the nine tRNA synthetase components of the complex. As part of an inquiry into the molecular and functional organization of this macromolecular assembly, we isolated the cDNA encoding the p38 non-synthetase component and determined its function. The 320 amino acid residue encoded protein has been shown to have no homolog in yeast, bacteria and archaea, according to the examination of the complete genomic sequences available. The p38 protein is a moderately hydrophobic protein, displays a putative leucine-zipper motif, and shares a sequence pattern with protein domains that are involved in protein-protein interactions. We used the yeast two-hybrid system to register protein connections between components of the complex. We performed an exhaustive search of interactive proteins, involving 10 of the 11 components of the complex. Twenty-one protein pairs have been unambiguously identified, leading to a global view of the topological arrangement of the subunits of the multisynthetase complex. In particular, p38 was found to associate with itself to form a dimer, but

also with p43, with the class I tRNA synthetases ArgRS and GlnRS, with the class II synthetases AspRS and LysRS, and with the bifunctional GluProRS. We generated a series of deletion mutants to localize the regions of p38 mediating the identified interactions. Mapping the interactive domains in p38 showed the specific association of p38 with its different protein partners. These findings suggest that p38, for which no homologous protein has been identified to date in organisms devoid of multisynthetase complexes, plays a pivotal role for the assembly of the subunits of the eukaryotic tRNA synthetase complex.

Rafferty, J. B., S. M. Ingleston, et al. (1998). "Structural similarities between Escherichia coli RuvA protein and other DNA-binding proteins and a mutational analysis of its binding to the holliday junction." Journal of Molecular Biology **278**(1): 105.

http://www.sciencedirect.com/science/article/B6WK7-45S49PS-F9/2/270634bb2840b4339922db0b97092d05

Comparison of the structure of Escherichia coli RuvA with other proteins in the Protein Data Bank gives insights into the probable modes of association of RuvA with the Holliday junction during homologous recombination. All three domains of the RuvA protein possess striking structural similarities to other DNA-binding proteins. Additionally, the second domain of RuvA contains two copies of the helix-hairpin-helix (HhH) structural motif, which has been implicated in non-sequence-specific DNA binding. The two copies of the motif are related by approximate 2-fold symmetry and may form a bidentate DNA-binding module. The results described provide support for the organization of the arms of the DNA in our RuvA/Holliday junction complex model and support the involvement of the HhH motifs in DNA binding.

Ribard, C., M. Rochet, et al. (2003). "Sub-families of [alpha]/[beta] Barrel Enzymes: A New Adenine Deaminase Family." Journal of Molecular Biology **334**(5): 1117.

http://www.sciencedirect.com/science/article/B6WK7-4B3G1K1-S/2/13c840c2f288d970beb01b7ee50d2e8e

No gene coding for an adenine deaminase has been described in eukaryotes. However, physiological and genetical evidence indicates that adenine deaminases are present in the ascomycetes. We have cloned and characterised the genes coding for the adenine deaminases of Aspergillus nidulans, Saccharomyces cerevisiae and Schizosaccharomyces pombe. The A. nidulans gene was expressed in Escherichia coli and the purified enzyme shows adenine but not adenosine deaminase activity. The open reading frames coded by the three genes are very similar and obviously related to the bacterial and eukaryotic adenosine deaminases rather than to the bacterial adenine deaminases. The latter are related to allantoinases, ureases and dihydroorotases. The fungal adenine deaminases and the homologous adenosine deaminases differ in a number of residues, some of these being clearly involved in substrate specificity. Other prokaryotic enzymes in the database, while clearly related to the above, do not fit into either sub-class, and may even have a different specificity. These results imply that adenine deaminases have appeared twice in the course of evolution, from different ancestral enzymes constructed both around the [alpha]/[beta] barrel scaffold.

Samuelson, J. C. and S.-y. Xu (2002). "Directed Evolution of Restriction Endonuclease BstYI to Achieve Increased Substrate Specificity." Journal of Molecular Biology **319**(3): 673.

http://www.sciencedirect.com/science/article/B6WK7-45Y7BYF-8/2/cf7a66cb60997c8c6c910cc2325180de

Restriction endonucleases have proven to be especially resistant to engineering altered substrate specificity, in part, due to the requirement of a cognate DNA methyltransferase for cellular DNA protection. The thermophilic restriction endonuclease BstYI recognizes and cleaves all hexanucleotide sequences described by 5'-R[darr]GATCY-3' (where R=A or G and Y=C or T). The recognition of a degenerate sequence is a relatively common feature of the more than 3000 characterized restriction endonucleases. However, very little is known concerning substrate recognition by such an enzyme. Our objective was to investigate the substrate specificity of BstYI by attempting to increase the specificity to recognition of only AGATCT. By a novel genetic selection/screening process, two BstYI variants were isolated with a preference for AGATCT cleavage. A fundamental element of the selection process is modification of the Escherichia coli host genomic DNA by the BglII N4-cytosine methyltransferase to protect AGATCT sites. The amino acid substitutions resulting in a partial change of specificity were identified and combined into one superior variant designated NN1. BstYI variant NN1 displays a 12-fold preference for cleavage of AGATCT over AGATCC or GGATCT. Moreover, cleavage of the GGATCC sequence is no longer detected. This study provides further evidence that laboratory evolution strategies offer a powerful alternative to structure-guided protein design.

Sanders, J. and P. Towner (1992). "Circle reopening in the Tetrahymena ribozyme resembles site-specific hydrolysis at the 3' splice site." Journal of Molecular Biology **223**(1): 351.

http://www.sciencedirect.com/science/article/B6WK7-4DM0XBC-3J/2/7d2216cb7ab929359cbc960ae82dc0db

The Tetrahymena intron, after splicing from its flanking exons, can mediate its own circularization. This is followed by site-specific hydrolysis of the phosphodiester bond formed during the circularization reaction. The structural components involved in recognition of this bond for hydrolysis have not been established. We have made base substitutions to the P9.0 pairing and at the 3'-terminal guanosine residue (G414) of the intron to investigate their effects on circle formation and reopening. We have found that disruption of either P9.0 pairing or binding of the terminal nucleotide result in the formation of a large circle, C-413:5E23 from precursor RNA molecules that have undergone hydrolysis at the 3' splice site. This circle is formed at the phosphodiester bond of the 5'-terminal guanosine residue of the upstream exon via nucleophilic attack by the 3'-terminal nucleotide of the intron. The large circle is novel since it can reopen eight bases downstream from the original circularization junction at a site resembling the normal 3' splice site, restoring a guanosine to the 3' terminus and re-establishing P9.0 pairing. The new 3' terminus of the intron is capable of recircularization at any of the three normal wild-type sites. We conclude that both P9.0 and the 3'-terminal guanosine residue are required for the selection of the phosphodiester bond hydrolysed during circle reopening.

Saxton, J. A. and S. L. Martin (1998). "Recombination between subtypes creates a mosaic lineage of LINE-1 that is expressed and actively retrotransposing in the mouse genome." Journal of Molecular Biology **280**(4): 611.

http://www.sciencedirect.com/science/article/B6WK7-45M7T42-2V/2/2a099a6f73d3f6378915ca7621e6d24d

LINE-1, or L1, elements are retrotransposons that have amplified to high-copy number during the evolution of mammals. L1 appears to amplify in waves, spawning large numbers of progeny such

that elements with distinct sequence features dominate the dispersal process in a given window of time. This process generates discrete subfamilies of L1 within mammalian genomes, with the oldest being remnants, or fossils, of earlier waves of amplification. In mice, at least three distinct subfamilies of L1 were distinguished by their unique 5' ends, A, F and V. These subfamilies amplified at distinct times in the evolution of mice, with A being the youngest and V the oldest; both V and F subfamilies were believed extinct. Recent data established that a variant of the F family, TF, is actively retrotransposing. We demonstrate here that members of the TF subfamily are abundantly expressed in mouse cells and encode the major protein constituent of L1 ribonucleoprotein particles. Although members of the TF subfamily are not as numerous in the genomes of laboratory mice as are members of the older A and F subfamilies, they appear to have been activated some time ago during mouse evolution, in the common ancestor of Mus spretus and Mus domesticus. Phylogenetic analysis demonstrates that this modern, active form of TF-type L1 has a composite evolutionary history, showing evidence of multiple recombinations between distinct L1 variants, including members of the A and F subfamilies.

Sekine, Y., K. Aihara, et al. (1999). "Linearization and transposition of circular molecules of insertion sequence IS3." Journal of Molecular Biology **294**(1): 21.

http://www.sciencedirect.com/science/article/B6WK7-45R86WM-38/2/d9662b6b6089ab804677ded3aaa47c04

IS3 transposase has been shown to promote production of characteristic circular and linear IS3 molecules from the IS3-carrying plasmid; IS3 circles have the entire IS3 sequence with terminal inverted repeats, IRL and IRR, which are separated by a three base-pair sequence originally flanking either end in the parental plasmid, whereas linear IS3 molecules have three nucleotide overhangs at their 5' ends. Here, we showed that a plasmid carrying an IS3 derivative, which is flanked by different sequences at both ends, generated IS3 circles and linear IS3 molecules owing to the action of transposase. Cloning and sequencing analyses of the linear molecules showed that each had the same 5'-protruding three nucleotide overhanging sequences at both ends, suggesting that the linear molecules were not generated from the parental plasmid by the two double-strand breaks at both end regions of IS3. The plasmid carrying IS3 with a two base-pair mutation in the terminal dinucleotide, which would be required for transposase to cleave the 3' end of IS3, could still generate linear molecules as well as circles. Plasmids bearing an IS3 circle were cleaved by transposase and gave linear molecules with the same 5'-protruding three nucleotide overhanging sequences. These show that the linear molecules are generated from IS3 circles via a double-strand break at the three base-pair intervening sequence. Plasmids carrying an IS3 circle with the two base-pair end mutation still were cleaved by transposase, though with reduced efficiencies, suggesting that IS3 transposase has the ability to cleave not only the 3' end of IS3, but a site three nucleotides from the 5' end of IS3. IS3 circles also were shown to transpose to the target plasmids. The end mutation almost completely inhibited this transposition, showing that the terminal dinucleotides are important for the transfer of the 3' end of IS3 to the target as well as for the end cleavage.

Shaikh, T. H., A. M. Roy, et al. (1997). "cDNAs derived from primary and small cytoplasmic Alu (scAlu) transcripts." Journal of Molecular Biology **271**(2): 222.

http://www.sciencedirect.com/science/article/B6WK7-45S94S1-S/2/08d921b440f985e80583a04211ebae84

We have isolated and sequenced twenty-six cDNAs derived from primary Alu transcripts. Most cDNAs (22/26) sequenced end in multiple T residues, known to be at the termination for RNA polymerase III-directed transcripts. We conclude that these cDNAs were derived from authentic,

RNA polymerase III-directed primary Alu transcripts. Sequence alignment of the cDNAs with Alu consensus sequences show that the cDNAs belong to different, previously described Alu subfamilies. The sequence variation observed in the 3' non-Alu regions of each of the cDNAs led us to conclude that they were derived from different genomic loci, thus demonstrating that multiple Alu loci are transcriptionally active. The subfamily distribution of the cDNAs suggests that transcriptional activity is biased towards evolutionarily younger Alu subfamilies, with a strong selection for the consensus sequence in the first 42 bases and the promoter B box. Sequence data from seven cDNAs derived from small cytoplasmic Alu (scAlu) transcripts, a processed form of Alu transcripts, also have a similar bias towards younger Alu subfamilies. About half of these cDNAs are due to processing or degradation, but the other half appear to be due to the formation of a cryptic RNA polymerase III termination signal in multiple loci. Using our sequence data, we have isolated a transcriptionally active genomic Alu element belonging to the Ya5 subfamily. In vitro transcription studies of this element suggest that its flanking sequences contribute to its transcriptional activity. The role of flanking sequences and other factors involved in transcriptional activity of Alu elements are discussed.

Singer, S. S., D. N. Mannel, et al. (2004). "From "Junk" to Gene: Curriculum vitae of a Primate Receptor Isoform Gene." Journal of Molecular Biology **341**(4): 883.

http://www.sciencedirect.com/science/article/B6WK7-4CVR26T-4/2/c53d6061966c438d3d4691fbf166c98e

Exonization of Alu retroposons awakens public opinion, particularly when causing genetic diseases. However, often neglected, alternative "Alu-exons" also carry the potential to greatly enhance genetic diversity by increasing the transcriptome of primates chiefly via alternative splicing. Here, we report a 5' exon generated from one of the two alternative transcripts in human tumor necrosis factor receptor gene type 2 (p75TNFR) that contains an ancient Alu-SINE, which provides an alternative N-terminal protein-coding domain. We follow the primate evolution over the past 63 million years to reconstruct the key events that gave rise to a novel receptor isoform. The Alu integration and start codon formation occurred between 58 and 40 million years ago (MYA) in the common ancestor of anthropoid primates. Yet a functional gene product could not be generated until a novel splice site and an open reading frame were introduced between 40 and 25 MYA on the catarrhine lineage (Old World monkeys including apes).

Turgeon, D., J.-S. Carrier, et al. (2000). "Isolation and characterization of the human UGT2B15 gene, localized within a cluster of UGT2B genes and pseudogenes on chromosome 4." Journal of Molecular Biology **295**(3): 489.

http://www.sciencedirect.com/science/article/B6WK7-45F4V0W-4D/2/6f9d4a0364ddd798bf7366f4c0f28287

Glucuronidation is a major pathway of androgen metabolism and is catalyzed by UDP-glucuronosyltransferase (UGT) enzymes. UGT2B15 and UGT2B17 are 95 % identical in primary structure, and are expressed in steroid target tissues where they conjugate C19 steroids. Despite the similarities, their regulation of expression are different; however, the promoter region and genomic structure of only the UGT2B17 gene have been characterizedX to date. To isolate the UGT2B15 gene and other novel steroid-conjugating UGT2B genes, eight P-1-derived artificial chromosomes (PAC) clones varying in length from 30 kb to 165 kb were isolated. The entire UGT2B15 gene was isolated and characterized from the PAC clone 21598 of 165 kb. The UGT2B15 and UGT2B17 genes are highly conserved, are both composed of six exons spanning approximately 25 kb, have identical exon sizes and have identical exon-intron boundaries. The homology between the two genes extend into the 5'-flanking region, and contain several

conserved putative cis-acting elements including Pbx-1, C/EBP, AP-1, Oct-1 and NF/[kappa]B. However, transfection studies revealed differences in basal promoter activity between the two genes, which correspond to regions containing non-conserved potential elements. The high degree of homology in the 5'-flanking region between the two genes is lost upstream of -1662 in UGT2B15, and suggests a site of genetic recombination involved in duplication of UGT2B genes. Fluorescence in situ hybridization mapped the UGT2B15 gene to chromosome 4q13.3-21.1. The other PAC clones isolated contain exons from the UGT2B4, UGT2B11 and UGT2B17 genes. Five novel exons, which are highly homologous to the exon 1 of known UGT2B genes, were also identified; however, these exons contain premature stop codons and represent the first recognized pseudogenes of the UGT2B family. The localization of highly homologous UGT2B genes and pseudogenes as a cluster on chromosome 4q13 reveals the complex nature of this gene locus, and other novel homologous UGT2B genes encoding steroid conjugating enzymes are likely to be found in this region of the genome.

Van Way, S. M., E. R. Hosking, et al. (2000). "Mot protein assembly into the bacterial flagellum: a model based on mutational analysis of the motB gene." Journal of Molecular Biology **297**(1): 7.

http://www.sciencedirect.com/science/article/B6WK7-460PM8T-2/2/b744c8af4793f295b2e2e9443f01e1fd

The 308 residue MotB protein anchors the stator complex of the Escherichia coli flagellar motor to the peptidoglycan of the cell wall. Together with MotA, it comprises the transmembrane channel that delivers protons to the motor. At the outset of the mutational analysis of MotB described here, we found that the non-motile phenotype of a [Delta]motAB strain was rescued better by a pmotA+B+ plasmid than the non-motile phenotype of a [Delta]motB strain was rescued by a pmotB+ plasmid. Transcription in each case was from the inducible tac promoter but relied on the native ribosome-binding site (RBS). This result confirms that translational coupling to motA is important for normal translation of the motB mRNA, since overproduction of MotA in trans did not improve complementation by pmotB. However, introduction of an optimized RBS into pmotB (to generate pmotBo) did. To dissect the function of the periplasmic domain of MotB, site-directed mutagenesis was used to replace Gln, Ser, and Tyr codons scattered throughout motB with amber (UAG) codons. Plasmid-borne motBam genes were introduced into supo, supE, and supF strains to see what motility defects were imposed by particular amber mutations and whether the defects could be suppressed by amber-suppressor tRNAs inserting the native or heterologous amino acids. Amber mutations at codon 268 or earlier in pmotB, and at codon 261 or earlier in pmotBo or pmotAB, eliminated motility. Thus, in agreement with the deletion analysis of motB by another laboratory, we conclude that the portion of MotB carboxyl-terminal to its peptidoglycan-binding motif (residues 161 to 264) is not essential. In strains containing supE or supF alleles, motility defects associated with motBam mutations were suppressed weakly, if at all, in pmotB. In contrast, motility defects conferred by most motBam mutations in pmotBo or pmotAB could be suppressed to a significant extent. However, the S18am, Q100am, Q112am, Q124am, Y201am, and Y208am mutations were still suppressed extremely poorly. Full-length MotB was present at very low levels in suppressor strains containing the first four mutations, but Y201am and Y208am were suppressed efficiently at the translational level. We suggest that a translational pause by suppressor tRNAs reading UAG at these two positions may divert the nascent polypeptide into an alternative folding pathway that traps MotB in a non-functional conformation. We further propose that MotA and MotB form a stable pre-assembly complex in the membrane. In this complex, MotB exists in a form that cannot associate with peptidoglycan and blocks the proton-conducting channel. Opening of the channel and attachment to the cell wall may occur when the complex collides with a flagellar basal body and MotA makes specific contacts with the C ring and/or the MS ring.

Vanamee, E. S., P.-c. Hsieh, et al. (2003). "Glucocorticoid Receptor-like Zn(Cys)4 Motifs in BslI Restriction Endonuclease." Journal of Molecular Biology **334**(3): 595.

http://www.sciencedirect.com/science/article/B6WK7-4B0KW8C-R/2/c1f75a029da1863e6a01571ac5c2ac82

BslI restriction endonuclease cleaves the symmetric sequence CCN7GG (where N=A, C, G or T). The enzyme is composed of two subunits, [alpha] and [beta], that form a heterotetramer ([alpha]2[beta]2) in solution. The [alpha] subunit is believed to be responsible for DNA recognition, while the [beta] subunit is thought to mediate cleavage. Here, for the first time, we provide experimental evidence that BslI binds Zn(II). Specifically, using X-ray absorption spectroscopic analysis we show that the [alpha] subunit of BslI contains two Zn(Cys)4-type zinc motifs similar to those in the DNA-binding domain of the glucocorticoid receptor. This conclusion is supported by genetic analysis of the zinc-binding motifs, whereby amino acid substitutions in the zinc finger motifs are demonstrated to abolish or impair cleavage activity. An additional putative zinc-binding motif was identified in the [beta] subunit, consistent with the X-ray absorption data. These data were corroborated by proton induced X-ray emission measurements showing that full BslI contains at least three fully occupied Zn sites per [alpha]/[beta] heterodimer. On the basis of these data, we propose a role for the BslI Zn motifs in protein-DNA as well as protein-protein interactions.

Washburn, R. S., Y. Wang, et al. (2003). "Role of E. coli Transcription-Repair Coupling Factor Mfd in Nun-mediated Transcription Termination." Journal of Molecular Biology **329**(4): 655.

http://www.sciencedirect.com/science/article/B6WK7-48PDMV4-6/2/8a43d1c7ce50778acdee26625b08b354

Phage HK022 Nun protein excludes phage [lambda] by binding nascent [lambda]-nut RNA and inducing termination and transcript release. In contrast, in a purified in vitro system, Nun arrests transcription on [lambda]DNA templates without dissociation of the transcription elongation complex (TEC). Our evidence indicates that transcription-repair coupling factor (Mfd) frees Nun-arrested RNA polymerase. The activity of Nun is enhanced in an mfd-null mutant, consistent with prolonged association of Nun with the TEC. Furthermore, expression of [lambda] nut RNA in the mfd mutant titrates Nun, allowing superinfecting [lambda] to form plaques. Finally, addition of Mfd releases a Nun-arrested transcription complex in vitro.

Wurth, C., R. M. Thomas, et al. (2001). "Folding and self-assembly of herpes simplex virus type 1 thymidine kinase." Journal of Molecular Biology **313**(3): 657.

http://www.sciencedirect.com/science/article/B6WK7-457CXXC-41/2/bf16ad2316eeaa3d59cd32e9f92ef91d

Thymidine kinase from herpes simplex virus type 1 (HSV1 TK) has been postulated to be a homodimer throughout the X-ray crystallography literature. Our study shows that HSV1 TK exists as a monomer-dimer equilibrium mixture in dilute aqueous solutions. In the presence of 150 mM NaCl, the equilibrium is characterized by a dissociation constant of 2.4 [mu]M; this constant was determined by analytical ultracentrifugation and gel filtration experiments. Dimerization seems to be unfavorable for enzymatic activity: dimers show inferior catalytic efficiency compared to the monomers. Moreover, soluble oligomers formed by self-assembly of TK in the absence of physiological salt concentrations are even enzymatically inactive.This study investigates enzymatic and structural relevance of the TK dimer in vitro. Dissociation of the dimers into

monomers is not accompanied by large overall changes in secondary or tertiary structure as shown by thermal and urea-induced unfolding studies monitored by circular dichroism and fluorescence spectroscopy. A disulfide-bridge mutant TK (V119C) was designed bearing two cysteine residues at the dimer interface in order to crosslink the two subunits covalently. Under reducing conditions, the properties of V119C and wild-type HSV1 TK (wt HSV1 TK) were identical in terms of expression yield, denaturing SDS PAGE gel electrophoresis, enzyme kinetics, CD spectra and thermal stability. Crosslinked V119C (V119Cox) was found to have an increased thermal stability with a tm value of 59.1(+/-0.5)[deg]C which is 16 deg. C higher than for the wild type protein. This is thought to be a consequence of the conformational restriction of the dimer interface. Furthermore, enzyme kinetic studies on V119Cox revealed a Km for thymidine of 0.2 [mu]M corresponding to wt HSV1 TK, but a significantly higher Km for ATP. The present findings raise the question whether the monomer, not the dimer, might be the active species in vivo.

Xiang, J., J. Sun, et al. (2001). "The importance of hinge sequence for loop function and catalytic activity in the reaction catalyzed by triosephosphate isomerase." Journal of Molecular Biology **307**(4): 1103.

http://www.sciencedirect.com/science/article/B6WK7-457D172-3R/2/7774d43fd186568d3606a16b55b63745

We have determined the sequence requirements for the N-terminal protein hinge of the active-site lid of triosephosphate isomerase. The codons for the hinge (PVW) were replaced with a genetic library of all possible 8000 amino acid combinations. The most active of these 8000 mutants were selected using in vivo complementation of a triosephosphate isomerase-deficient strain of Escherichia coli, DF502. Approximately 0.3 % of the mutants complement DF502 with an activity that is between 10 and 70 % of wild-type activity. They all contain Pro at the first position. Furthermore, the sequences of these hinge mutants reveal that hydrophobic packing is very important for efficient formation of the enediol intermediate. However, the reduced catalytic activities observed are not due to increased rates of loop opening. To explore the relationship between the N-terminal and C-terminal hinges, three semi-active mutants from the N-terminal hinge selection experiment (PLH, PHS and PTF), and six active C-terminal hinge mutants from previous work (NSS, LWA, YSL, KTK, NPN, KVA) were combined to form 18 "double-hinge" mutants. The activities of these mutants suggest that the N-terminal and C-terminal hinge structures affect one another. It appears that specific side-chain interactions are important for forming a catalytically active enzyme, but not for preventing release of the unstable enediol intermediate from the active site of the enzyme. The independence of intermediate release on amino acid sequence is consistent with the absence of a "universal" hinge sequence in structurally related enzymes

Xie, G.-x., X. Han, et al. (2003). "Gene Structure, Dual-promoters and mRNA Alternative Splicing of the Human and Mouse Regulator of G Protein Signaling GAIP/RGS19." Journal of Molecular Biology **325**(4): 721.

http://www.sciencedirect.com/science/article/B6WK7-47HPP2H-F/2/9bc4e1a87ef0daf06f0eecf0f8f6b89f

Here we report the gene structure and transcription regulation of the human and mouse G protein-signaling regulator GAIP/RGS19. The GAIP/RGS19 gene is adjacent to and in an opposite orientation to the opioid-receptor-like receptor 1 (ORL1) gene. In both human and mouse, the GAIP/RGS19 gene is composed of seven exons. The first two exons are under the control of two different promoters and are alternatively employed to start the transcription of two 5' distinctive mRNAs. The two promoters appear to compete with and inhibit each other. We have

also identified in mice an alternatively spliced short GAIP/RGS19 mRNA that lacks the exon 2 region and utilizes an ATG in exon 3 as its translation initiation codon. As a result, the short GAIP/RGS19 protein does not have the N-terminal 22 amino acid residues of a full-length isoform. GAIP/RGS19 alternative splicing patterns are differentially expressed in various tissues. The mRNA alternative splicing to produce multiple isoforms may play a significant role in regulating the function and selectivity of GAIP/RGS19.

Zaccolo, M. and E. Gherardi (1999). "The effect of high-frequency random mutagenesis on in vitro protein evolution: a study on TEM-1 [beta]-lactamase." Journal of Molecular Biology **285**(2): 775.

http://www.sciencedirect.com/science/article/B6WK7-45R88CK-WM/2/c20f3feff31f16143a5bff7f39621d95

For a number of years a major limitation in genetic analysis of protein function has been the inability to introduce multiple substitutions at distant sites that would enable the selection of clusters of mutations required for improved or novel biological functions. In order to achieve this, we have recently developed a novel mutagenesis procedure in which the triphosphate derivatives of a pyrimidine (6-(2-deoxy-[beta]--ribofuranosyl)-3,4-dihydro-8H-pyrimido-[4,5-c][1,2]oxazin-7-one; dP) and a purine (8-oxo-2'-deoxyguanosine; 8-oxodG) nucleoside analogue are employed in DNA synthesis reactions in vitro. The procedure allows control of the mutational load and can yield frequencies of amino acid residue substitutions at least one order of magnitude greater than those previously achieved. Here we report the results of an experiment in which we have hypermutated the bacterial enzyme TEM-1 [beta]-lactamase and selected small pools (5) of clones for enzymatic activity against the [beta]-lactam antibiotic cefotaxime. The experiment resulted in the isolation of a number of TEM-1 mutants with greatly improved activity against cefotaxime. Among these, clone 3D.5 (E104K:M182T:G238S) exhibited a minimum inhibitory concentration for cefotaxime 20,000-fold higher than wild-type TEM-1 and a catalytic efficiency (kcat/Km) 2383 times higher than the wild-type enzyme. Thus, small pools of hypermutated sequences enabled the selection of one of the most active extended [beta]-lactamases described so far. These results argue against the accepted view that multiple rounds of low-rate mutagenesis and stepwise selection are essential for in vitro protein evolution and extend the scope of directed molecular evolution to proteins for which no genetic selection is available.

Zhu, Z., J. C. Samuelson, et al. (2004). "Engineering Strand-specific DNA Nicking Enzymes from the Type IIS Restriction Endonucleases BsaI, BsmBI, and BsmAI." Journal of Molecular Biology **337**(3): 573.

http://www.sciencedirect.com/science/article/B6WK7-4BVSYB4-5/2/a87c82a47e0bcbb8cb5cfe0529f389bb

More than 80 type IIA/IIS restriction endonucleases with different recognition specificities are now known. In contrast, only a limited number of strand-specific nicking endonucleases are currently available. To overcome this limitation, a novel genetic screening method was devised to convert type IIS restriction endonucleases into strand-specific nicking endonucleases. The genetic screen consisted of four steps: (1) random mutagenesis to create a plasmid library, each bearing an inactivated endonuclease gene; (2) restriction digestion of plasmids containing the wild-type and the mutagenized endonuclease gene; (3) back-crosses with the wild-type gene by ligation to the wild-type N-terminal or C-terminal fragment; (4) transformation of the ligated DNA into a pre-modified host and screening for nicking endonuclease activity in total cell culture or cell extracts of the transformants. Nt.BsaI and Nb.BsaI nicking endonucleases were isolated from BsaI using this genetic screen. In addition, site-directed mutagenesis was carried out to isolate BsaI nicking variants with minimal double-stranded DNA cleavage activity. The equivalent amino acid

substitutions were introduced into BsmBI and BsmAI restriction endonucleases with similar recognition sequence and significant amino acid sequence identity and their nicking variants were successfully isolated. This work provides strong evidence that some type IIS restriction endonucleases carry two separate active sites. When one of the active sites is inactivated, the type IIS restriction endonuclease may nick only one strand.

Zhu, Z., J. Zhou, et al. (2003). "Isolation of BsoBI Restriction Endonuclease Variants with Altered Substrate Specificity." Journal of Molecular Biology **330**(2): 359.

http://www.sciencedirect.com/science/article/B6WK7-48XD3BG-M/2/dd5cf5c4437972bb5953e0c9f6af33ec

BsoBI is a thermophilic restriction endonuclease that cleaves the degenerate DNA sequence C/PyCGPuG (where/=the cleavage site and Py=C or T, Pu=A or G). In the BsoBI-DNA co-crystal structure the D246 residue makes a water-mediated hydrogen bond to N6 of the degenerate base adenine and was proposed to make a complementary bond to O6 of the alternative guanine residue. To investigate the substrate specificity conferred by D246 and to potentially alter BsoBI specificity, the D246 residue was changed to the other 19 amino acids. Variants D246A, D246C, D246E, D246R, D246S, D246T, and D246Y were purified and their cleavage activity determined. Variants D246A, D246S, and D246T display 0.2% to 0.7% of the wild-type cleavage activity. However, the substrate specificity of the three variants is altered significantly. D246A, D246S, and D246T cleave CTCGAG sites poorly. In filter binding assays using oligonucleotides, wild-type BsoBI shows almost equal affinity for CTCGAG and CCCGGG sites. In contrast, the D246A variant shows 70-fold greater binding affinity for the CCCGGG substrate. Recycled mutagenesis was carried out on the D246A variant, and revertants with enhanced activity were isolated by their dark blue phenotype on a dinD:lacZ DNA damage indicator strain. Most of the amino acid substitutions present within the revertants were located outside the DNA-protein interface. This study demonstrates that endonuclease mutants with altered specificity and non-lethal activity can be evolved towards more active variants using a laboratory evolution strategy.

Journal of Pharmacological and Toxicological Methods (2)

Cotreau, M. M., L. L. von Moltke, et al. (2000). "Methodologies to study the induction of rat hepatic and intestinal cytochrome P450 3A at the mRNA, protein, and catalytic activity level." Journal of Pharmacological and Toxicological Methods **43**(1): 41.

http://www.sciencedirect.com/science/article/B6T8S-41PP0J6-6/2/6e9142ddaecb9a6a3ee1ba22bd48972b

Studies were conducted to characterize assays for the isolation and quantitation of rat cytochrome P450 (CYP) 3A isoforms from hepatic and intestinal tissues. Isolated intestinal microsomes were analyzed for their alkaline phosphatase activity and CYP 3A immunoreactivity. The involvement of CYP 3A in the in vitro hydroxylation of midazolam (MDZ) was also evaluated using isoform specific chemical and antibody inhibitors. The effect of glycerol (a common constituent of the microsomal reconstitution buffer) concentration on in vitro MDZ hydroxylation was also investigated. Additionally, to verify that the intestinal preparation was adequate for use in studies investigating the induction of CYP3A at the MRNA, protein, and catalytic activity within

a single animal, a separate induction study was carried out with the CYP 3A inducer dexamethasone (DEX). A reverse transcription-polymerase chain reaction (RT-PCR) assay and a quantitative Western blotting method were used to reliably detect differences in CYP 3A mRNA and immunoreactivity between DEX- and vehicle (VH)-treated tissues. The in vitro hydroxylation of MDZ evaluated CYP 3A catalytic activity and identified increases in CYP 3A activity caused by DEX in comparison to VH. Collectively, these described techniques provide an experimental model to study xenobiotic induction of rat hepatic and intestinal CYP 3A from the molecular to the catalytic level in individual rats without the need for pooling of tissue.

Davies, A., E. Whiting, et al. (1995). "The application of the human beta-globin gene locus control region and murine erythroleukemia cell system to the expression and pharmacological characterization of human endothelin receptor subtypes." Journal of Pharmacological and Toxicological Methods **33**(3): 153.

http://www.sciencedirect.com/science/article/B6T8S-3YMWF3D-F/2/deda4e36ab68af617fb165a2284a3798

The cDNAs encoding both A and B subtypes of the human endothelin receptor have been inserted into mammalian cell expression vectors that utilize the human globin gene, locus control region. These constructs have been introduced into murine erythroleukemia cells and inducible high level expression of the receptors has been achieved (~1.5-pM/mg membrane protein and ~13,500 binding sites/cell for both receptor subtypes). Cell lines expressing these receptors were obtained on a rapid time scale (3-4 weeks), facilitated by the need for the analysis of only small numbers of cell clones/receptor (~6). Competitive binding assays with endothelin-1 gave IC50s of 130 +/- 30 pM for endothelin-A receptor and 160+/-30 pM for endothelin-B receptor. Similar studies with the different isoforms of endothelin, sarafatoxin-S6b and -S6c, BQ123 and BQ3020, all gave the expected selectivity profiles. The IC50s for all compounds were in close agreement with those reported for native receptors. Thus, this expression system, which has several advantages over other described expression systems, is capable of rapidly providing large quantities of receptor for detailed pharmacological analyses or drug screening. In addition, the expressed receptors display the expected pharmacological profiles in the absence of any complicating, competing interactions from other subtypes or binding sites.

Journal of Photochemistry and Photobiology B: Biology (1)

Piette, J. (1992). "Molecular analysis of mutations induced by 4'-hydroxymethyl-4,5',8-trimethylpsoralen and UVA in the mouse HPRT gene." Journal of Photochemistry and Photobiology B: Biology **12**(1): 37.

http://www.sciencedirect.com/science/article/B6TH0-44K8208-5/2/9b362dbc93a487920381ee71cb3d2d35

The effects of the reaction photosensitized by 4'-hydroxymethyl-4,5',8-trimethylpsoralen (HMT) on a mouse lymphoma cell line have been examined. Using the hypoxanthine phosphoribosyltransferase (HPRT) locus as target gene, a mutagenic effect of the photoreaction can be detected concomitantly with a loss of cell viability. Isolation of HPRT deficient clones has permitted a molecular characterization of the mutational pattern induced by the photosensitization

reaction mediated by HMT. Southern blotting analysis demonstrated that the HPRT deficiency could not be correlated with gene deletions larger than 300 bp. Using polymerase chain reaction on both DNA and cDNA, amplification products have been cloned into M13mp18 and sequenced. Base transversions targeted on thymine residues have been located in exon 2, 3, 8 and 9 together with spontaneous frameshift mutations occurring in a run of guanine residues in exon 3. HPRT deficiencies owing to mutations arising in the HPRT promoter region have also been observed. Dot and Northern blot analysis revealed that the photoreaction could lead to either a reduced level of gene transcription or to a complete absence of HPRT m-RNA. Using polymerase chain reaction (PCR) amplification and agarose gel electrophoresis, deletions in the HPRT promoter have been observed and correlated to deficient enzyme expression.

Journal of Plant Physiology (1)

Ouziad, F., U. Hildebrandt, et al. "Differential gene expressions in arbuscular mycorrhizal-colonized tomato grown under heavy metal stress." Journal of Plant Physiology **In Press, Corrected Proof** http://www.sciencedirect.com/science/article/B7GJ7-4FDJ73D-3/2/3c64f58eabfda0d5c4df315c914ef08d

SummaryWhen tomato was grown in either "Breinigerberg" soil, which has a high content of Zn and of other heavy metals or in non-polluted soil enriched with upto 1 mM CdCl2, plants colonized with the arbuscular mycorrhizal fungus (AMF) Glomus intraradices grew distinctly better than non-mycorrhizal controls. An analysis of differential mRNA transcript formations was performed on several plant genes coding for products potentially involved in heavy metal tolerance. Northern blot analyses indicated that the mRNA from either roots or leaves was not differentially expressed in the case of LePCS1 (coding for phytochelatin synthase), Lemt1, Lemt3 and Lemt4 (for metallothioneins) or LeNramp2 (for a broad range heavy metal transporter) in both mycorrhizal and non-mycorrhizal plants, grown either with or without heavy metals. In contrast, Lemt2 was strongly expressed only in non-AMF-colonized roots, and only after growth in the Breinigerberg soil or in the presence of high CdCl2-concentrations. AMF colonization distinctly reduced the level of Lemt2 transcripts. This was also the case for the root specific LeNramp1 transporter, however, only after growth in the Breinigerberg soil, but not under Cd-stress. Likewise, the levels of LeNramp3 transcripts were reduced by the AMF colonization in roots, but not in leaves. Quantitative Real-Time RT-PCR-experiments performed with Lemt2, LeNramp1 and LeNramp3 largely corroborated the Northern analysis data. In situ hybridization experiments with Lemt2 and LeNramp1 showed that both genes were strongly expressed throughout the plant cells in non-colonized roots, whereas colonized roots revealed only few signals restricted to some parenchyma cells. All the data suggest that the transcript levels of some, but not all genes of the Nramp or mt family are elevated under heavy metal stress. AMF colonization results in a down-regulation of these genes, presumably due to the fact that the content of heavy metals is lower in mycorrhizal than in non-colonized roots. A suppression subtractive hybridization (SSH) library from hyphae of the AMF G. intraradices grown in high versus low Zn++ provided none of the genes which were down-regulated at the plant side (mt or Nramp genes). In contrast, several gene sequences coding for enzymes potentially catalysing the detoxification of reactive oxygen species were found. Thus the fungal cells in the symbiosis may primarily have to cope with the heavy metal-induced oxidative stress.

Aberham, C., C. Pendl, et al. (2001). "A quantitative, internally controlled real-time PCR Assay for the detection of parvovirus B19 DNA." Journal of Virological Methods **92**(2): 183.

http://www.sciencedirect.com/science/article/B6T96-42D81PP-9/2/fde3d3e7f7f43cb0f896041e1ff48bdd

Parvovirus B19 is an erythrovirus causing diverse clinical manifestations ranging from asymptomatic or mild, to more severe outcomes in, for example, immune-compromised patients. B19 is spread primarily via the respiratory route, but it can also be transmitted via blood and blood products. Viral loads in blood or plasma donations amount up to 1011 genome equivalents/ml. Therefore, screening of plasma for fractionation for the presence of B19 and removal of highly loaded donations is a way to limit considerably the input of B19 into production pools and to improve further the safety of plasma products.An assay for the quantitative detection of B19 DNA, based on real-time PCR using ABI Prism SDS7700 (TaqMan) is described here. This assay allows precise quantitation of viral loads over 7 orders of magnitude. An exogenous internal control (internal quality marker) is included in each individual sample to prevent false negative results. A linearized plasmid is used as an internal quality marker that contains the identical sequence of the B19 target sequence but with an altered probe hybridization site. This allows co-amplification of B19 and internal quality marker and co-detection of FAM (6-carboxyfluorescein) or VIC labeled probes respectively. The assay is validated according to current guidelines (of the International Conference on Harmonization, Paul Ehrlich Institute, and the Council of Europe) and is optimized for high throughput screening.

Agnes, F., J. M. Crance, et al. (1994). "Separate detection of the two complementary RNA strands of hepatitis A virus." Journal of Virological Methods **49**(3): 323.

http://www.sciencedirect.com/science/article/B6T96-476TY1K-8/2/edb199dcce304e64fed3c2484ef8ad1f

The minus strand of hepatitis A virus can be detected specifically by reverse transcription and polymerase chain reaction amplification in infected cell culture extracts. Several controls gave evidence that the amplified fragment actually used the minus strand as initial template. Non-thermostable reverse transcriptase was not efficient for this purpose because of self-priming of the positive-stranded viral RNA during the reverse transcription step. This problem was overcome by the use of the thermostable rTth DNA polymerase that also has reverse transcriptase activity in the presence of Mn2+.

Albrecht, B., N. D. Collins, et al. (1998). "Quantification of human T-cell lymphotropic virus type 1 proviral load by quantitative competitive polymerase chain reaction." Journal of Virological Methods **75**(2): 123.

http://www.sciencedirect.com/science/article/B6T96-3V5F5S6-1/2/d33602ffbb9310033bf5828a1d6013b4

The polymerase chain reaction (PCR) has been established as a highly sensitive technique for detection of viral DNA or RNA. However, due to inherent limitations of PCR the amount of amplified product often does not correlate with the initial amount of template DNA. This is

particularly true for PCR detection of viral infections that are characterized by low in vivo viral copy numbers in certain stages of the infection, such as human T-cell lymphotropic virus type 1 (HTLV-1) and simian T-cell lymphotropic virus type 1 (STLV-1). Therefore, we developed a quantitative competitive polymerase chain reaction (qcPCR) for detection of HTLV-1 and STLV-1 proviral DNA. The assay was optimized using an infectious HTLV-1 clone, ACH, HTLV-1 infected cell lines, MT-2.6 and HUT-102 and STLV-1 infected lines Kia and Matsu. Applicability of this system was demonstrated by determining HTLV-1 proviral load in peripheral blood mononuclear cells (PBMC) of human subjects with HTLV-1 associated diseases and an asymptomatic carrier as well as rabbits infected experimentally. This qcPCR method, the first designed specifically for HTLV-1 and STLV-1, will provide an important tool for pathogenesis studies of HTLV-1 and for evaluating the efficacy of antiviral drugs and vaccines against the viral infection using animal models.

Alcaraz, C., F. Rodriguez, et al. (1995). "Highly specific confirmatory Western blot test for African swine fever virus antibody detection using the recombinant virus protein p54." Journal of Virological Methods **52**(1-2): 111.

http://www.sciencedirect.com/science/article/B6T96-3YXC151-W/2/4eb43f695bf6fc6097bd9155e7b760e0

A Western blot technique using a recombinant protein has been developed to confirm positive results obtained in African swine fever (ASF)-specific antibody detection by ELISA. The new confirmatory Western blot is based on the use of protein p54, one of the most antigenic ASF virus structural proteins, expressed in Escherichia coli fused to the N-terminus of MS2 polymerase. The recombinant Western blot assay was highly specific and equally sensitive for ASF virus-infected pigs detection as the conventional Western blot, which uses virus-induced proteins ranging in molecular weight between 23 and 35 kDa. The novel Western blot assay provides a simpler interpretation of the test, eliminates the possibility of false-positive reactions produced by cellular compounds that contaminate the antigen employed in the conventional technique, and avoids the use of live virus in antigen production.

Argaw, T., A. Ritzhaupt, et al. (2002). "Development of a real time quantitative PCR assay for detection of porcine endogenous retrovirus." Journal of Virological Methods **106**(1): 97.

http://www.sciencedirect.com/science/article/B6T96-46TBF6J-H/2/ab392041d9a6b7f599146874bab1b8f6

Real time PCR technology was applied to the development of assays for detection and quantitation of porcine endogenous retrovirus (PERV) RNA and DNA sequences in tissues and cells of human or animal origin. A plasmid construct encoding the PERV-pol gene or the in vitro transcribed RNA derived from the plasmid (cRNA) serves as a standard template for amplification of a 178 bp fragment. This study showed that the detection of this target sequence was linear over a range from 20 copies to 2 million copies of the plasmid and from 100 copies to 1 million copies of the cRNA. In addition, amplification of the target sequence was not inhibited by the presence of exogenous genomic DNA. These results demonstrate that a real time (TaqMan-based) PCR or RT-PCR assay can provide a sensitive, reproducible, and robust method for detecting and quantifying PERV DNA or RNA sequences in samples of human or guinea pig origin.

Ault, G. S., C. F. Ryschkewitsch, et al. (1994). "Type-specific amplification of viral DNA using touchdown and hot start PCR." Journal of Virological Methods **46**(2): 145.

http://www.sciencedirect.com/science/article/B6T96-476F69S-BD/2/caa598ec4d1318cace2bb44a0c627ea5

Two types of JC virus (JCV) are found in infected brain and kidney tissues. A highly reliable PCR assay to determine viral type in tissue is presented. This type-specific system is analogous to allele-specific PCR used to detect point mutations in cellular genes. Specific amplification of two fragments, using four pairs of type-specific primers, is based on a single nucleotide difference at the 3'-ends of the primers. A combination of three conditions in the PCR reaction was required for specificity: 'hot start', a ramped ('touchdown') cycle profile, and a slightly lowered molar concentration of the specific primers and dNTPs. Efficient yield of PCR product is not lost under these conditions, and even the least selective mismatches (C:A and T:G) provided specific amplification. Type-specific restriction enzyme sites within the amplified fragments confirm type designation.

Avellon, A., P. Perez, et al. (2001). "Rapid and sensitive diagnosis of human adenovirus infections by a generic polymerase chain reaction." Journal of Virological Methods **92**(2): 113.

http://www.sciencedirect.com/science/article/B6T96-42D81PP-2/2/540b7580d8d9f11b8c1865872705eb3c

A new adenovirus specific nested polymerase chain reaction (PCR) method is described. It was designed inside the hexon protein gene of the adenovirus genome, and was able to detect DNA of all 47 human adenovirus types in a wide range of clinical samples. A sensitive internal control system able to assure proper analytical conditions for the amplification of as few as 100 molecules of a heterologous DNA was included to avoid false negative results. Sensitivity was estimated at about 10 molecules per tube of a plasmid containing an insert of the first amplification product. The method was able to detect adenovirus infection in 31/43 conjunctival scrapings from patients with acute kerato conjunctivitis 10/40 nasopharyngeal aspirates from patients admitted to hospital with acute respiratory disease and 2/26 urine samples from patients with haemorrhagic cystitis with better sensitivity than cell culture or rapid diagnosis by antigen detection by immunofluorescence (IF) in the case of respiratory specimens. Only two of 17 stools positive for a group F adenovirus specific latex immunoassay were PCR negative. The internal control system avoided a false negative result on another two stool samples. In conclusion, the method described below was shown to be useful for rapid diagnosis of adenovirus infections with higher sensitivity than antigen detection by IF.

Baines, J. E., R. M. McGovern, et al. (2005). "Consensus-degenerate hybrid oligonucleotide primers (CODEHOP) for the detection of novel papillomaviruses and their application to esophageal and tonsillar carcinomas." Journal of Virological Methods **123**(1): 81.

http://www.sciencedirect.com/science/article/B6T96-4DNB3BK-2/2/42b0ea14114390dc20621805c2b654de

Human papillomaviruses (HPVs) have been identified in 99% of cervical tumors. Considerable speculation exists about the role of HPV in cancer at other sites, such as skin, esophagus, and lung. One barrier to the study of HPV in extragenital tumors may be the inability to detect novel HPV types. Our objective was to design broad-range primers for detecting papillomaviruses from any group in the HPV Sequence Database. Complete L1 protein sequences were aligned and 11

blocks of conserved protein sequences were identified. Primers were designed in the corresponding nucleotide regions of five blocks. One pair of primers, ME and MH, derived from blocks E and H, appeared promising when compared with established consensus primers. To improve the sensitivity for detection of multiple papillomavirus types, ME/MH primers were split into several primers with the 5' regions matched to individual papillomavirus supergroups. Compared with the commonly used MY09/11 primer set, our primer sets were broader in range and more sensitive against most papillomavirus types tested. Application of these primers to esophageal and tonsillar carcinomas identified no novel HPV types but confirmed a high level of sensitivity for detecting HPV from these clinical samples. These primers should facilitate the search for novel papillomaviruses.

Barbezange, C. and V. Jestin (2002). "Development of a RT-nested PCR test detecting pigeon Paramyxovirus-1 directly from organs of infected animals." Journal of Virological Methods **106**(2): 197.

http://www.sciencedirect.com/science/article/B6T96-46WSX8S-2/2/5213504c066b45784a6c0b93533807ca

A RT-nested PCR that amplifies part of the conserved nucleoprotein gene of avian Paramyxovirus type 1 is described. The technique allowed the detection of pigeon Paramyxovirus type 1 (pPMV-1) virus directly from a wide range of infected chicken and pigeon organs, and should be able to detect typical Newcastle disease viruses too. Compared with the reference method, the developed RT-nested PCR was found more sensitive, as it was able to detect virus genome in infected pigeon organs at late stage of infection, when virus isolation failed. Such a molecular technique represents an alternative method of diagnosis for research purposes on pPMV-1 variants, for example to study pathogenesis aspects of the infection or to assess the efficacy of vaccines.

Barlic-Maganja, D. and J. Grom (2001). "Highly sensitive one-tube RT-PCR and microplate hybridisation assay for the detection and for the discrimination of classical swine fever virus from other pestiviruses." Journal of Virological Methods **95**(1-2): 101.

http://www.sciencedirect.com/science/article/B6T96-433NRB5-B/2/a24675b98a618b42aeb421941d4670b5

Rapid, sensitive and specific laboratory diagnostic methods are necessary to confirm outbreaks of classical swine fever. The detection of classical swine fever virus (CSFV) and its discrimination from other pestiviruses can be achieved by virus isolation on cell culture, antigen detection, or molecular methods. To reduce the time and the number of steps in the diagnostic procedure a sensitive and rapid detection method based on specific amplification of the pestiviral RNA by one-step reverse transcription-polymerase chain reaction (RT-PCR) followed by detection and differentiation of the amplification products by pestivirus-, bovine viral diarrhoea virus- (BVDV-) and CSFV-specific capture probe hybridisation and colorimetric assay in microwell plates (enzyme liked immunosorbent assay (ELISA)) was developed. Two different methods using two gene regions for pestivirus RT-PCR amplification were carried out. One pair of primers was selected from the 5'-UTR region and the second one from the gene region coding for Npro, C and E0 proteins. The designed oligonucleotide primers were used for several pestivirus reference strains as well as for some field isolates detection in cell culture supernatants and in clinical specimens. The specificity and sensitivity of both methods were compared using EZ rTth RNA PCR kit and ACCESS RT-PCR system for combined RT-PCR assay. The use of one-step RT-PCR eliminates the additional manipulations that are generally required for a two reaction system and limits the risk of carry-over contamination. Labelling of PCR products with digoxigenin (DIG)

during the amplification reaction enables colorimetric assessment of hybridisation reactions. For solution hybridisation pestivirus-, BVDV- and CSFV-specific biotin-labelled capture probes were used. By serial dilutions of DIG-labelled PCR products the RT-PCR-ELISA was found to be 100-times more sensitive than the conventional agarose gel electrophoresis. Higher sensitivity of RT-PCR-ELISA detection using specific biotin-labelled probes offers the opportunity to eliminate strain specific nested PCR and to overcome the problems with contamination and false positive results.

Barlough, J., N. East, et al. (1994). "Double-nested polymerase chain reaction for detection of caprine arthritis-encephalitis virus proviral DNA in blood, milk, and tissues of infected goats." Journal of Virological Methods **50**(1-3): 101.

http://www.sciencedirect.com/science/article/B6T96-47DKYF8-C/2/56659fd9129ad03f9351de6729c6c776

A nested polymerase chain reaction (PCR) for detecting proviral DNA of caprine arthritis-encephalitis virus (CAEV) in biological samples was developed. Primers for both gag and pol sequences of the CAEV genome were included in a single tube for simultaneous amplification ('double' PCR), and the resulting bands were resolved visually in ethidium bromide-stained agarose gels. Internal gag and pol probes were used to verify the identity of the amplified products by non-radioactive Southern hybridization. Final confirmation of the identity of representative PCR bands was provided by DNA sequence analysis. A comparison between the PCR and an antibody ELISA (with recombinant CAEV p28 as target) using 141 caprine blood samples indicated very strong agreement between the two assays ([kappa] = 0.912). Four of 7 goats with indeterminate ELISA results were PCR-positive as were 5 of 40 (12.5%) seronegative goats, most probably indicating delayed seroconversion. Eleven of 27 goats (41%) PCR-positive on blood had detectable CAEV proviral DNA in milk. Proviral DNA was also detected in lung, mesenteric lymph node, bone marrow, synovial membrane, and mammary gland of a seropositive, clinically affected goat, but not in equivalent tissues of a healthy seronegative goat.

Beaulieux, F., M. M. Berger, et al. (2003). "RNA extraction and RT-PCR procedures adapted for the detection of enterovirus sequences from frozen and paraffin-embedded formalin-fixed spinal cord samples." Journal of Virological Methods **107**(2): 115.

http://www.sciencedirect.com/science/article/B6T96-47DTDTF-3/2/2adec925f3d102f950d4be0508ecb015

Methods for detecting enterovirus RNA in both paraffin-embedded, formalin-fixed and frozen spinal cord sections from amyotrophic lateral sclerosis (ALS) patients were established. A proteinase K digestion following the deparaffinization procedure was required for the fixed spinal cord sections, whereas only one step of crushing in phosphate buffered saline was necessary for the frozen samples prior to the extraction of the RNA. With an optimized reverse transcription and PCR procedure, enterovirus RNA could be detected from frozen and fixed archival spinal cord samples.

Bellau-Pujol, S., A. Vabret, et al. "Development of three multiplex RT-PCR assays for the detection of 12 respiratory RNA viruses." Journal of Virological Methods **In Press, Corrected Proof**
http://www.sciencedirect.com/science/article/B6T96-4FJTNTM-2/2/0ba5a40b32fe1ff28df50d75b2fab22a

Three multiplex hemi-nested RT-PCR assays were developed to detect simultaneously 12 RNA respiratory viruses: influenza viruses A, B and C, human respiratory syncytial virus (hRSV), human metapneumovirus (hMPV), parainfluenza virus types 1-4 (PIV-1, -2, -3 and -4), human coronavirus OC43 and 229E (HCoV) and rhinovirus (hRV). An internal amplification control was included in one of the RT-PCR assays. The RT-PCR multiplex 1 and the hemi-nested multiplex 1 detected 1 and 0.1 TCID50 of RSV A, respectively, and 0.01 and 0.001 TCID50 of influenza virus A/H3N2, respectively. Two hundred and three nasal aspirates from hospitalised children were retrospectively tested in comparison with two conventional methods: direct immunofluorescence assay and viral isolation technique. Almost all samples (89/91) that were positive by immunofluorescence assay and/or viral isolation technique were detected by the multiplex assay. This method also detected an additional 85 viruses and 33 co-infections. The overall sensitivity (98%), rapidity and enhanced efficiency of these multiplex hemi-nested RT-PCR assays suggest that they would be a significant improvement over conventional methods for the detection of a broad spectrum of respiratory viruses.

Belliot, G. M., R. L. Fankhauser, et al. (2001). "Characterization of 'Norwalk-like viruses' and astroviruses by liquid hybridization assay." Journal of Virological Methods **91**(2): 119.

http://www.sciencedirect.com/science/article/B6T96-426XYG8-3/2/ee396f5d131df9dded538957d49b01ce

'Norwalk-like viruses' (NLVs) and human astroviruses are causative agents of gastroenteritis in all age-groups. The typing of these agents is generally done by nucleotide sequencing, blot hybridization, or enzyme immunoassay. These techniques are expensive, time-consuming, and sometimes require scarce reagents, which limits the typing of NLVs and astroviruses to a few reference laboratories. This report describes a liquid hybridization assay that uses broadly reactive probes whose sequences are based on data from specimens in collections available at CDC and GenBank. Two astrovirus genogroup-specific probes were designed and tested successfully on 26 wild strains from all serotypes. Fourteen GII and 16 GI representative NLV strains were typed without cross-hybridization by using P1B- and P2A-specific probes, described previously, and new P2B- and P1A-specific probes. Analysis of the specificity of the probes, the effect of the mismatches during hybridization, and the sensitivity of hybridization assay demonstrates this method to be a rapid and simple technique for molecular typing of NLVs and preliminary characterization of astroviruses.

Bennett, J. M., S. Kaye, et al. (1999). "A quantitative PCR method for the assay of HIV-1 provirus load in peripheral blood mononuclear cells." Journal of Virological Methods **83**(1-2): 11.

http://www.sciencedirect.com/science/article/B6T96-3XVPG88-2/2/2e45f7793c78e80a591462826d37427c

The use of high activity antiretroviral therapies (HAART) to treat HIV-infected patients frequently results in the long-term suppression of plasma virus RNA loads below levels detectable by current assays. The measurement of provirus DNA load in peripheral blood mononuclear cells provides a means of continuing to monitor the efficacy of treatment and the decline in reservoirs of latent virus. A quantitative PCR assay was developed for HIV-1 provirus using a three-point internal calibrator system to give high reproducibility and accuracy at the low copy numbers of provirus seen in clinical samples. Provirus DNA copies are related to cell number in the samples using a fluorescent dye-binding assay for measurement of input DNA. The assay agreed closely with an end-point dilution PCR and gave accurate quantification of extracts from an HIV-1 infected continuous cell line containing known provirus copy numbers. The inclusion of a second primer set in the LTR region of the HIV-1 genome, optimised to non-clade-B virus strains

improved the detection and quantification of samples from patients infected with genetically divergent virus strains. Application of the assay to clinical trial patients showed no relationship between changes in provirus DNA loads and plasma virus RNA and changes in provirus load over 24 weeks were small.

Bettinger, D., C. Mougin, et al. (1994). "Rapid detection of active cytomegalovirus infection by in situ polymerase chain reaction on MRC5 cells inoculated with blood specimens." Journal of Virological Methods **49**(1): 59.

http://www.sciencedirect.com/science/article/B6T96-476F6X3-KS/2/4277fbf8076a971214cbc06ee1d9a158

An in situ polymerase chain reaction was developed to amplify immediate early genes of human cytomegalovirus in cells cultured in a 96 well plate and infected with leukocytes. The technical parameters enabling optimal detection of the DNA sequences were defined. The key to this method is the fixation of cells, which facilitates the access of the PCR mixture into the cell nuclei and preserves cell morphology. Such a technique could have wide application for the detection and identification of other infectious viruses in cultured cells very early after inoculation of clinical samples.

Beuret, C. (2004). "Simultaneous detection of enteric viruses by multiplex real-time RT-PCR." Journal of Virological Methods **115**(1): 1.

http://www.sciencedirect.com/science/article/B6T96-49V3JJG-1/2/1cf1f7ec80bbf36619d849b77af86662

A multiplex real-time RT-PCR protocol for the simultaneous detection of noroviruses ("Norwalk-like viruses") of genogroups I and II, human astroviruses and enteroviruses is described. The protocol was developed and evaluated using the LightCycler(TM) and corresponding SYBR Green reagents. New primers were designed within conserved genome regions to optimize the detection range of virus subtypes of each genus. To enable the development of a multiplex PCR assay within one tube (capillary), similar mastermix- and cycling-conditions were respected for each individual primer system. Subsequent melting curve analysis allowed the determination of possible dual-contaminations of entero- and noro- or astroviruses by the formation of dual peaks. Special care was taken to minimize the loss of sensitivity, since the detection of small viral contaminations is a crucial parameter especially for food analysis. The multiplex assay was compared successfully to the single SYBR Green assay, and revealed to be at least 10 times more sensitive than the one obtained with an endpoint PCR thermocycler protocol published previously.

Binder, T., W. Siegert, et al. (1999). "Identification of human cytomegalovirus variants by analysis of single strand conformation polymorphism and DNA sequencing of the envelope glycoprotein B gene region-distribution frequency in liver transplant recipients." Journal of Virological Methods **78**(1-2): 153.

http://www.sciencedirect.com/science/article/B6T96-3W07P76-G/2/dc26e56fe1796ed67a375466047c1a9e

Single strand conformation polymorphism analysis (SSCP) of PCR-amplified DNA and

subsequent DNA sequencing of human cytomegalovirus (HCMV) glycoprotein B (gB) gene were applied to identify known HCMV strains and to detect new virus variants. 61 HCMV PCR positive patients were studied out of a cohort of 410 patients after liver transplantation (LTX). SSCP was able to distinguish between strains Davis, AD169, and Towne, and in addition could identify five new virus variants (Berlin B, C, E, F, and H). Their frequency, gB and gH types were determined. Simultaneous infections with two or three strains or variants, as well as a switch from one virus to another virus were observed during long-term follow-up. No correlation between the occurrence of certain virus strains or gB types and defined clinical manifestations of HCMV infection after LTX was drawn.

Black, E. M., J. P. Lowings, et al. (2002). "A rapid RT-PCR method to differentiate six established genotypes of rabies and rabies-related viruses using TaqMan(TM) technology." Journal of Virological Methods **105**(1): 25.

http://www.sciencedirect.com/science/article/B6T96-45TTWC2-4/2/84fb96b7a1635f9735ea69436c30ec10

A rapid and sensitive reverse transcriptase-polymerase chain reaction (RT-PCR) assay incorporating TaqMan(TM) probes has been developed that can distinguish among the six established rabies and rabies-related virus genotypes. TaqMan(TM) probes were designed and validated against 106 rabies and rabies-related virus isolates, one isolate of the Australian bat Lyssaviruses (genotype 7), and 18 other non-rabies viruses important in the veterinary field. The N gene was used as the target for the probes as it is well conserved and has been intensively used to genotype rabies isolates. Additionally, it was found to contain regions specific to each genotype conducive to probe design. The RT-PCR assay described amplifies a portion of the nucleoprotein gene of all 107 rabies and rabies-related viruses, but none of the other viruses tested. Inclusion of TaqMan(TM)-genotype-specific probes in the RT-PCR assay permits rapid identification of the virus present. By combining RT-PCR with TaqMan(TM) genotyping probes suspect rabies virus isolates can be identified in a single closed tube system that prevents potential PCR-product carry over contamination.

Bockstahler, L. E., P. G. Carney, et al. (1992). "Detection of Junin virus by the polymerase chain reaction." Journal of Virological Methods **39**(1-2): 231.

http://www.sciencedirect.com/science/article/B6T96-476F6MB-HT/2/d5e47bbe329918cde17901be1436f7dd

Argentine hemorrhagic fever is an often fatal human disease caused by Junin virus, an RNA-containing virus and member of the Arenavirus family. This virus was detected in vitro by the polymerase chain reaction (PCR) procedure. A pair of Junin virus-specific PCR DNA oligonucleotide primers and an oligonucleotide probe were designed from a known portion of the viral RNA sequence. RNA was isolated from Junin virus-infected monkey kidney cells and used to produce complementary DNA (cDNA) by reverse transcription. A DNA segment, 151 +/- 24 bp long, was amplified from this cDNA and characterized by agarose gel electrophoresis and Southern blot hybridization with the Junin virus-specific DNA probe. Sensitivity experiments showed that Junin virus could be detected with nanogram quantities of RNA isolated from virus-infected cells. The rapid and sensitive assay described here may contribute towards the development of a procedure for the early diagnosis of Argentine hemorrhagic fever.

Boerner, B., W. Weigelt, et al. (1999). "A sensitive and specific PCR/Southern blot assay for detection of bovine herpesvirus 4 in calves infected experimentally." Journal of Virological Methods **83**(1-2): 169.

http://www.sciencedirect.com/science/article/B6T96-3XVPG88-M/2/7c0dfd4d4d7210d5a3602dbfff0b6e9e

A PCR/Southern blot assay for detection of bovine herpesvirus 4 (BHV-4) in the background of bovine cellular DNA was developed. A BHV-4 specific sequence within the gene coding for the glycoprotein B (gB) was selected for primer sequences to guarantee the specificity of the assay. With a detection limit of six molecules BHV-4 DNA in the background of 1 [mu]g of cellular DNA (equals about 150000 bovine cells) this PCR/Southern blot assay represents a highly sensitive method for detection of BHV-4 DNA. At low concentrations of BHV-4 genomes, this assay also allows to estimate the copy number of BHV-4: a distinction between fewer than 6, 6-59 and more than 60 BHV-4 genomes/100 [mu]l DNA suspension was possible. Tissue and blood samples of two calves, infected experimentally with BHV-4 were examined for the prevalence of BHV-4 DNA 130 days post infection. Ten days before taking samples, one of the calves was immuno-suppressed with dexamethasone. In both calves, BHV-4 DNA was detected in the leucocyte fraction of the blood, and beyond that in lower quantities in the spleen and the kidney of the immuno-suppressed calf. It is assumed that a latent BHV-4 infection was activated after application of dexamethasone and that the leucocyte fraction of the blood represents one site of latency of BHV-4 in cattle.

Boni, J. and J. Schupbach (1993). "Primer extension analysis provides a sensitive tool for the identification of PCR-amplified DNA from HIV-1." Journal of Virological Methods **42**(2-3): 309.

http://www.sciencedirect.com/science/article/B6T96-476RM72-2J/2/16688fe329aa2ea32a615cf7e04e8d9a

Primer extension analysis was evaluated as a means to identify PCR-amplified DNA from HIV-1. Solution hybridization with radioactive labeled oligonucleotides followed by an extension reaction with Klenow fragment of Escherichia coli DNA polymerase I and subsequent separation on denaturing polyacrylamide gels reveals single stranded DNA products of the predicted size. The specificity of the reaction is further demonstrated by specific endonuclease digestion. The analysis is more sensitive than Southern blotting and about equally sensitive as Slot blot analysis when double-stranded DNA probes are used for hybridization. With end-labeled oligonucleotide probes, primer extension analysis proved an order of magnitude more sensitive than membrane hybridization. The analysis also allows quantitation of amplified DNA from 1 pg to about 1 ng of DNA product. Under the conditions described for amplification, primer extension analysis is capable of detecting a single HIV-1 plasmid DNA molecule in the presence of 1 [mu]g of total DNA. 3'-end mismatching of the oligonucleotide probe does not result in a significantly altered detection limit. Primer extension analysis can also be carried out with at least three different PCR-amplified DNAs in the same reaction tube.

Boom, R., C. Sol, et al. (2000). "Detection and quantitation of human cytomegalovirus DNA in faeces." Journal of Virological Methods **84**(1): 1.

http://www.sciencedirect.com/science/article/B6T96-3Y21VDG-1/2/9c147c239ca178c36e64f171c29e57b8

The development and performance of a robust and sensitive PCR assay are described for the

detection and quantitation of human cytomegalovirus DNA in human faecal specimens. In this assay, CMV DNA was purified by an optimised DNA extraction protocol together with internal control DNA that monitored both DNA extraction efficiency and PCR efficiency. The lower detection limit of the assay was reached at about 100 CMV particles per ml of (25-50%) faecal suspension. CMV DNA could be quantitated in the range of about 300-100000 molecules per ml of faecal suspension. CMV DNA loads obtained in clinical faeces specimens suggest that the assay can be used to monitor the efficacy of antiviral treatment. Reconstruction experiments that monitored the efficiency of DNA extraction of a preliminary DNA extraction protocol, showed low DNA yields for 9% of the specimens (n=78). In all cases, low DNA extraction efficiency seemed to be due to a component present in faeces that prevented DNA binding to silica particles, presumably by competitive binding. Choosing the right ratio of silica particles to faeces specimen solved this problem. Similarly, reconstruction experiments showed that the strong PCR inhibition that was observed in 8% of the specimens could effectively be relieved by the inclusion of [alpha]-casein in the PCR mixtures.

Boonham, N., L. G. Perez, et al. (2004). "Development of a real-time RT-PCR assay for the detection of Potato spindle tuber viroid." Journal of Virological Methods **116**(2): 139.

http://www.sciencedirect.com/science/article/B6T96-4B958S0-1/2/960177b13ab6d627eaef729d0be24bf0

Potato spindle tuber viroid (PSTVd) is a quarantine pathogen in the European Union and causes damaging diseases of solanaceous crops. Under the EU Plant Health directive 2000/29/EC, countries must have the ability to detect and identify accurately and rapidly the introduction of harmful organisms in plants or plant products; furthermore, if the quarantine pathogen is found, be able to survey extensively for it. In this respect, PSTVd poses an interesting technical problem, since its RNA does not code for any proteins and thus any diagnostic method must be based on the detection of the RNA and be suitable for scaling up to testing large sample numbers. With this in mind a one-tube real-time RT-PCR assay based on TaqMan(TM) chemistry was developed. Investigations were carried out into various aspects of the assay relevant to the efficient amplification of targets that have a significant amount of secondary structure such as viroids. Thus comparisons were made of reverse transcription temperature, concentration and type of reverse transcriptase, RNA denaturation, sample purity and single versus two-tube reaction format. The assay developed was shown to be able to detect a wide range of isolates of PSTVd and in comparison with a chemi-luminescent hybridisation system was shown to be 1000-fold more sensitive. A further significant advantage of this assay format compared with hybridisation is that it is suitable for scaling up to large sample numbers using robotic liquid handling systems.

Bootman, J. S. and P. A. Kitchin (1992). "An international collaborative study to assess a set of reference reagents for HIV-1 PCR." Journal of Virological Methods **37**(1): 23.

http://www.sciencedirect.com/science/article/B6T96-476F5XR-52/2/e431ef2b94b8d76f1b0e7033094e40ba

An international collaborative study was performed to evaluate a set of PCR reference reagents for HIV diagnosis. Twenty-six laboratories from 9 countries analysed a proficiency panel of 10 coded DNA samples using the PCR reference reagents and protocols. For comparison, these coded samples were then assessed using a laboratory's own 'in-house' reagents and methodologies. The objectives of the study were: (i) to assess inter-laboratory variation of PCR sensitivity, (ii) to evaluate the DNA 'carryover' problem and frequency of false negative results and (iii) to examine the utility of the complete set of reagents and templates to act as reference preparations for HIV PCR.Using the reference reagents, 46% of laboratories reported no false

positive results in any of their assays of the negative controls. The remaining laboratories all reported a false positive result(s) in at least one assay. The overall false positive result rate for the study was 9.3%. In contrast, an overall false negative result rate of 7.4% was observed, with some laboratories recording negative results even for samples containing 10000 molecules of target DNA.The level of absolute sensitivity may be assessed accurately only from the 12 laboratories that obtained no false positive results. All 12 laboratories detected the sample containing 10 molecules of template DNA and 9 out of the 12 laboratories detected the sample containing 1 molecule. This is in close agreement with the theoretical detection rate based on a statistical probability model for the detection of a single molecule.These characterised reference reagents were at least as sensitive as any of the 'in-house' reagents and methodologies applied, including nested PCR. The complete set of characterised reference reagents is now available for quality control assessment of HIV-1 PCR from the MRC ADP.

Boriskin, Y. S., J. C. Booth, et al. (1993). "Rapid detection of mumps virus by the polymerase chain reaction." Journal of Virological Methods **42**(1): 23.

http://www.sciencedirect.com/science/article/B6T96-47YGSVX-4/2/03c0453462ef9d8a8a4d7e2087948693

A procedure for detecting mumps virus in under 48 h was developed using the PCR. The sensitivity of the PCR amplification reaction and of the detection of the PCR product was significantly improved by: (i) enriching for viral template RNAs by overnight culture of the virus in Vero cells and (ii) substitution of polyacrylamide gel analysis for agarose gel electrophoresis. The technique was capable of detecting 1-20 infectious units of virus or an equivalent of 1-10 pg of mumps virus-specific plasmid DNA.

Briddon, R. W., A. G. Prescott, et al. (1993). "Rapid production of full-length, infectious geminivirus clones by abutting primer PCR (AbP-PCR)." Journal of Virological Methods **43**(1): 7.

http://www.sciencedirect.com/science/article/B6T96-476F666-8K/2/68fb7c8742f2e6189b54c24e8d228262

The application of the polymerase chain reaction (PCR) method of DNA amplification for the isolation of full-length, infectious clones of geminiviruses is described. Non-overlapping, abutting 20-mer oligonucleotide primers were used to produce a linear product from the circular geminivirus genomic template. Clones of African cassava mosaic virus (ACMV) DNA A, obtained by this method, were infectious following mechanical inoculation (in the presence of ACMV DNA B) onto Nicotiana benthamiana. Normal ACMV symptoms resulted and typical geminate viral particles were detected by electron microscopy. The use of PCR for the detection and production of full-length, infectious geminivirus clones is discussed.

Bruni, R., C. Argentini, et al. (1995). "A PCR-based strategy for rapid mapping of hepadnavirus integrated sequences in hepatocellular carcinomas." Journal of Virological Methods **52**(3): 347.

http://www.sciencedirect.com/science/article/B6T96-3YXC14C-C/2/5aef364fd8afd7ef3fce0500c4187b0a

A methodology based on polymerase chain reaction (PCR) and restriction analysis for rapid mapping of woodchuck hepatitis virus (WHV) integrations in hepatocellular carcinoma (HCC)

tissues is described. Conventional PCR with viral primer pairs is not suitable for mapping WHV-integrated regions because the presence of minimum amounts of non-integrated (PCR amplifiable) WHV genome and replicative intermediates cannot be excluded. The first relevant part of the strategy is the identification of the cellular sequences flanking the WHV integration in order to select one (or more) integration-specific primer. The cellular flanking sequence can be rapidly obtained by means of inverse-PCR amplification of the viral/cellular junction and sequencing of the product. Mapping of the integrated regions is carried out by fixed flanking primer PCR (FFP-PCR) using the cellular primer as a 'fixed' primer in PCR in association with each of an available set of WHV primers. Amplification of episomal WHV sequences is thus avoided. PCR products can also undergo restriction analysis. PCR-positive viral primers and specific WHV restriction sites are assembled into a map, based on the size and restriction pattern of the PCR products. The results of WHV integration mapping in a woodchuck HCC are described.

Cameron-Wilson, C. L., P. Muir, et al. (2003). "Evaluation of a line probe assay for identification of hepatitis B virus precore variants in serum from chronic hepatitis B carriers." Journal of Virological Methods **114**(1): 97.

http://www.sciencedirect.com/science/article/B6T96-49SWFWP-7/2/bde013aadafe321e685e3e429fb19b76

A prototype line probe assay (LiPA) for identifying hepatitis B virus (HBV) precore variants (INNO-LiPA HBV precore) was evaluated using a panel of 50 sera from 46 patients with HBV infection. The assay detected sequence variations detected commonly in the precore promoter region and in amino acid codons 28 and 29 of the precore gene. There was strong agreement between INNO-LiPA HBV precore results and those of a codon 28 point mutation assay (PMA), with identical results obtained in 40 of 43 sera (93%) typeable by both assays (kappa coefficient ([kappa])=0.90). In addition, the precore codon 29 sequence identified by the INNO-LiPA HBV precore was confirmed by nucleotide sequencing in all seven samples analysed. However, the INNO-LiPA HBV precore identified precore promoter sequences much less efficiently. The prototype assay could identify codon 28/29 sequences from as little as 10 HBV genome equivalents in 10 [mu]l serum, and in experiments using artificially prepared mixtures of variants could identify a minor component constituting 2.5% of the total viral DNA population. The INNO-LiPA HBV precore was also straightforward technically and rapid, and is therefore likely to be useful for epidemiological investigations into the prevalence, distribution and clinical significance of HBV precore variants.

Casas, I., A. Tenorio, et al. (1997). "Detection of enteroviral RNA and specific DNA of herpesviruses by multiplex genome amplification." Journal of Virological Methods **66**(1): 39.

http://www.sciencedirect.com/science/article/B6T96-3RYCMYM-18/2/a8b89a2b6019a196e061ff4c236aae9a

A reverse transcription (RT) multiplex polymerase chain reaction (PCR) assay was developed to allow rapid, sensitive and simultaneous detection of enteroviral RNA and herpesviral DNA specific sequences in a single tube. The method involves a reverse transcription step followed by a multiplex nested PCR in which the combination of primers amplifies cDNA from enteroviruses and specific herpesviruses DNA. Nested amplification utilises primers designed to anneal into the amplification product from the first reaction. Individual viruses were then detected and differentiated by the size of their PCR products determined using ethidium bromide stained agarose gels. To exclude false negatives due to sample inhibitors an internal amplification control, a cloned fragment of DNA from Pseudorabies virus (PRV DNA) was included in the

reaction mixture. Detection levels between 0.01 and 0.001 TCID50 of prototype strains of Polio and Coxsackie type B viruses and between 1 and 100 molecules of cloned-DNA of herpesviruses prototype strains were achieved. The RT multiplex PCR method proved capable of detecting enteroviral RNA or herpesviral DNA in cerebro spinal fluid (CSF) samples from patients with aetiologically well characterized encephalitis or aseptic meningitis.

Chatellard, P., R. Sahli, et al. (1998). "Single tube competitive PCR for quantitation of CMV DNA in the blood of HIV+ and solid organ transplant patients." Journal of Virological Methods **71**(2): 137.

http://www.sciencedirect.com/science/article/B6T96-3VCVDKK-1/2/b46f708cdced2d2252040882fee76527

Cytomegalovirus (CMV) infection is a major cause of morbidity and mortality in transplant and HIV-infected patients. However, CMV can also cause asymptomatic infection. An elevated blood viral load as assessed by various methods appears to be a predictor for symptomatic infections, and can be used to identify patients at the highest risk of developing CMV disease. We developed a single tube competitive quantitative PCR assay for CMV DNA, using as a competitor a plasmid carrying the target sequence for amplification with an internal deletion. The analysis of data from repeated extractions and amplifications of samples showed that the coefficient of variation of the assay was typically less than 20%. Clinical samples from 14 HIV-infected and 13 solid organ transplant patients were analyzed. Widely varying CMV DNA levels were found in leukocytes, with a positive correlation with the measure of infectivity in the leukocytes by quantitative culture on fibroblasts. The highest CMV DNA content in leukocytes was found in two patients with presumptive CMV disease. In HIV patients, the amount of DNA in leukocytes was much larger than in solid organ transplant recipients, when standardized for infectivity. Although based on a very limited number of patients, this observation probably points to a difference in the biology of CMV infection in these two categories of susceptible individuals. CMV DNA was also found in the plasma of some of the patients with a high CMV DNA leukocyte load. The present test should be useful for identifying patients at high risk of developing CMV disease, for monitoring therapeutic efficacy of antiviral regimens and to improve the understanding the pathogenesis of CMV infection.

Chimside, E. D. and W. J. M. Spaan (1990). "Reverse transcription and cDNA amplification by the polymerase chain reaction of equine arteritis virus (EAV)." Journal of Virological Methods **30**(2): 133.

http://www.sciencedirect.com/science/article/B6T96-476KWJH-34/2/c882ab4e649025070a2e728af800f2c8

A technique is described for the amplification and specific identification of equine arteritis virus (EAV) nucleotide sequences. The polymerase chain reaction (PCR) was evaluated initially by amplification of cloned virus specific cDNA sequences prior to amplification of single-stranded (ss) cDNA produced by reverse transcription (RT) of viral genomic RNA. Three separate primer pairs were used for RT/PCR of EAV genomic RNA, each pair producing only one band in agarose gels of the predicted size from the genomic nucleotide sequence. The viral origin of cDNA products was confirmed by hybridisation analysis with EAV-specific probes. RT/PCR analysis of clinical material indicates the methodology is sensitive enough to detect 600 pfu/ml EAV in seminal plasma.

Chin Yuan, C., W. Miley, et al. (2001). "A quantification of human cells using an ERV-3 real time PCR assay." Journal of Virological Methods **91**(2): 109.

http://www.sciencedirect.com/science/article/B6T96-426XYG8-2/2/c6f338eb172e5c82ee0b71a1554d9fde

A novel approach to quantifying human cells using a real time PCR assay was developed. The target sequence used in the assay is a 135 bp segment within the unique 1.7 kb Hind III / Pst I fragment of the ERV-3 envelope gene. ERV-3 is a full-length human endogenous retrovirus present in known copy number in all human cells. The detection range of ERV-3 by real time PCR is from 106 to 101. The precision described, sensitivity and specificity of the assay indicate that the ERV-3 sequence is an accurate cell quantitation marker. The quantitative ERV-3 assay enables simple, fast, and reproducible detection and quantitation of the cell number. The assay can be used to determine the sample DNA conditions and also it can be used to adjust the quantitative DNA measurements of other target gene assays relative to the number of cell equivelants.

Contorni, M. and P. Leoncini (1993). "Typing of human papillomavirus DNAs by restriction endonuclease mapping of the PCR products." Journal of Virological Methods **41**(1): 29.

http://www.sciencedirect.com/science/article/B6T96-476RMYJ-6C/2/8e2abca7118a98dc596152c434d950f0

The polymerase chain reaction (PCR) for the diagnosis of human papillomavirus (HPV) infections, and in particular for the study of cervical HPV-associated lesions, is used widely. We identified a novel set of universal primers that are able to amplify a fragment spanning the E1 open reading frame (ORF) from different mucosotropic HPV types. A restriction endonuclease digestion of the amplified products is suggested for accurate typing. In particular, AluI digestion of the amplified fragments yields a distinctive fragment pattern for each 'high-risk' (16, 18, 31 and 33) HPV sequence, thus distinguishing them from 'low-risk' (6b and 11) HPV sequences.

Davidson, I. and M. Malkinson (1996). "A non-radioactive method for identifying enzyme-amplified products of the reticuloendotheliosis proviral env and LTR genes using psoralen-biotin labelled probes." Journal of Virological Methods **59**(1-2): 113.

http://www.sciencedirect.com/science/article/B6T96-3VYV049-V/2/53bd806cd3e82f6562a721458a51bdaf

A novel polymerase chain reaction (PCR) system based on the env gene of reticuloendotheliosis virus (REV) strain REV-A for the detection of proviral DNA is described. The designed PCR product of 807 bp was identified using an internal probe of 278 bp produced by nested PCR from REV-infected DNA CEF. The env-gene PCR was then compared with the previously described PCR for proviral REV-long terminal repeat and the PCR product served also as the probe. The probes were labelled with the psoralen-biotin system by photoactivation and the southern blot hybridization signal was detected colorimetricaly. The advantages of using a non-radioactive means of probe labelling were demonstrated clearly in that study, as well as the effective labeling of probes with psoralen-biotin and the simple colorimetric method of detection. The env-gene PCR detected all eleven REV strains used in the study. These included three REV prototype strains and eight Israeli REV isolates. Both PCR systems had similar levels of sensitivity.

Desario, C., N. Decaro, et al. "Canine parvovirus infection: Which diagnostic test for virus?" Journal of Virological Methods **In Press, Corrected Proof**
http://www.sciencedirect.com/science/article/B6T96-4FRHD09-1/2/6391ff1332d2ceb835611b30a558f7db

Five laboratory tests for diagnosis of canine parvovirus type 2 (CPV-2) infection were employed to test 89 faecal samples collected from dogs with diarrhoea. The tests analysed were immunochromatography (IC), haemagglutination (HA), virus isolation (VI), conventional and real-time PCR. IC, HA, VI and conventional or real-time PCR were able, respectively, to detect CPV-2 antigen or nucleic acid in 41, 50, 54, 68 and 73 of the samples. The best correlation was found between conventional and real-time PCR, with an overall agreement of 94.38%. Sixty-eight samples that tested positive by HA, VI or conventional PCR were subjected to antigenic and/or genetic analyses of the CPV-2 strains by monoclonal antibody (MAb), restriction fragment-length polymorphism (RFLP) and/or sequence analyses. In sum, out of the 68 strains analysed, 26 were characterised as CPV-2a, 18 as CPV-2b and 24 as a CPV-2 Glu-426 mutant recently identified in Italy.

Dewhurst-Maridor, G., V. Simonet, et al. (2004). "Development of a quantitative TaqMan RT-PCR for respiratory syncytial virus." Journal of Virological Methods **120**(1): 41.

http://www.sciencedirect.com/science/article/B6T96-4CHGDDP-1/2/3ff11d621d2f4d6514dcece3411c97ec

Respiratory syncytial virus (RSV) is a ubiquitous RNA virus of the family Paramyxoviridae that may interfere with graft tolerance and with other interstitial lung diseases. The low viral titre observed in the immunodeficient transplanted patients requires a highly sensitive detection method. Although different tests already exist for the detection of RSV, reverse transcription-polymerase chain reaction (RT-PCR) has been shown to have the best sensitivity. In this study, a SYBR Green assay was established for the detection of RSV A and RSV B in a common screening test, and two quantitative TaqMan RT-PCRs were developed to quantify both RSV subgroups separately. Standard dilutions obtained from RSV cell infections were included in each test, and the assay was normalised using a housekeeping gene. RSV was found in 16% of the transplanted patients tested. The quantitative TaqMan assay is fast, reproducible, specific and very sensitive, and could facilitate considerably the detection of RSV virus. This would in-turn facilitate studies on the role of RSV in graft rejection.

Dougherty, R. M., P. E. Phillips, et al. (1993). "Restriction endonuclease digestion eliminates product contamination in reverse transcribed polymerase chain reaction." Journal of Virological Methods **41**(2): 235.

http://www.sciencedirect.com/science/article/B6T96-476F64G-80/2/51693b3c879ce42330fb13fe790d5a64

Restriction endonuclease digestion was used to eliminate false-positive signals caused by polymerase chain reaction (PCR) product DNA contamination in a reverse transcribed (RT) PCR for amplifying rubella virus (RV) RNA sequences. A restriction enzyme selected to cut the PCR product DNA between, but not within, the primer binding sites was used to digest reaction mixtures after reverse transcription but before PCR amplification. Because restriction enzymes generally react only with specific double-strand sequences, contaminating DNA was rendered inactive while reverse-transcribed single strand cDNA was amplified. Assays showed that restriction enzyme digestion reduced template activity of product DNA by a factor of 107, while

leaving sensitivity of the RT-PCR unaffected.

Ebeling, S. B., H. P. Eric Borst, et al. (2003). "Development and application of quantitative real time PCR and RT-PCR assays that discriminate between the full-length and truncated herpes simplex virus thymidine kinase gene." Journal of Virological Methods **109**(2): 177.

http://www.sciencedirect.com/science/article/B6T96-484V26H-1/2/17f6f3a7e735f57fbe9f39d15243200e

Allogeneic donor T lymphocytes manipulated genetically to express the herpes simplex virus thymidine kinase (HSV-TK) gene have emerged as promising tools to alter the balance between graft versus host disease and graft versus leukemia after allogeneic stem cell transplantation, since they can be eliminated selectively in vivo with ganciclovir. Recently, it was reported that in SFCMM-3, an HSV-TK-encoding retroviral vector, two cryptic splice sites in the HSV-TK sequence led to the generation of an HSV-TK splice variant ([Delta]HSV-TK) that encodes a ganciclovir-resistant gene product. In order to quantify wtHSV-TK and [Delta]HSV-TK RNA levels we have developed two real time Taqman PCR assays. We demonstrate that the sensitivity of both PCR assays is 10-4. It was found that the splice variant is generated in the packaging cell line and results in approximately 4.8+/-1.9% of virions that contain [Delta]HSV-TK RNA. After transduction of human T cells no significant increase in [Delta]HSV-TK RNA could be detected. Thus, at maximum 4.2+/-1.2% of T cells transduced with SFCMM-3 will be resistant to ganciclovir due to this mechanism only. Together, these assays provide a powerful method to monitor patients in future clinical trials.

Erker, J. C., S. M. Desai, et al. (1998). "Rapid detection of GB virus C RNA by reverse transcription-polymerase chain reaction (RT-PCR) using primers derived from the 5'nontranslated region." Journal of Virological Methods **70**(1): 1.

http://www.sciencedirect.com/science/article/B6T96-3VW3057-1/2/1f25822de976214f53e39e96ee7dcc10

A simple reverse transcription-polymerase chain reaction (RT-PCR) procedure for the detection of GB virus C (GBV-C) RNA in serum or plasma is described. In this method, total nucleic acid, extracted from a small volume of human plasma, is reverse transcribed using random hexamers. An aliquot of cDNA is then utilized in PCR employing GBV-C specific primers designed to highly conserved regions of the 5'nontranslated region (NTR). For additional sensitivity, a second round of nested amplification is performed. Reactions are analyzed on an agarose gel and samples showing an ethidium bromide stained band of the appropriate size in the first and second amplification, or in the second amplification only, are designated to be positive. This protocol allows for the rapid and sensitive detection of GBV-C infection in human plasma or serum.

Erker, J. C., S. M. Desai, et al. (1999). "Rapid detection of Hepatitis E virus RNA by reverse transcription-polymerase chain reaction using universal oligonucleotide primers." Journal of Virological Methods **81**(1-2): 109.

http://www.sciencedirect.com/science/article/B6T96-3XMPJNX-G/2/6e14405581559722cb6294105d75e4ab

A rapid reverse transcription-polymerase chain reaction (RT-PCR) procedure for the detection of

Hepatitis E virus (HEV) RNA in serum is described. Total nucleic acids are extracted from a small volume of human serum and reverse transcribed using random hexamers. An aliquot of cDNA is then utilized in nested PCR employing degenerate HEV consensus primers. These primers are designed to sequences conserved between the Burma, Mexico, and US HEV strains, generating amplicons within each of the three open reading frames. Reactions are analyzed by agarose gel electrophoresis and samples showing an ethidium bromide stained band of the appropriate size in the first and second amplification, or in the second amplification only, are designated as positive. This protocol allows for the rapid and sensitive detection of HEV infection in human serum.

Eun, A. J.-C., M.-L. Seoh, et al. (2000). "Simultaneous quantitation of two orchid viruses by the TaqMan(R) real-time RT-PCR." Journal of Virological Methods **87**(1-2): 151.

http://www.sciencedirect.com/science/article/B6T96-40GJ2JM-J/2/4f08efce75bb16c36484b88e0b6ac30a

Simultaneous quantitation of two orchid viruses, cymbidium mosaic potexvirus (CymMV) and odontoglossum ringspot tobamovirus (ORSV), were carried out using the TaqMan(R) real-time RT-PCR, a novel detection technique that combines RT-PCR with the power of fluorescent detection. Four TaqMan(R) probes were synthesized, targeting at the RNA-dependent RNA polymerase (RdRp) and coat protein (CP) genes of both viruses. The reporter dye FAM (6-carboxyfluorescein) was used to label the 5' terminus of probes specific to CymMV, while TET (tetrachloro-6-carboxyfluorescein) was used for the ORSV probes. TAMRA (6-carboxy-tetramethyl-rhodamine), which was attached at the 3' terminus of each probe, was used as the universal quencher. With increasing amounts of standard RNA templates, the respective threshold cycle (CT) values were determined and a linear relationship was established between these CT values and the logarithm of initial template amounts. The amounts of starting templates in mixed-infected Oncidium flowers and leaves were estimated from the standard curves. As little as 104 copies or 5 fg each of CymMV and ORSV could be detected simultaneously with either the RdRp or CP gene as the target. This system offers a sensitive, high throughput and rapid method for plant virus detection.

Evans, P. C., J. Gray, et al. (1998). "Optimisation of the polymerase chain reaction and dot-blot hybridisation for detecting cytomegalovirus DNA in urine: comparison with detection of early antigen fluorescent foci and culture." Journal of Virological Methods **73**(1): 41.

http://www.sciencedirect.com/science/article/B6T96-3T2PCND-5/2/d3c00709a679180bccdcf47c6b8e4730

Rapid, sensitive and specific assays are required for the diagnosis of CMV infection following transplantation. We describe our experience in developing assays for detecting CMV in urine. Conventional preparation of probes cloned after amplification in E. coli led to contamination with E. coli nucleic acids; these hybridised to E. coli DNA present in urine and produced false positive results. Two CMV probes (Hind III and gL) hybridised to human DNA despite high stringency; these probes were thus unsuitable for detecting viral nucleic acids in clinical samples. A PCR derived probe from the immediate early gene of CMV detected dot-blotted CMV DNA specifically. Optimal preparation of urine for detection of CMV DNA was as follows; four freeze/thaw cycles and ultracentrifugation before in vitro proteinase K/SDS treatment, phenol:chloroform extraction, heat denaturation and direct application onto a nylon membrane. However, dot-blot hybridisation was a poor test for CMV in urine; it had low sensitivity and specificity compared with virus isolation and DEAFF. Single round PCR of a 293bp region of CMV DNA was sensitive and specific to CMV targets. However, undiluted urine contained PCR inhibitors that could only be

partly removed by using PEG precipitation. PCR of CMV DNA from urine was specific but was insensitive compared to conventional culture and DEAFF. A significant proportion of urine samples were toxic in conventional culture and DEAFF tests but, PCR of CMV DNA from urine is insensitive and despite its specificity is unlikely to be advantageous in clinical practice even when DEAFF or culture prove unreliable.

Fabre, F., C. Kervarrec, et al. (2003). "Improvement of Barley yellow dwarf virus-PAV detection in single aphids using a fluorescent real time RT-PCR." Journal of Virological Methods **110**(1): 51.

http://www.sciencedirect.com/science/article/B6T96-48CNW0D-1/2/439cbec68940d7e63b490ad51961bc19

One of the major factors determining the incidence of Barley yellow dwarf virus (BYDV) on autumn-sown cereals is the viruliferous state of immigrant winged aphids. This variable is assessed routinely using the enzyme-linked immunosorbant assay (ELISA). However, the threshold for virus detection by ELISA can lead to false negative results for aphids carrying less than 106 particles. Although molecular detection techniques enabling the detection of lower virus quantities in samples are available, the relatively laborious sample preparation and data analysis have restricted their use in routine applications. A gel-free real-time one-step reverse transcription polymerase chain reaction (RT-PCR) protocol is described for specific detection and quantitation of BYDV-PAV, the most widespread BYDV species in Western Europe. This new assay, based on TaqMan(R) technology, detects and quantifies from 102 to 108 BYDV-PAV RNA copies. This test is 10 and 103 times more sensitive than the standard RT-PCR and ELISA assays published previously for BYDV-PAV detection and significantly improves virus detection in single aphids. Extraction of nucleic acids from aphids using either phenol/chloroform or chelatin resin-based protocols allow the use of pooled samples or of a small part (up to 1/1600th) of a single aphid extract for efficient BYDV-PAV detection.

Falcone, E., E. D'Amore, et al. (1997). "Rapid diagnosis of avian infectious bronchitis virus by the polymerase chain reaction." Journal of Virological Methods **64**(2): 125.

http://www.sciencedirect.com/science/article/B6T96-3RJ9BMF-2/2/8f8a7aebc8132e8848af2ec6d2901c8c

A simple, sensitive and specific polymerase chain reaction (PCR) procedure was developed in order to detect infectious bronchitis virus (IBV) directly in tissue samples. Viral RNA was extracted from allantoic fluids and cell cultures infected experimentally with different strains of IBV and from tissues of naturally infected birds. Viral RNA was then amplified and identified by a nested RT-PCR assay using two sets of primers flanking a well-conserved region of the nucleocapsid gene. The selected IBV nucleocapsid sequence was detected successfully by simple direct electrophoresis of amplified material.

Fang, G., G. Zhu, et al. (1998). "Minimizing DNA recombination during long RT-PCR." Journal of Virological Methods **76**(1-2): 139.

http://www.sciencedirect.com/science/article/B6T96-3V5V5SV-H/2/7c71936eb89a8bdcb4722309fe5e2d2b

Recent developments have made it possible to reverse transcribe RNA and amplify cDNA

molecules of >10 kb in length, including the HIV-1 genome. To use long reverse transcription combined with polymerase chain reaction (RT-PCR) to best advantage, it is necessary to determine the frequency of recombination during the combined procedure and then take steps to reduce it. We investigated the requirements for minimizing DNA recombination during long RT-PCR of HIV-1 by experimenting with three different aspects of the procedure: conditions for RT, conditions for PCR, and the molar ratios of different templates. We used two distinct HIV-1 strains as templates and strain-specific probes to detect recombination. The data showed that strategies aimed at completing DNA strand synthesis and the addition of proofreading function to the PCR were most effective in reducing recombination during the combined procedure. This study demonstrated that by adjusting reaction conditions, the recombination frequency during RT-PCR can be controlled and greatly reduced.

Feucht, H. H., B. Zollner, et al. (1995). "Comparison of conventional autoradiography with a new DNA enzyme immunoassay for the detection of hepatitis C virus-polymerase chain reaction amplification products." Journal of Virological Methods **55**(1): 105.

http://www.sciencedirect.com/science/article/B6T96-3YYTF0W-19/2/ae45973e875d9693bcf16d31f7f60463

The detection of HCV-PCR amplification products by DNA enzyme immunoassay (DEIA) was compared with conventional hybridization carried out with a 32P-labelled oligonucleotide probe. The detection limit of both methods was shown to be between 100 pg and 1 ng of amplicon. All serum samples of 40 HCV-seropositive patients were positive after PCR in autoradiography, but only 38 with the DELA technique (sensitivity 95%). There were no false-positive reactions by either method. The advantage of the DEIA method was the fast and non-radioactive detection of HCV amplicons. DELA combines the specificity of the hybridization event with the speed of an ELISA procedure and is suitable for HCV-PCR.

Formiga-Cruz, M., A. Hundesa, et al. (2005). "Nested multiplex PCR assay for detection of human enteric viruses in shellfish and sewage." Journal of Virological Methods **125**(2): 111.

http://www.sciencedirect.com/science/article/B6T96-4FFH1XG-2/2/3f8dd23d017b88125eeccf9122d6e4fc

Environmental samples and contaminated shellfish present frequently low concentrations of more than one viral species. For this reason, a nested multiplex RT-PCR was developed for the detection of adenoviruses, enteroviruses and hepatitis A viruses in different environmental samples such as urban sewage and shellfish. This assay will save time and cost for detection of these enteric viruses with a smaller sample volume, which otherwise can be a limiting factor in routine analysis. The limit of detection was approximately 1 copy for adenovirus and 10 copies for enterovirus and hepatitis A virus per PCR reaction using titrated cell-cultured viruses as template material. In shellfish and environmental samples, this multiplex PCR was optimized to detect all three viruses simultaneously when the concentration of each virus was equal or lower than 1000 copies per PCR reaction. This is the level found predominantly in the environment and in shellfish when the numbers of fecal bacterial and phage indicators are low. The detection of human adenoviruses by PCR has been suggested as a molecular index of fecal contamination of human origin in the environment and food and the multiplex assay developed may be a tool for evaluating the presence of viral contamination in shellfish and water and to expand microbiological control to include viral markers.

Forslund, O., H. Ly, et al. (2003). "Improved detection of cutaneous human papillomavirus DNA by single tube nested 'hanging droplet' PCR." Journal of Virological Methods **110**(2): 129.

http://www.sciencedirect.com/science/article/B6T96-48KMMH5-1/2/21b0818017e2050923d968ba9fd138bf

A single tube nested 'hanging droplet' PCR was developed for detection of cutaneous human papillomavirus (HPV) DNA of the phylogenetic group B1. The nested PCR was compared with a single round PCR method by testing 56 fresh biopsies from Australian skin tumour patients. HPV DNA was detected in 64% (36/56) of the biopsies by nested PCR and in 30% (17/56) by single round PCR (PP=0.003], squamous cell carcinoma [43% (7/16) vs. 25% (4/16)] and in solar keratosis [93% (13/14) vs. 57% (8/14); P=0.038]. The nested PCR and the single round PCR system detected 26 and 11 different HPV types/putative types/subtypes, respectively. Multiple types were found in eight samples by the nested PCR and two samples by single round PCR. The nested HPV PCR is more sensitive and capable of amplifying a broad spectrum of HPV types from skin tumours, but further improvements are needed before all HPV infections in skin can be detected by a single assay.

Forsman, A., D. Uzameckis, et al. (2003). "Single-tube nested quantitative PCR: a rational and sensitive technique for detection of retroviral DNA. Application to RERV-H/HRV-5 and confirmation of its rabbit origin." Journal of Virological Methods **111**(1): 1.

http://www.sciencedirect.com/science/article/B6T96-48NKTJ2-2/2/e7668d7aed4a8cd8be75c568f8c0011f

It was reported earlier that a few patients suffering from non-Hodgkin's lymphoma had low amounts of DNA from the so-called fifth human exogenous retrovirus, HRV-5. A sensitive and rational method for large-scale screening for HRV-5 DNA was therefore developed. It is a single-tube nested quantitative PCR (stnQPCR), which uses two functionally isolated primer pairs and one probe target distinct from related endogenous retroviral sequences, yet encompassing known HRV-5 variation, allowing optimal use of sequence conservation. DNA from lymphoma, myeloma, and follicular dendritic cell lines was tested for HRV-5 positivity, as was DNA from whole blood of blood donors, non-Hodgkin's lymphoma and systemic lupus erythematosus patients, as well as DNA from lymph node biopsies of rheumatoid arthritis patients with lymphoma. One blood donor, one systemic lupus erythematosus patient, two previously known positive non-Hodgkin's lymphoma patients, and one rheumatoid arthritis lymphoma patient, came out positive. They had 24, 2, 148, 480 and 30 proviral copies per [mu]g of DNA from PBMC or lymphoma tissue, respectively. During the completion of this work it was reported that HRV-5 is a rabbit endogenous retrovirus (RERV-H), and that HRV-5 positivity was due to presence of rabbit DNA. DNA from six RERV-H/HRV-5 positive samples was therefore retested. Three also contained rabbit mitochondrial DNA. A search for HRV-5 antibodies using synthetic peptides was negative in sera from three RERV-H/HRV-5 positive individuals, as well as in 144 other sera, according with a noninfectious origin of the RERV-H/HRV-5 DNA in human samples. A search for possible sources of rabbit DNA contamination was negative. Methods for prevention of PCR contamination were strictly adhered to. Three samples from RERV-H/HRV-5 positive individuals positive at the Uppsala laboratory were retested at one or two other laboratories, and all three were positive. Two other samples, which were positive in the Riga laboratory, were tested also in London and also found positive. One non-Hodgkin's lymphoma patient was RERV-H/HRV-5 positive in four consecutive samples, showing that positivity was a property of that patient. It is concluded that the stnQPCR developed to detect and quantify minute amounts of RERV-H/HRV-5 DNA is a principle which can be applied widely and HRV-5 is a RERV-H. Its presence in a few human blood samples could not be explained.

Forsyth, M. A., S. Parida, et al. (2003). "Rinderpest virus lineage differentiation using RT-PCR and SNAP-ELISA." Journal of Virological Methods **107**(1): 29.

http://www.sciencedirect.com/science/article/B6T96-474DS2H-2/2/2d11968dd2eeb7be200157d2e733cbdb

An RT-PCR/ELISA system has been developed that detects and differentiates Rinderpest virus (RPV) from the other closely related morbillivirus of ruminants, Peste des petits Ruminants virus (PPRV). In addition, using lineage specific probes, it is possible to determine whether the virus sample is wild-type or vaccine, and the likely origin of the outbreak if it is wild-type. It involves carrying out a RT-PCR with one digoxygenin (Dig)-labelled primer followed by a hybridisation step with a virus-specific, biotin-labelled, probe. The hybridisation step is carried out in an ELISA format on a streptavidin-coated plate. The DIG-labelled products are detected using a specific anti-DIG monoclonal antibody and an anti-mouse horseradish peroxidase conjugate. The hybridisation step replaces nucleotide sequencing or nested PCR for confirmation of the identity of DNA product. The assay is fast and easy to carry out and can give semi-quantitative estimates of the virus content of samples.

Foster, T. P., G. V. Rybachuk, et al. (1998). "Expression of the enhanced green fluorescent protein by herpes simplex virus type 1 (HSV-1) as an in vitro or in vivo marker for virus entry and replication." Journal of Virological Methods **75**(2): 151.

http://www.sciencedirect.com/science/article/B6T96-3V5F5S6-3/2/cdcbe9ca99cc3181143189332fa1b1ef

Glycoprotein K (gK) is involved in membrane fusion phenomena during infectious virus production and egress and is an important determinant for neurovirulence. To assess better the in vitro and in vivo roles of gK in virus replication, a recombinant virus was constructed expressing an engineered enhanced green fluorescent protein (EGFP) under the control of the human cytomegalovirus immediate early gene promoter (HCMV-IEP) inserted in place of the gK gene. The EGFP gene insertion was confirmed by diagnostic polymerase chain reaction (PCR), and the presence of the EGFP protein was detected by western immunoblot analysis using anti-GFP monoclonal antibody. Fluorescence microscopy revealed that virus infected cells emitted bright fluorescence when examined using filters for fluorescein. Fluorescence emission was detected as early as 4 h post-infection. Fluorescence intensity increased over time and was stable at late times after infection at which point viral plaques continued to emit bright green fluorescence. The amount of fluorescence emitted by virus infected Vero cells was monitored by fluorescence cytometry using a FACS cytometer. At an MOI of 3, all infected cells emitted strong green fluorescence as quantified by cytometry at 48 h post-infection. The [Delta]gK-EGFP expressing recombinant virus will enable the determination of the role of gK in virus entry and egress as well as the role of gK in the molecular pathogenesis of herpes simplex virus type 1 (HSV-1).

Fuessel Haws, A. L., Q. He, et al. (2004). "Nested PCR with the PGMY09/11 and GP5+/6+ primer sets improves detection of HPV DNA in cervical samples." Journal of Virological Methods **122**(1): 87.

http://www.sciencedirect.com/science/article/B6T96-4DDTMGF-1/2/ac059cf432fa0b44655c9edccf53a55e

Based on epidemiological and research evidence, HPV has a causal role in cervical carcinogenesis. Several HPV detection methods exist to date; the most commonly used method for detection of genital HPVs consists of nested PCR using the MY09/11 and GP5+/6+ primer

sets (MY/GP+). Recently, the PGMY09/11 primer set, a modified version of the MY09/11 primer set, was introduced for single PCR and was found to detect a wider range of HPV types. The next logical step was taken and the efficacy of nested PCR using the PGMY09/11 and GP5+/6+ primer sets (PGMY/GP+) to detect HPV in cervical samples was evaluated.In this comparative study, nested PCR using the novel PGMY/GP+ primer set combination was found to be more type sensitive than the nested PCR with the MY/GP+ primer sets, detecting a wider range of HPV types, low copy HPVs, and better characterizing samples infected with multiple strains of HPV. Standardization and use of the PGMY/GP+ PCR system could aid physicians in providing more efficient HPV screening and better treatment for patients.

Fujioka, S., H. Koide, et al. (1995). "Analysis of enterovirus genotypes using single-strand conformation polymorphisms of polymerase chain reaction products." Journal of Virological Methods **51**(2-3): 253.

http://www.sciencedirect.com/science/article/B6T96-402KYYX-D/2/d910471a8c50421b04c3852185606e15

Enterovirus genotypes were identified rapidly by reverse transcription-polymerase chain reaction (RT-PCR) followed by single-strand conformation polymorphism (SSCP) analysis. The primer pair was chosen from the highly conserved sequence at the 5' non-coding region of enterovirus genomes. RT-PCR amplified a 154 bp sequence in all samples from 14 serotypes of enteroviruses, including group A and B Coxsackie viruses, echoviruses and polioviruses. SSCP analysis of these products revealed different electrophoretic profiles. Thus, SSCP analysis will be useful for differentiating the genotypes of enteroviruses, and may be applicable for rapid diagnosis of enteroviral infection.

Gehringer, H., K. Von der Helm, et al. (2003). "Development and evaluation of a phenotypic assay monitoring resistance formation to protease inhibitors in HIV-1-infected patients." Journal of Virological Methods **109**(2): 143.

http://www.sciencedirect.com/science/article/B6T96-484VJJM-1/2/e41b6217f067fcd97d1cd3c98143f8f2

A novel phenotypic assay, based on recombinant expression of the HIV-1-protease was developed and evaluated; it monitors the formation of resistance to protease inhibitors. The HIV-1 protease-encoding region from the blood sample of patients was amplified, ligated into the expression vector pBD2, and recombinantly expressed in Escherichia coli TG1 cells. The resulting recombinant enzyme was purified by a newly developed one-step acid extraction protocol. The protease activity was determined in presence of five selected HIV protease inhibitors and the 50% inhibitory concentration (IC50) to the respective protease inhibitors determined. The degree of resistance was expressed in terms of x-fold increase in IC50 compared to the IC50 value of an HIV-1 wild type protease preparation. The established test system showed a reproducible recombinant expression of each individual patients' HIV-1 protease population. Samples of nine clinically well characterised HIV-1-infected patients with varying degrees of resistance were analysed. There was a good correlation between clinical parameters and the results obtained by this phenotypic assay. For the majority of patients a blind genotypic analysis of the patients' protease domain revealed a fair correlation to the results of the phenotypic assay. In a minority of patients our phenotypic results diverged from the genotypic ones. This novel phenotypic assay can be carried out within 8-10 days, and offers a significant advantage in time to the current employed phenotypic tests.

Gibbs, A. and A. Mackenzie (1997). "A primer pair for amplifying part of the genome of all potyvirids by RT-PCR." Journal of Virological Methods **63**(1-2): 9.

http://www.sciencedirect.com/science/article/B6T96-3RHM7T6-T/2/98d98dc0e4883bedac56c3fdd03157cf

Sequence analysis was used to design a pair of degenerate oligonucleotide primers that amplified a 1.6-2.1 kbp fragment from the 3' end of the genome (virion protein gene and part of the NIb gene) of 17 species of the Potyviridae ('potyvirids'); 11 potyviruses, 2 bymoviruses, 2 macluraviruses, an ipomovirus and a rymovirus. The 'potyvirid primer 1' hybridizes to the 3' terminal poly-A region of the genome, and 'potyvirid primer 2' to the genomic region encoding the-GNNSGQ-motif of the NIb protein. Database searches showed that the potyvirid 2 primer is specific for potyvirids. Associated analyses indicated that the published amino acid sequence of part of the wheat streak mosaic rymovirus NIb protein is probably incorrect in part.

Gibson, K. M., K. A. McLean, et al. (1991). "A simple and rapid method for detecting human immunodeficiency virus by PCR." Journal of Virological Methods **32**(2-3): 277.

http://www.sciencedirect.com/science/article/B6T96-476KXFR-JG/2/fe551045ebeffc936ebbfa04546eb4de

A simple, sensitive and specific method using the polymerase chain reaction (PCR) for amplification of human immunodeficiency virus type 1 (HIV-1) is described. The method involves minimal manipulations. Peripheral blood mononuclear cells (PBMC) were prepared by a rapid Ficoll-Paque gradient method. Lymphocytes were lysed in PCR buffer containing Proteinase K and detergents, and subjected to amplification under stringent conditions, using two primer pairs. Amplified DNA sequences were hybridized with a 3'-end labelled probe, electrophoresed on agarose gels and visualised by ethidium bromide staining. Identification of amplified HIV-1 proviral DNA sequences was confirmed by autoradiography. HIV-1 sequences were amplified in all samples from 103 HIV-1 seropositive individuals, but not in 40 HIV-1 seronegative controls. The absence of contamination may be attributable in part to minimisation of manipulations before amplification.

Gough, K. C., W. Cockburn, et al. (1999). "Selection of phage-display peptides that bind to cucumber mosaic virus coat protein." Journal of Virological Methods **79**(2): 169.

http://www.sciencedirect.com/science/article/B6T96-3WJDTS6-6/2/8d9b46546fa13509ce4d2304d087dacf

Several discrete peptides that bind specifically to the coat protein of cucumber mosaic virus (CMV) were isolated from a diverse phage library displaying random nonapeptides on the major coat protein VIII. Enrichment was shown by polyclonal phage enzyme linked immunosorbent assay (ELISA) after three rounds of selection. Sequencing of the genes encoding 10 of these peptides revealed an absence of any conserved motifs, although nine of them contained a high proportion of proline residues. Some of the selected peptides were displayed at the N-terminus of thioredoxin and expressed in the cytoplasm of Escherichia coli. Both the phage-displayed and thioredoxin-fusion versions of the peptides could detect purified CMV and CMV present in crude leaf extracts from infected plants. By dot blot analysis, a thioredoxin-peptide fusion could readily detect as little as 5 ng of CMV. The peptides did not bind to other plant viruses. These peptides

have been shown to be specific and highly sensitive tools in the detection of CMV and, as well as their diagnostic potential, they could form the basis for a novel disease resistance strategy.

Gouvea, V., S. J. Cohen, et al. (1997). "Identification of hepatitis E virus in clinical specimens: amplification of hydroxyapatite-purified virus RNA and restriction endonuclease analysis." Journal of Virological Methods **69**(1-2): 53.

http://www.sciencedirect.com/science/article/B6T96-3S2BMBF-7/2/187546e9a6dc5b99c6e2ddc12dddb55e

A multi-site nested reverse transcription and polymerase chain reaction (RT-PCR) followed by restriction endonuclease analysis (REA) was developed to identify hepatitis E virus (HEV) in clinical specimens. Four sets of primers were selected to amplify regions in the HEV genome supposed to encode the helicase, polymerase, and parts of the viral capsid protein. Digestion of the nested PCR products with HinfI, HaeII, AvaII, BglI, KpnI, SmaI, or EcoRI generated readily recognizable profiles that confirm the HEV sequences and/or distinguish the unique Mexico genotype (our positive control) from all other isolates (Asian genotype). In addition, the hydroxyapatite (HA) adsorption method was compared to other adsorption and extraction methods widely used to purify viral RNA from clinical specimens for RT-PCR. All methods presented the same sensitivity of recovery of HEV RNA, but only the adsorption methods efficiently removed fecal enzymatic inhibitors. The HA method gave the best results and was the most economic in terms of time, cost, manipulations and reagents. The method was validated by screening a small number of serum and fecal specimens available from patients with acute non-A,B,C hepatitis in Nepal. HEV RNA was identified in half (5/11) of the fecal specimens obtained from patients with evidence of recent HEV infection, but in none of the 14 patients without a serological marker for hepatitis E.

Gouvea, V., N. Santos, et al. (1994). "Identification of Norwalk virus in artificially seeded shellfish and selected foods." Journal of Virological Methods **48**(2-3): 177.

http://www.sciencedirect.com/science/article/B6T96-476F6FR-DM/2/5bb703002ffee89b1144607dde0dbdb6

A rotavirus dsRNA purification protocol was adapted to extract Norwalk ssRNA from artificially contaminated shellfish, and a sensitive reverse transcription-polymerase chain reaction assay for Norwalk virus was devised to identify an estimated 20-200 genomic copies. The technique includes deproteinization with guanidinium isothiocyanate, adsorption of RNA to hydroxyapatite, and sequential precipitation with cetyltrimethylammonium bromide and ethanol. The protocol allows high recovery of viral RNA free of enzymatic inhibitors from oysters, clams, and a variety of food matrices. Norwalk virus sequences were copied and amplified by using primers selected from the polymerase gene. Digestion of the amplified products with restriction enzymes ensured the specificity of the test. This rapid and sensitive assay may significantly improve the prospect for the routine screening of the uncultivatable Norwalk virus in food stuffs.

Grankvist, O., L. Walther, et al. (1996). "Nested PCR assays with novel primers yield greater sensitivity to Tanzanian HIV-1 samples than a commercial PCR detection kit." Journal of Virological Methods **62**(2): 131.

http://www.sciencedirect.com/science/article/B6T96-3RHM7T6-

H/2/64445824136310710ca8f94cbe9f017f

To investigate the efficacy of the SK431/SK145 primer pair and two nested primer assays in amplifying African HIV-1 samples, a total of 35 Tanzanian PBMC samples were examined. These were assayed by two HIV-1 specific nested in-house PCR assays and a commercial HIV-1 PCR kit (GeneAmpTM) using SK431/SK145 as the primer pair. One of the nested PCR assays has been evaluated previously (old assay), whereas the modified assay was constructed from the HIV-1 sequence alignment released in August 1993. The modified nested primer assay showed increased sensitivity in the gag and env regions compared to the old nested primer assay. However, both the old and the modified nested primer assays displayed higher sensitivity for the detection of Tanzanian HIV-1 proviruses than the GeneAmpTMassay. When two regions were used (gag and env) as targets for the amplification, the modified nested primer assay detected 97.1% (34/35) of the proteinase K lysed samples, compared to 68.6% (24/35) using the SK431/SK145 primer pair ($P < 0.01$**). The results indicate that the SK431/SK145 primer pair may be less suitable when HIV-1 samples from Africa are analysed. The results also show that continuous modification of primer sequences can improve and maintain high sensitivity for the detection of highly divergent HIV-1 strains.

Gratzl, S., C. Moroni, et al. (1997). "Quantification of HIV-1 viral RNA and proviral DNA by isotopic competitive PCR." Journal of Virological Methods **66**(2): 269.

http://www.sciencedirect.com/science/article/B6T96-3RYCMYM-8/2/c7a8302dd1ca779cfecce4db7bbba2c7

A quantitative isotopic competitive PCR (icPCR) assay was established using 32P-labeled primers targeting the HIV-1 gag gene followed by quantification using a phosphoimager. The detection limit varied from 3 to 10 molecules of DNA and 10 to 100 molecules of RNA per reaction. The icPCR quantification of HIV-1 DNA copies correlated well with the cell number of 8E5/LAV cells bearing a single provirus (r2 = 0.95). Provirus quantification was applied to overnight infected donor PBMCs, thereby determining infectious virus titres in culture supernatants as a rapid alternative to limiting dilution culture. Parallel quantification of the HIV-1 RNA indicated the infectious virus fraction to be 0.3%. In 39 HIV-1-infected patients with clinical stages A (n = 17), B (n = 15), and C (n = 7), the HIV-1 RNA in the plasma was determined ranging from 100 to 90600 RNA copies/ml. The results of icPCR and a commercial assay (ROCHE Amplicor HIV-1 Monitor) correlated well (r = 0.97). In 13 additional patients, the plasma viral load per ml was compared with the proviral load per 106 PBMC showing a viral excess of 10-1000-fold (mean of 85, r = 0.7, $P < 0.01$). It is concluded that icPCR is suitable for the measurement of proviral and viral load in experimental and clinical settings.

Gravitt, P. E., C. Peyton, et al. (2003). "Reproducibility of HPV 16 and HPV 18 viral load quantitation using TaqMan real-time PCR assays." Journal of Virological Methods **112**(1-2): 23.

http://www.sciencedirect.com/science/article/B6T96-497RGSC-3/2/c55bd22df6d30913b5deec124ef5ed8f

A reproducibility study was designed to assess within-assay, between-day, and interlaboratory variability of three real-time PCR assays targeting HPV 16, HPV 18, and the human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) pseudogenes. Fifteen HPV 16 and fifteen HPV 18 cervical swab samples were amplified in triplicate by GAPDH and HPV 16 and by GAPDH and HPV 18 assays, respectively. All samples were amplified undiluted and at a 1:10 dilution on 2 separate days in the same laboratory, and the same samples were amplified in a

separate laboratory. HPV 16 and HPV 18 normalized viral load is reported as the number of HPV genomes per 20000 GAPDH copies. The analytic specificity of the HPV 16 and 18 assays was 100 and 97%, respectively. The intraclass correlation coefficients (ICC) were 0.99, 0.97, and 0.98 for HPV 16, HPV 18, and GAPDH, respectively, indicating that the variability due to experimental error was very low. Ten-fold differences in viral load could be readily discriminated across a six order of magnitude dynamic range (ca. 5-5 x 106 copies). Power of discrimination was increased at higher target concentrations (>5000 copies). The correlation of normalized HPV 16 and 18 viral load was high between the two laboratories (Spearman rho ([rho])=0.96 and 0.87, respectively). These HPV 16 and HPV 18 quantitative PCR assays with GAPDH normalization are reproducibly quantitative over a broad linear dynamic range allowing for application in epidemiologic studies for measurement of viral load.

Greening, G. E., L. Woodfield, et al. (1999). "RT-PCR and chemiluminescent ELISA for detection of enteroviruses." Journal of Virological Methods **82**(2): 157.

http://www.sciencedirect.com/science/article/B6T96-3XT0BN2-7/2/149fa3cfe9d254a79e6a213cd2cab2cd

Reverse transcription followed by polymerase chain reaction amplification (RT-PCR) is now used commonly to detect the presence of enteric RNA viruses in environmental samples. A sensitive, non-isotopic microtitre plate hybridisation assay was developed and applied for detection of enteroviruses in environmental samples. Following reverse transcription, viral cDNA was labelled with digoxigenin (DIG)-dUTP during the PCR amplification step. The labelled PCR products were then hybridised with enterovirus-specific biotinylated oligonucleotide probe and captured in streptavidin-coated microtitre wells. Hybridised enteroviral PCR products were detected by an anti-digoxigenin peroxidase conjugate using either a colourimetric or a chemiluminescent substrate and automated measurement. Standard curves were established for poliovirus and other enteroviruses. The chemiluminescent assay was more sensitive than the colourimetric assay for detection of poliovirus, and was specific for enteroviruses. The chemiluminescent ELISA assay was used to confirm the presence of enteroviruses in environmental water samples.

Grimm, A. C. and G. Shay Fout (2002). "Development of a molecular method to identify hepatitis E virus in water." Journal of Virological Methods **101**(1-2): 175.

http://www.sciencedirect.com/science/article/B6T96-44PVRKC-1/2/e7b6f2e02418d20b85f1e0d2d50bb872

Hepatitis E virus (HEV) causes an infectious form of hepatitis associated with contaminated water. By analyzing the sequence of several HEV isolates, a reverse transcription-polymerase chain reaction method was developed and optimized that should be able to identify all of the known HEV strains. When tested under laboratory conditions, this method was able to detect low levels of five diverse HEV variants. In addition, internal controls were constructed so that any PCR inhibition could be detected. Finally, virus-spiked environmental water samples were analyzed successfully with these assays.

Hammond, R. W., J. M. Crosslin, et al. (1999). "Differentiation of closely related but biologically distinct cherry isolates of Prunus necrotic ringspot virus by polymerase chain reaction." Journal of Virological Methods **80**(2): 203.

http://www.sciencedirect.com/science/article/B6T96-3X29D70-C/2/18edff2a89bc88f27b8881698af3c946

Prunus necrotic ringspot ilarvirus (PNRSV) exists as a number of biologically distinct variants which differ in host specificity, serology, and pathology. Previous nucleotide sequence alignment and phylogenetic analysis of cloned reverse transcription-polymerase chain reaction (RT-PCR) products of several biologically distinct sweet cherry isolates revealed correlations between symptom type and the nucleotide and amino acid sequences of the 3a (putative movement protein) and 3b (coat protein) open reading frames. Based upon this analysis, RT-PCR assays have been developed that can identify isolates displaying different symptoms and serotypes. The incorporation of primers in a multiplex PCR protocol permits rapid detection and discrimination among the strains. The results of PCR amplification using type-specific primers that amplify a portion of the coat protein gene demonstrate that the primer-selection procedure developed for PNRSV constitutes a reliable method of viral strain discrimination in cherry for disease control and will also be useful for examining biological diversity within the PNRSV virus group.

Hauptli, D., L. Bruckner, et al. (1997). "Use of reverse transcriptase polymerase chain reaction for detection of vaccine contamination by avian leukosis virus." Journal of Virological Methods **66**(1): 71.

http://www.sciencedirect.com/science/article/B6T96-3RYCMYM-1C/2/18f6ba294c42adbeeda2604566560edd

A reverse transcriptase polymerase chain reaction (RT-PCR) for avian leukosis virus (ALV) was developed for the detection of contamination of vaccines produced in embryonated eggs and cell cultures derived from chicken. ALV is highly pathogenic and induces a wide spectrum of disease in infected animals. ALV can be divided into five subgroups (A-E). The envelope glycoprotein (env gp85) is the main antigen determinant and responsible for subgroup classification. Viral RNA of all subgroups (A-E) was isolated and amplified using three sets of primers. Subsequently, restriction endonuclease analysis confirmed the product identity and discriminated between subgroups. In specific pathogen free (SPF) eggs experimentally inoculated with ALV, viral RNA was found in allantoic fluids, as well as in vaccines spiked with different subgroups of ALV. No adventitious virus was detected in commercially available preparations. This system provides a rapid and specific in vitro method for the detection of ALV RNA as an extraneous agent and may be applied for quality control of avian vaccines.

Hawrami, K. and J. Breuer (1999). "Development of a flurogenic polymerase chain reaction assay (TaqMan) for the detection and quantitation of varicella zoster virus." Journal of Virological Methods **79**(1): 33.

http://www.sciencedirect.com/science/article/B6T96-3W7XC6H-4/2/9bbe0ae1f4316bf4978918f709a3e76a

A TaqMan based polymerase chain reaction (PCR) assay was developed for the detection and quantitation of varicella zoster virus (VZV). This method enables simple, reproducible, sensitive and specific detection and quantification of VZV. The TaqMan assay was able to detect four copies of VZV and did not cross-react with other herpesviruses DNA. The assay has several advantages over conventional PCR. First, in the TaqMan assay there is no need for gel electrophoresis and contact with hazardous chemicals. Second, the method is rapid allowing the analysis of 92 samples within minutes after completion of PCR. Finally, the incorporation of a specific probe into the PCR reaction enhances the sensitivity and specificity of the method

compared with conventional PCR. The TaqMan system could, therefore, be a useful tool for the epidemiological and diagnostic investigation of VZV.

Hays, J. P. and S. H. Myint (1998). "PCR sequencing of the spike genes of geographically and chronologically distinct human coronaviruses 229E." Journal of Virological Methods **75**(2): 179.

http://www.sciencedirect.com/science/article/B6T96-3V5F5S6-6/2/db84d5fc2a47343679b811d468d045f2

A reverse transcription nested PCR (RT-PCR) sequencing methodology was developed and used to generate sequence data from the spike genes of three geographically and chronologically distinct human coronaviruses 229E. These three coronaviruses were isolated originally from the USA in the 1960s (human coronavirus 229E strain ATCC VR-74), the UK in the 1990s (human coronavirus 229E LRI 281) and Ghana (human coronavirus 229E A162). Upon translation and alignment with the published spike protein sequence of human coronavirus 229E 'LP' (isolated in the UK in the 1970s), it was found that variation within the translated protein sequences was rather limited. In particular, minimal variation was observed between the translated spike protein sequence of human coronaviruses 229E LP and ATCC VR-74 (1/1012 amino acid differences), whilst most variation was observed between the translated spike protein sequence of human coronaviruses 229E LP and A162 (47/1012 amino acid changes). Further, the translated spike protein sequence of human coronavirus 229E A162 showed three clusters of amino acid changes, situated within the 5' half of the translated spike protein sequence.

Heinemeyer, T., A. Klingenhoff, et al. (1997). "A sensitive method for the detection of murine C-type retroviruses." Journal of Virological Methods **63**(1-2): 155.

http://www.sciencedirect.com/science/article/B6T96-3RHM7T6-1B/2/5f203004dd348500779dd345dc7a37fb

A RT-PCR assay was developed for group-specific detection of murine C-type retroviruses using a nested set of degenerated primers. To distinguish exogenous viruses from related, but silent endogenous viruses, a DNAse I pretreatment of supernatants is applied. This is followed by a heat inactivation/denaturation step. The PCR method is ultrasensitive, which enables the detection of 100 attogram of MoMuLV proviral DNA or up to 1-10 infectious mouse C-type retroviruses in 10 [mu]l supernatant of infected cells. The high specificity of the method allows the differentiation between mouse C-type retroviruses and related retroviruses of the A, B, and D type and C-type retroviruses found in other species. It serves as a valuable tool for the screening of animal cell cultures for contaminations with mouse retroviruses, e.g. hybridomas or recombinant cell lines producing foreign proteins.

Hofmann, M. A. (2003). "Construction of an infectious chimeric classical swine fever virus containing the 5'UTR of bovine viral diarrhea virus, and its application as a universal internal positive control in real-time RT-PCR." Journal of Virological Methods **114**(1): 77.

http://www.sciencedirect.com/science/article/B6T96-49SWFWP-9/2/ecf6aa0b9aa5ce4e7ebac259e29b85cc

RT-PCR is used widely as a diagnostic method to detect and differentiate pestiviruses. The construction of two chimeric classical swine fever virus (CSFV) recombinants based on a marker

virus constructed previously [J. Virol. 72 (1998) 5318-5322] is described. These viruses, termed vA187CAT_5UTRBVD and vA187CAT_IRESBVD, contain the entire 5' untranslated region (5'UTR) or the internal ribosome entry site (IRES) of bovine viral diarrhea virus (BVDV), respectively. Both chimeric viruses proved to be infectious in cell culture. Hence, the 5'UTR as well as the IRES element only of BVDV can substitute for the corresponding genome region of CSFV.Next, two sets of primers and corresponding dual-labeled TaqMan(R) probes were designed; one detecting specifically a conserved but CSFV-specific area within the 5'UTR of wild-type CSFV, the other one targeting the CAT gene inserted in vA187CAT_5UTRBVD. The two primer/probe sets were combined in a closed-tube multiplex one-step RT-PCR. To monitor the entire extraction and detection process limited amounts of vA187CAT_5UTRBVD were added directly to clinical samples before RNA extraction. The multiplex RT-PCR proved to be as sensitive as the single primer/probe set method, but allowed the validation of each sample tested individually, based on the detection of the CAT marker gene. vA187CAT_5UTRBVD was also used successfully for foot-and-mouth disease virus (FMDV) TaqMan(R) RT-PCR. Therefore, it is considered a universal internal positive control for RT-PCR assays to exclude loss of RNA during extraction, or failure of amplification due to inhibitory substances present in the sample.

Hofner, M. C., W. C. Carpenter, et al. (1993). "Detection of foot-and-mouth disease virus RNA in clinical samples and cell culture isolates by amplification of the capsid coding region." Journal of Virological Methods **42**(1): 53.

http://www.sciencedirect.com/science/article/B6T96-47YGSVX-7/2/fb924bcb48df04e1e516d9f231b84406

Foot-and-mouth disease is one of the most economically important virus diseases of livestock. Two important requirements for the control of this disease are rapid laboratory diagnosis and epidemiological investigation. The use of the polymerase chain reaction method (PCR) to amplify specific nucleic acid regions offers the unique possibility of combining swift viral detection with the production of genetic material suitable for sequencing and other methods of molecular epidemiological analysis. The sequencing of the region of foot-and-mouth disease virus (FMDV) genome encoding the capsid proteins of the virus (~ 2260 bps), provides valuable information that adds to the molecular characterization of an isolate. This paper describes the use of the PCR for the amplification of this region of the FMDV genome from bovine clinical samples and cell culture isolates. Suitable pairs of oligonucleotide primers were selected from the published sequence of FMDV type O1, Kaufbeuren. One primer set amplified 2091 bps of the capsid coding region of all seven serotypes of FMDV. The other primer set amplified 216 bp from this region of FMDV type O1, BFS 1860, in nucleic acid extracts from several clinical samples. Nucleic acid extracts from the picornaviruses, bovine enterovirus and swine vesicular disease virus, which affect the same animals, were not amplified. Direct sequencing was carried out on the amplified fragments and showed that the PCR products were>98% homologous to published FMDV sequences.

Houng, H.-S. H., R. Chung-Ming Chen, et al. (2001). "Development of a fluorogenic RT-PCR system for quantitative identification of dengue virus serotypes 1-4 using conserved and serotype-specific 3' noncoding sequences." Journal of Virological Methods **95**(1-2): 19.

http://www.sciencedirect.com/science/article/B6T96-433NRB5-3/2/fe9854a050a05acce7c4cbad7e48f6a1

A fluorogenic reverse transcriptase-polymerase chain reaction (RT-PCR) system was developed for use as a rapid diagnostic test for determining dengue viremia. The dengue virus 3'-noncoding sequence was utilized to formulate serotype-specific RT-PCR assays for quantitative

identification of the four different dengue virus serotypes. A generic RT primer set containing two dengue specific anti-sense primers (DV-L1 and DV-L2) could be used to transcribe extracted viral RNA of all four dengue virus types to complimentary DNA (cDNA). The resultant dengue viral cDNA could be quantitatively identified at the serotype level by the 5'-3' exonuclease assay using four serotype-specific sense primers. The fluorogenic dengue type-specific RT-PCR can detect each of the four dengue types at similar low detection limits, i.e. 20-50 plaque forming units per milliliter of serum. Two panels with four dengue reference serotypes and 134 clinical samples were used to validate detection sensitivity and specificity of the dengue serotype RT-PCR assay, using virus isolation in cell culture as the criterion standard. By analyzing sera samples from Puerto Rico that were collected from 1999 through 2000, the assay demonstrated high level detection sensitivity and specificity of 92.8 and 92.4%, respectively, for all four dengue virus serotypes.

Houng, H.-S. H., D. Hritz, et al. (2000). "Quantitative detection of dengue 2 virus using fluorogenic RT-PCR based on 3'-noncoding sequence." Journal of Virological Methods **86**(1): 1.

http://www.sciencedirect.com/science/article/B6T96-3YS34HX-1/2/0a5f4885616059f11995aef5f257f642

A fluorescent DNA probe (DV2.P1) specific to the conserved distal 3'-noncoding region (nucleotides 10653-10678) of dengue 2 virus and a pair of flanking primers (DV2.L1 and DV2.U2) were designed to formulate a dengue 2-specific fluorogenic polymerase chain reaction (PCR). In addition, DV2.L1 was also used as a reverse transcription (RT) primer to generate superior cDNA from dengue viral RNA. Optimal assay conditions with zero background were established to detect low levels of dengue 2 virus from clinical specimens. The range of dengue virus detection in spiked human sera was determined to be from 10 to106 infectious virions per milliliter (plaque forming units determined using Vero cell line). Dengue 2 virus isolates from different geographic regions can be detected universally and identified by the fluorogenic RT-PCR assay. Moreover, the assay is specific for dengue 2 virus and does not recognize other related flaviviruses, including dengue serotypes 1, 3 and 4, Japanese encephalitis, St. Louis encephalitis, yellow fever, and Kunjin viruses. The assay also detected efficiently immunocomplexed dengue viruses. In practice, the fluorogenic RT-PCR assay detected readily viremia in sera collected from individuals ill with dengue fever. The rise and fall of dengue 2 virus concentrations in rhesus monkeys, reflecting viral proliferation and clearance, was also clearly illustrated by the assay.

Houng, H.-S. H., D. Norwood, et al. (2004). "Development and evaluation of an efficient 3'-noncoding region based SARS coronavirus (SARS-CoV) RT-PCR assay for detection of SARS-CoV infections." Journal of Virological Methods **120**(1): 33.

http://www.sciencedirect.com/science/article/B6T96-4CJVJR6-1/2/905fb4fa8e6218acd28373a8e9e90b64

The severe acute respiratory syndrome (SARS) epidemic originating from China in 2002 was caused by a previously uncharacterized coronavirus that could be identified by specific RT-PCR amplification. Efforts to control future SARS outbreaks depend on the accurate and early identification of SARS-CoV infected patients. A real-time fluorogenic RT-PCR assay based on the 3'-noncoding region (3'-NCR) of SARS-CoV genome was developed as a quantitative SARS diagnostic tool. The ideal amplification efficiency of a sensitive SARS-CoV RT-PCR assay should yield an E value (PCR product concentration increase per amplification cycle) equal to 2.0. It was demonstrated that the 3'-NCR SARS-CoV based RT-PCR reactions could be formulated to reach excellent E values of 1.81, or 91% amplification efficacy. The SARS-CoV cDNA preparations derived from viral RNA extract and the cloned recombinant plasmid both exhibit the identical

amplification characteristics, i.e. amplification efficacy using the same PCR formulation developed in this study. The viral genomic copy (or genomic equivalences, GE) per infectious unit (GE/pfu) of SARS-CoV used in this study was also established to be approximate 1200-1600:1. The assay's detection sensitivity could reach 0.005 pfu or 6-8 GE per assay. It was preliminarily demonstrated that the assay could efficiently detect SARS-CoV from clinical specimens of SARS probable and suspected patients identified in Taiwan. The 3'-NCR based SARS-CoV assay demonstrated 100% diagnostic specificity testing samples of patients with acute respiratory disease from a non-SARS epidemic region.

Hoyland, J. A., J. A. Dixon, et al. (2003). "A comparison of in situ hybridisation, reverse transcriptase-polymerase chain reaction (RT-PCR) and in situ-RT-PCR for the detection of canine distemper virus RNA in Paget's disease." Journal of Virological Methods **109**(2): 253.

http://www.sciencedirect.com/science/article/B6T96-488G8YB-2/2/8749aa4932edd03dc0163ff7236499ce

Previous evidence implicating Paramyxoviruses in the aetiopathology of Paget's disease of bone has proved controversial. Whilst several groups have demonstrated Paramyxoviruses using techniques such as in situ hybridisation (ISH), reverse transcriptase-polymerase chain reaction (RT-PCR), and in situ-RT-PCR (IS-RT-PCR), others have found no evidence of viruses using only RT-PCR. To investigate this latter finding, we have now compared detection of canine distemper virus by ISH, RT-PCR (three different methods) and IS-RT-PCR, in 10 patients with Paget's disease, and samples of non-diseased bone from four patients. Canine distemper virus was detectable in six of the samples by ISH, but only in five of the samples by RT-PCR, using one of the methods. Neither of the other RT-PCR methods detected canine distemper virus. IS-RT-PCR demonstrated canine distemper virus in all 10 samples. There was no evidence of virus in the control samples. We have shown that the ability to detect canine distemper virus in bone is dependent on the technique used. IS-RT-PCR clearly showed that canine distemper virus was present in 100% of Pagetic samples, whereas canine distemper virus was only found in 60% by ISH and in 50% using one particular RT-PCR method. These results provide conclusive evidence that canine distemper virus is present within Pagetic bone, and provide a possible explanation for the failure of some groups to detect Paramyxovirus sequences. These findings also have wider implications for other studies investigating viral expression.

Ijzerman, M. M., D. R. Dahling, et al. (1997). "A method to remove environmental inhibitors prior to the detection of waterborne enteric viruses by reverse transcription-polymerase chain reaction." Journal of Virological Methods **63**(1-2): 145.

http://www.sciencedirect.com/science/article/B6T96-3RHM7T6-19/2/d082502d074d3579f7dfa4541d72c8a4

A method was developed to remove environmental inhibitors from sample concentrates prior to detection of human enteric viruses using the reverse transcription-polymerase chain reaction (RT-PCR). Environmental inhibitors, concentrated along with viruses during water sample processing, are removed by the method through a series of steps that includes dialysis, solvent extraction, ultrafiltration and glass purification. The method was tested by spiking sodium phosphate with poliovirus type 1 with or without humic or fulvic acids and then measuring virus recovery by plaque assay and RT-PCR. Results of the study indicated that (i) 90% of the spiked virus could be recovered from samples at the end of the ultrafiltration step, (ii) virus was detected in the final eluate of samples containing as much as 0.5 mg of humic acid or 5.0 mg of fulvic acid, and (iii) as little as 0.06 plaque forming units (PFU) was detectable per RT-PCR reaction. These results indicate that the described purification method along with RT-PCR is a feasible approach for

detecting waterborne human enteric viruses in the presence of interfering substances.

Inoshima, Y., A. Morooka, et al. (2000). "Detection and diagnosis of parapoxvirus by the polymerase chain reaction." Journal of Virological Methods **84**(2): 201.

http://www.sciencedirect.com/science/article/B6T96-3YDG9HS-C/2/f99b5e663728bccc0f6b91033a27867b

The genus Parapoxvirus includes four members, bovine papular stomatitis virus (BPSV), pseudocowpox virus (PCPV), orf virus (ORFV) and parapoxvirus of red deer in New Zealand (PVNZ). A set of primers for polymerase chain reaction (PCR) was designed to detect viral DNA from cells infected with each of the four parapoxviruses. The set of primers resulted in the amplification of appropriately sized products from cells infected with BPSV, PCPV, ORFV and PVNZ, respectively. The PCR method was applied for the detection of seven field isolates of parapoxvirus from cattle, sheep and free-ranging wild Japanese serows. The expected size of DNA was amplified from cells infected with each of the seven isolates. No specific PCR products were detected from vaccinia virus-, fowlpox virus- and mock-infected cells. Moreover, by a semi-nested PCR with an inner primer and Southern blot analysis, viral DNA was detected from lesions of clinically affected cattle, sheep and Japanese serows. These results suggested that the PCR method used in this study was specific for the detection of parapoxviruses and thus useful for diagnosis of parapoxvirus infections, especially in discrimination from diseases with similar clinical symptoms.

Ito, T., H. Ieki, et al. (2002). "Simultaneous detection of six citrus viroids and Apple stem grooving virus from citrus plants by multiplex reverse transcription polymerase chain reaction." Journal of Virological Methods **106**(2): 235.

http://www.sciencedirect.com/science/article/B6T96-46WSX8S-1/2/58a09d09703fa70372a5ba9d5e86230c

We developed a multiplex reverse transcription polymerase chain reaction (RT-PCR) to detect six citrus viroids: Citrus exocortis viroid (CEVd), Citrus bent leaf viroid (CBLVd), Hop stunt viroid (HSVd), Citrus viroid III (CVd-III), Citrus viroid IV (CVd-IV) and Citrus viroid OS (CVd-OS) and Apple stem grooving virus (ASGV, synonym: Citrus tatter leaf virus (CTLV)) from citrus plants. The multiplex RT-PCR was also designed to distinguish CVd-I-LSS (a distinct variant of CBLVd) from CBLVd. By the multiplex RT-PCR, one to eight fragments specific to the pathogens were simultaneously amplified from one sample and identified by their specific molecular sizes in 6% polyacrylamide gel electrophoresis. The results of the multiplex RT-PCR were consistent with those of other diagnoses, such as uniplex RT-PCR, to detect each of the pathogens. The multiplex RT-PCR provides a simple and rapid method for detecting various viroids and ASGV in citrus plants, which will help diagnose many citrus plants at a time.

Jacquot, E., M. Tribodet, et al. (2005). "A single nucleotide polymorphism-based technique for specific characterization of YO and YN isolates of Potato virus Y (PVY)." Journal of Virological Methods **125**(1): 83.

http://www.sciencedirect.com/science/article/B6T96-4FFH1XG-1/2/9cbcec844d2200fffb8c12c19f0afd84

One of the most important properties used to classify Potato virus Y (PVY) isolates is their ability to induce (PVYN) or not (PVYO) veinal necrosis symptoms on the indicator host plant Nicotiana tabacum cv. Xanthi. As an alternative to biological assays, several serological and molecular detection tools have been developed for PVY detection and characterization and these have evolved as our knowledge of PVY has improved. However, the assays that have been previously published are all based on the use of neutral markers (antigenic determinants, sequence data, recombination sites or restriction enzyme cleavage sites), which are unlinked to the biological property being characterized (e.g. veinal necrosis). Using the recently identified molecular determinants of the tobacco leaf necrosis symptom induced by PVYN isolates, a one-step fluorescent [TaqMan[trademark]] RT-PCR assay, based on a single nucleotide polymorphism (SNP) linked to the necrosis property of PVY isolates, has been designed. This assay reliably detects and distinguishes PVYN and PVYO isolates. The method is simple (leaf soak extraction process, gel-free, no post-PCR manipulations), rapid (96 tests in less than 3 h from plants sampling to diagnostic results), sensitive (threshold in a range of 104-105 PVY copies), reliable (correctly assigns 42 PVY isolates in their respective group) and allows co-detection of mixed samples containing close to equivalent PVYN and PVYO quantities. All these characteristics suggest that the newly developed SNP assay could be used to reliably classify PVY isolates, as a substitute for biological assays performed on N. tabacum cv. Xanthi.

Jordens, J. Z., S. Lanham, et al. (2000). "Amplification with molecular beacon primers and reverse line blotting for the detection and typing of human papillomaviruses." Journal of Virological Methods **89**(1-2): 29.

http://www.sciencedirect.com/science/article/B6T96-429RNYH-4/2/4deb02669f9ef56632dd6a8d9523d8e1

A novel method for the detection and typing of human papillomavirus (HPV) was developed using molecular beacon primers. The method is based on the use of HPV-specific primers containing a hairpin loop structure in which fluorescent donor and quencher groups are held in close proximity such that fluorescence is quenched. Amplification of the target sequence results in the opening of the loop and the resulting fluorescence can be detected on a sequence detector system (SDS) 7700 (Applied Biosystems), as used for TaqMan(TM) assays. Fluorescent amplicons were identified on the SDS 7700 and then typed by a single hybridisation with specific probes immobilised in lines on a nylon membrane and detected on a fluorescent scanner. This novel beacon primer method compared well with conventional PCR for cervical scrape specimens. The combination of the beacon primer method and reverse line blotting should enable large-scale population studies of HPV infection.

Kanematsu, S., T. Hibi, et al. (1991). "Comparison of nonradioactive cDNA probes for detection of potato spindle tuber viroid by dot-blot hybridization assay." Journal of Virological Methods **35**(2): 189.

http://www.sciencedirect.com/science/article/B6T96-476RM29-11/2/2ec189aaa939c16854589fee60f6800d

Six nonradioactive cDNA probes were compared for their sensitivities for detecting potato spindle tuber viroid (PSTVd) by dot-blot hybridization assay. Three biotinylated PSTVd cDNA probes, labeled by photoactivation with photobiotin, by nick translation or by random priming with biotinylated deoxyribonucleotides, were all capable of detecting 20 pg of purified PSTVd by a colorimetric assay and 2-20 pg by a chemiluminescent assay. Digoxigeninlabeled probe was able to detect 200 pg of purified PSTVd. Two biotinylated probes prepared with polymerase chain reaction (PCR) incorporating biotinylated dUTP or dATP were the most sensitive: 0.2-2 pg of PSTVd was detectable by both assays. All six probes could detect PSTVd also in extracts of

infected tomato leaves at a dilution of up to 1/250-1/1250. These nonradioactive probes are equal to radioactive probes in their sensitivity, and the biotinylated probes produced with PCR amplification are particularly suitable for practical diagnosis, as they are sensitive and rapidly prepared in large quantities.

Kato, H., E. Orito, et al. (2001). "Determination of hepatitis B virus genotype G by polymerase chain reaction with hemi-nested primers." Journal of Virological Methods **98**(2): 153.

http://www.sciencedirect.com/science/article/B6T96-441N4XX-7/2/832c9c9efcf60538867ea86e644c8315

Hepatitis B virus (HBV) has been classified into six genotypes designated A-F by sequence divergence in the entire genome exceeding 8%. Very recently, the seventh genotype was reported and named genotype G. HBV genotype G is distinct from genomes of the other six genotypes in that it possesses an insertion of 36 nucleotides in the core gene, and has been found so far in France and the United States. A method for determining HBV genotype G was developed by polymerase chain reaction (PCR) with primers deduced from the 36-nucleotide (nt) insertion in five isolates of HBV genotype G the sequences of which have been deposited in DNA databases. The validity of this method, for specifically detecting HBV genotype G, was verified on a panel consisting of 142 HBV isolates of six major genotypes and four of genotype G. A total of 540 sera containing HBV in Japan covering symptom free carriers and patients with a spectrum of chronic liver disease were tested by this method, but not a single HBV genotype G sample was found. A possible method for serological determination of hepatitis B surface antigen of genotype G is suggested, without amplification or sequencing nucleotides, which would expand epidemiological and clinical researches on HBV genotype G.

Kato, H., E. Orito, et al. (2003). "Frequent coinfection with hepatitis B virus strains of distinct genotypes detected by hybridization with type-specific probes immobilized on a solid-phase support." Journal of Virological Methods **110**(1): 29.

http://www.sciencedirect.com/science/article/B6T96-48B0RKS-1/2/ea1b60574eee9bfdd94e8a79cb120c39

A genotype-specific probes assay (GSPA) was developed for distinguishing the seven genotypes (A-G) of hepatitis B virus (HBV). Nucleotide (nt) sequences corresponding to preS1 region were amplified by PCR with a primer labeled with biotin, and delivered to eight wells on which complementary sequences specific to one or other genotype had been immobilized. Thereafter, hybridization of HBV DNA sequences amplified from the test serum was detected by colorimetry. When 256 sera from HBV carriers in Bangladesh, Cameroon, Japan, South Africa, USA and Uzbekistan were subjected to GSPA, genotypes were concordant with those of ELISA with monoclonal antibodies to epitopes on preS2-region products in 242 (94.6%) of them; 8 sera (3.1%) were not genotypeable by either method. Cloning analysis confirmed the presence of two distinct HBV genotypes in the seven selected sera with coinfection. There were 7 (2.7%) sera with discordant genotyping results between GSPA and ELISA. When HBV DNA clones propagated from these sera were sequenced and analyzed phylogenetically, the genotypes determined by GSPA were verified. Coinfection with HBV strains of two distinct genotypes was identified by GSPA in 28 (10.9%) sera, while it was suggested by ELISA in only 2 (0.8%) sera. The GSPA method would be particularly useful for detecting the coinfection with distinct HBV genotypes of any clinical relevance, which seems to be more frequent than reported previously.

Kato, S., Y. Hiraishi, et al. (1998). "A plaque hybridization assay for quantifying and cloning infectious human immunodeficiency virus type 1 virions." Journal of Virological Methods **72**(1): 1.

http://www.sciencedirect.com/science/article/B6T96-3SXDXRR-1/2/f51fff8e04c825e5117efdc82ec50011

A biological method was developed for quantifying and cloning of infectious virions of human immunodeficiency virus type 1 (HIV-1). Virus preparations were mixed with permissive cells for binding, and the cells were cast in an agarose gel. After incubation for 9 days viral particles released from infected cells propagating from each initially infected cell were transferred on nylon membrane and subjected to hybridization using a radioactive HIV-1 DNA probe. Infectious centers of HIV-1 were detected as hybridization spots on autoradiographs regardless of cytopathic effects or syncytium formation. Three different CD4+ cell lines (MT-4, MOLT-4 and U937) and peripheral blood mononuclear cells from healthy donors were used as recipient cells. Infectious virions were recovered from a portion of agarose gel corresponding to each hybridization spot. This assay is suitable for quantifying infectious HIV-1 virions with different cell tropisms and for investigating the relationship between the phenotype and genotype of HIV-1 at a clonal level.

Kay, P., K. Meehan, et al. (2002). "The use of nested polymerase chain reaction and restriction fragment length polymorphism for the detection and typing of mucosal human papillomaviruses in samples containing low copy numbers of viral DNA." Journal of Virological Methods **105**(1): 159.

http://www.sciencedirect.com/science/article/B6T96-460DN3D-2/2/8d3a222d9b4ec8b3c8d97c518fd09b80

Mucosal human papillomaviruses (HPVs) that infect the genital area have also been shown to infect the oral cavity. In this study a restriction fragment length polymorphism (RFLP) method was developed on a nested polymerase chain reaction (PCR) product to identify ten high risk HPV types 16, 18, 31, 33, 35, 45, 51, 52, 58 and 59 as well as the low risk HPV 11. HPV DNA was detected in 23/31 (74%) of buccal specimens using a sensitive nested PCR employing degenerate consensus primers (Williamson and Rybicki, 1991). Consensus PCR using the PGMy09/11 primers. was able to detect HPV in only 29% of the specimens that had tested positive using the nested HPV PCR primers. HPV 11 type specific primers detected HPV 11 DNA in only 66% of the specimens showing HPV 11 DNA by means of nested PCR and RFLP. A Genbank search revealed that the PCR primers could detect a wide range of mucosal HPV types including types HPV 70, 72 and 73 which have all been isolated from immunocompromised patients. Of the 23 buccal specimens that were positive for HPV DNA, 13 were single infections, five were dual infections and three were triple infections. The HPV types identified by RFLP were: HPV 11 (18/23), HPV 18 (8/23), HPV 16 (3/23), and HPV 33 (1/23). HPV 13 (2/23) was identified by direct sequencing of the inner amplicon of the PCR product.

Kaye, S., C. Loveday, et al. (1991). "Storage and preservation of whole blood samples for use in detection of human immunodeficiency virus type-1 by the polymerase chain reaction." Journal of Virological Methods **35**(2): 217.

http://www.sciencedirect.com/science/article/B6T96-476RM29-14/2/ca3b2230e241dea9b5bde9cab36a6b2b

Methods used in the diagnosis of human immunodeficiency virus type-1 (HIV-1) infection by the polymerase chain reaction (PCR) usually require the separation of lymphocytes from a whole-

blood sample within 24 hours of patient sampling. A method is described in which blood samples are mixed with a cryopreservative ('Glycigel'), stored frozen, and DNA suitable for use in an HIV PCR recovered. Samples can be stored at --20[deg]C for up to 3 months and still give positive results with all samples from infected patients; storage at --80[deg]C for at least 3 months shows no loss of titre. The method shows no loss of sensitivity compared to previously described sample preparation methods. Deglycerolised Glycigel supernatants were found to be suitable for conventional anti-HIV-1 serological studies and loss of sensitivity only represented the dilution effect due to sample preparation. Application of the method as a means of storing samples frozen at the point of sampling and transporting them to a central laboratory for processing is demonstrated using samples taken from HIV-1-infected mothers and their babies.

Khare, M., M. Sharland, et al. (2004). "Use of serial maternal urine cytomegalovirus PCR to detect primary CMV infection in seronegative pregnant women." Journal of Virological Methods **119**(1): 31.

http://www.sciencedirect.com/science/article/B6T96-4C4X49B-1/2/481324f9b43b650ff596845f3a38616a

The aim of the study was to determine if serial maternal urine polymerase chain reaction (PCR) tests can detect primary CMV infection during pregnancy. This was a prospective study conducted from 1 January 1999 to 31 December 1999 in an antenatal clinic setting of a teaching hospital. The study group included women who were CMV IgG negative and aged At first attendance, 1549 (42%) women were CMV IgG negative. Of the 696 eligible women, 609 (88%) participated in the urine PCR study. PCR was performed on 2263 urine samples (median of 4/pregnancy). Primary CMV infection was identified in one woman by urine PCR at 36 weeks (baby CMV negative). Cord blood samples were available from 152/609 infants (25%). Seroconversion was noted in only one woman. Replies to the questionnaire were received from 264/609 women (43%): 214 (81%) had little or no anxiety, and 220 (83%) felt reassured by their study participation. Serial urine PCR is a feasible method of detecting primary maternal CMV infection during pregnancy which has potential for evaluation in further studies.

Kim, Y., S. M. Gharaibeh, et al. (2002). "Comparison and verification of quantitative competitive reverse transcription polymerase chain reaction (QC-RT-PCR) and real time RT-PCR for avian leukosis virus subgroup J." Journal of Virological Methods **102**(1-2): 1.

http://www.sciencedirect.com/science/article/B6T96-456T3KB-1/2/6e9c9b6a0052a9435c1a9243adcf070c

Avian leukosis virus subgroup J (ALV-J) infections cause significant economic losses because of increased mortality, tumor production, decreased production, and cost for eradication. Current quantification methods for ALV-J expressed by TCID50 are difficult to determine because of the lack of cytopathic effect in cell cultures and non-specificity of currently available antigen-capture ELISA tests. In this study, a one-tube fluorescent probe based real time RT-PCR method was developed for quantification of ALV-J and compared with available quantification methods. Cell lysates with different TCID50s determined by cell culture and antigen capture ELISA (ag-ELISA) were used for one-tube real time RT-PCR using fluorogenic probe and quantitative competitive RT-PCR (QC-RT-PCR). The results of QC-RT-PCR and real time RT-PCR were highly correlated to the TCID50s determined by conventional culture methods. They were also very specific, sensitive, easy to perform, reproducible, and rapid compared with conventional methods. These RT-PCR based quantification methods of ALV-J viral RNA will be useful for virological and pathogenesis studies.

Klein, S. A., S. Karsten, et al. (2003). "Comparison of TaqMan(TM) real-time PCR and p24 Elisa for quantification of in vitro HIV-1 replication." Journal of Virological Methods **107**(2): 169.

http://www.sciencedirect.com/science/article/B6T96-47DTDTF-9/2/b94c148adc8c912c06fe39684545b9df

In this study, TaqMan(TM) PCR was used to assess viral replication of HIV-1 infected cells in vitro. This PCR technique was compared with p24 ELISA as a standard method to monitor HIV-1 replication in cell culture. Hut78 T-lymphoblastoid cells were infected with different titres of HIV-1IIIb (MOI 0.05-0.0005). The course of HIV-1 replication was monitored by determination of p24 concentrations by ELISA in cell culture supernatants and by quantitation of HIV-1 gag RNA by TaqMan(TM) RT-PCR. Additionally, the number of HIV-1 proviral copies was assessed by TaqMan(TM) PCR. Monitoring of HIV-1 replication by p24 ELISA and TaqMan(TM) RT-PCR revealed comparable kinetics of infection. Both methods provided similar data on the exponential increase and on plateauing of HIV-1 replication. Furthermore, both methods were equally sensitive. However, a 7 log linearity of TaqMan(TM) HIV-1 gag PCR was demonstrated without dilution of the specimen, in contrast to p24 ELISA, where because of its narrow range of detectable p24 concentrations, sample dilution was necessary. Although determination of the number of proviral copies by TaqMan(TM) PCR does not measure HIV-1 replication, the kinetics of proviral copy number following in vitro inoculation of cells with HIV-1 was nearly the same as the kinetics of HIV-1 RNA copy numbers. In conclusion, TaqMan(TM) real-time RT-PCR was demonstrated as a reliable and sensitive tool to quantify and monitor HIV-1 replication in cell culture. It is suggested, therefore, that this technique be an alternative method to monitor HIV-1 replication in vitro.

Koch, N., N. Yahi, et al. (1999). "Genetic polymorphism near HIV-1 reverse transcriptase resistance-associated codons is a major obstacle for the line probe assay as an alternative method to sequence analysis." Journal of Virological Methods **80**(1): 25.

http://www.sciencedirect.com/science/article/B6T96-3WM5CT3-4/2/92f54634868a5eb138d5389ecd3ae41d

The performance of the line probe assay (LIPA) for the detection of mutations conferring resistance to nucleoside inhibitors of HIV-1 reverse transcriptase was evaluated in comparison with sequence analysis. The tests were undertaken on plasma samples from 63 patients (61 receiving combination therapy and 2 without treatment at the time of inclusion). In 27 cases (43%) which included codons 41, 69, 70, 74, 184 and 215, the sequence of the RT gene was distinct from the hybridization probes used in LIPA. Correspondingly, LIPA gave uninterpretable results in 15, 30 and 41% of cases for codons 184, 215 and 41, respectively. Overall, the concordance between LIPA and sequence analysis varied from 52% (codons 41 and 215) to 85% (codon 70). These data show that the polymorphism of the nucleotide sequence near resistance-associated codons is a major shortcoming of LIPA.

Koch, W. C. (1995). "A synthetic parvovirus B19 capsid protein can replace viral antigen in antibody-capture enzyme immunoassays." Journal of Virological Methods **55**(1): 67.

http://www.sciencedirect.com/science/article/B6T96-3YYTF0W-16/2/706a803b814c4e78c220b3b597852ab7

To establish a renewable source of parvovirus B19 antigens for diagnostic tests, gene sequences for the viral capsid proteins, VP1 and VP2, were cloned into baculovirus expression vectors and the recombinant viruses used to infect Sf9 insect cells. Cell lysates examined by immunoblotting demonstrated reactive proteins corresponding to the expected sizes of native VP1 (83 kDa) and VP2 (58 kDa). The VP2 protein was produced efficiently in quantity and self-assembled into empty capsids as shown by density equilibration in a CsCl step gradient. The VP2 protein was purified and used as an antigen in antibody-capture enzyme immunoassays for the detection of B19 IgG and IgM antibodies. Compared to a standard antibody-capture EIA based on whole viral antigen, the VP2-EIA gave a sensitivity of 100% and specificity of 97% in detection of B19 IgM in 138 patients suspected of B19 infection. No IgM-positive specimens were missed. IgG detection yielded a sensitivity of 100% and specificity of 96% in the same population. Recombinant VP2 capsid proteins expressed in baculovirus-infected insect cells can substitute for serum-derived B19 virus in standard antibody-capture EIA for the detection of B19 IgG and IgM with comparable results.

Koskiniemi, M., L. Mannonen, et al. (1997). "Luminometric microplate hybridization for detection of varicella-zoster virus PCR product from cerebrospinal fluid." Journal of Virological Methods **63**(1-2): 71.

http://www.sciencedirect.com/science/article/B6T96-3RHM7T6-11/2/27d2c8daff233d32eaee2eab70a91d62

We modified and optimized a new microplate hybridization assay to detect the varciella-zoster virus (VZV) PCR product, and studied cerebrospinal fluid (CSF) samples of 287 patients with meningitis, encephalitis or other neurological diseases or symptoms. Specific antibodies to VZV and reference antigens were determined by enzyme immunoassay from serum and CSF, they were then compared with clinical findings and with the results obtained by VZV-PCR using different detection methods for VZV-specific amplified DNA. VZV DNA was found in the CSF of 25 patients using the microplate hybridization assay and chemiluminescence detection for amplified DNA. All 25 CSF samples were also positive in Southern blotting. Among the patients, 10 had chickenpox, 4 had shingles, and 11 had no rash at all. The detection rate of VZV-specific DNA by microplate hybridization was 30% higher than that obtained by conventional agarose gel electrophoresis. In most patients the diagnosis was confirmed by demonstrating specific intrathecal antibody production to VZV but not to other viruses. These results indicate the presence of VZV in the central nervous system (CNS) in many patients with chickenpox or shingles, and even in patients without a rash. The microplate hybridization assay based on chemiluminescence detection improves considerably the detection rate of the VZV-PCR product compared to agarose gel electrophoresis and will add to the list of recognized VZV infections in the CNS. It is especially useful in cases where there is no cutaneous manifestation.

Kumar, R., N. Vandegraaff, et al. (2002). "Evaluation of PCR-based methods for the quantitation of integrated HIV-1 DNA." Journal of Virological Methods **105**(2): 233.

http://www.sciencedirect.com/science/article/B6T96-4619PYS-2/2/50ec536b8842a95e00e4b09d1a2a1017

Integration of HIV-1 DNA is essential both for productive viral replication and for viral persistence in patients. Methods to measure specifically proviral HIV DNA are required for investigating the mechanisms of HIV integration, for screening novel integrase inhibitors in cell culture and for monitoring levels of persistent integrated viral DNA in patients. In this report, the linker primer polymerase chain reaction (LP-PCR) and Alu-PCR methods for the quantitation of integrated HIV-1 DNA have been modified and evaluated. Each of the two modified assays allowed the

quantitative detection of 4 copies of integrated HIV DNA in presence of 2 x 105 cell-equivalents of human chromosomal DNA. The results show that proper DNA isolation procedures and the inclusion of appropriate controls in these assays are important for the accurate quantitation of integrated HIV DNA. With further improvements, it should be possible to use these methods as diagnostic tools to monitor closely the efficacy of antiretroviral therapy.

Kuno, G. (1998). "Universal diagnostic RT-PCR protocol for arboviruses." Journal of Virological Methods **72**(1): 27.

http://www.sciencedirect.com/science/article/B6T96-3SXDXRR-4/2/86a293289d7bd9826c94221c46474c83

A selected number of PCR protocols were evaluated to determine if they could serve as a universal protocol for detecting and identifying all arboviruses. In this study, four parameters that affect the efficacy of RT-PCR (RNA extraction method, choice of reverse transcriptase, choice of DNA polymerase and thermocycling program) were evaluated in combination. The most optimal combination of those parameters employed use of silica gel membrane spin column, RAV-2 reverse transcriptase, Tth DNA polymerase, and a simple modification of a published thermocycling program. By this modified protocol, viral RNA could be amplified satisfactorily with more than 50 pairs of primers designed for diagnosis of arboviruses representing five families. The sensitivity and specificity obtained by this universal protocol were comparable to those obtained by the original protocol for each primer pair tested; and for some primers, improved sensitivity was observed. It was also found that a simple modification of a suggested protocol of a commercial RT-PCR kit could produce nearly identical results and serve as another universal protocol. With the use of a universal diagnostic reverse transcriptase-polymerase chain reaction (RT-PCR) protocol, simultaneous screening of clinical or biological specimens against a large number of RNA viruses belonging to many families can be performed more efficiently for etiologic determination in the situations complicated by the difficulty of differential diagnosis. Furthermore, such a universal protocol facilitates reducing the cost of PCR-based diagnostic operation and standardizing the qualities of PCR-based diagnosis within an institution or among collaborating institutions. A logical strategy is to conduct diagnosis in two stages by using broadly group-reactive primers in the first stage to narrow the range of possible etiologic agents and using virus-specific primers in the second stage for identification. Before such a strategy is employed, however, more group-reactive primers for a large number of arboviruses, for which no such primers currently exist, must be made available. Furthermore, the best pair or pairs of primers need to be selected for each virus for the second stage of the strategy.

Laassri, M., V. Chizhikov, et al. (2003). "Detection and discrimination of orthopoxviruses using microarrays of immobilized oligonucleotides." Journal of Virological Methods **112**(1-2): 67.

http://www.sciencedirect.com/science/article/B6T96-496G002-1/2/007ea0d0150c0536163d989adc6b4e14

Variola virus (VARV), causing smallpox, is a potential biological weapon. Methods to detect VARV rapidly and to differentiate it from other viruses causing similar clinical syndromes are needed urgently. We have developed a new microarray-based method that detects simultaneously and discriminates four orthopoxvirus (OPV) species pathogenic for humans (variola, monkeypox, cowpox, and vaccinia viruses) and distinguishes them from chickenpox virus (varicella-zoster virus or VZV). The OPV gene C23L/B29R, encoding the CC-chemokine binding protein, was sequenced for 41 strains of seven species of orthopox viruses obtained from different geographical regions. Those C23L/B29R sequences and the ORF 62 sequences from 13 strains of VZV (selected from GenBank) were used to design oligonucleotide probes that were

immobilized on an aldehyde-coated glass surface (a total of 57 probes). The microchip contained several unique 13-21 bases long oligonucleotide probes specific to each virus species to ensure redundancy and robustness of the assay. A region approximately 1100 bases long was amplified from samples of viral DNA and fluorescently labeled with Cy5-modified dNTPs, and single-stranded DNA was prepared by strand separation. Hybridization was carried out under plastic coverslips, resulting in a fluorescent pattern that was quantified using a confocal laser scanner. 49 known and blinded samples of OPV DNA, representing different OPV species, and two VZV strains were tested. The oligonucleotide microarray hybridization technique identified reliably and correctly all samples. This new procedure takes only 3 h, and it can be used for parallel testing of multiple samples.

Leary, T. P., J. C. Erker, et al. (1999). "Optimized PCR assay for the detection of TT virus." Journal of Virological Methods **82**(2): 109.

http://www.sciencedirect.com/science/article/B6T96-3XT0BN2-1/2/218fc9f1a094d1bcbb614bf4c889a922

A polymerase chain reaction (PCR)-based procedure for the detection of TT virus DNA is described. In this method, total nucleic acid extracted from a small volume of serum or plasma is utilized as a template in PCR employing TT virus specific primers designed to highly conserved regions of the virus genome. Additional sensitivity is obtained by carrying out a second round of amplification. Reactions are analyzed by agarose gel electrophoresis, and samples having an ethidium bromide stainable fragment of the appropriate size in the first and/or second amplification are designated as positive. This protocol allows for the rapid and sensitive detection of TT virus in human plasma or serum.

Leary, T. P., J. C. Erker, et al. (2002). "Detection of reovirus by reverse transcription-polymerase chain reaction using primers corresponding to conserved regions of the viral L1 genome segment." Journal of Virological Methods **104**(2): 161.

http://www.sciencedirect.com/science/article/B6T96-45NPJB7-2/2/6c1eba241a205166b4ff2f88b41d233b

A rapid, reverse transcription-polymerase chain reaction (RT-PCR) procedure for the detection of reovirus RNA in cell culture is described. Total nucleic acids are extracted from a small volume of cell culture supernatant and reverse transcribed using random hexamers. An aliquot of cDNA is then utilized in nested PCR. The PCR primers correspond to sequences conserved between prototype reovirus strains type 1 Lang, type 2 Jones, and type 3 Dearing, as well as those of several reovirus field-isolate strains. Reactions are analyzed by agarose gel electrophoresis, and samples showing a band of the appropriate size in the first and second amplification, or in the second amplification alone, are designated as positive. This protocol allows for the rapid and sensitive detection of reovirus in cell culture. The RT-PCR methods described below can easily be adapted to the amplification of reovirus from other media, including preserved tissues, clinical specimens, and water.

Lednicky, J. A. and J. S. Butel (1997). "A coupled PCR and restriction digest method for the detection and analysis of the SV40 regulatory region in infected-cell lysates and clinical samples." Journal of Virological Methods **64**(1): 1.

http://www.sciencedirect.com/science/article/B6T96-3RHM7T6-1T/2/5e1cc0d23751272b6e2e2c9f33c73422

The polymerase chain reaction (PCR) is an increasingly popular analytical tool for the detection of virus sequences in laboratory preparations as well as in human clinical samples. In studies involving papovaviruses SV40, BK virus (BKV), and JC virus (JCV), one of the primary targets for analysis is the viral regulatory region, as that section of the papovavirus genome is distinct. A primary concern with PCR-based studies is whether amplified DNA sequences may be derived from laboratory contaminants. Recognizing that common sources of PCR contamination are the positive control templates, we devised a facile method to distinguish between real and false-positive PCR-amplified SV40 regulatory region DNAs. SV40 constructs that had been engineered to contain different combinations of 72-basepair (bp) enhancer elements and 21-bp repeats, as well as two introduced unique restriction enzyme sites, were used as positive control templates for PCR amplification. Cleavage of PCR-amplified DNA identifies products from the engineered control plasmids. The procedure is rapid, simple and cost-effective. We also report that primer sets predicted to be specific for the SV40 regulatory region can be used to amplify BKV and JCV regulatory region sequences under conditions of reduced stringency.

Lee, K., Y.-G. Kim, et al. (2003). "Shuttle PCR-based cloning of the infectious adeno-associated virus type 5 genome." Journal of Virological Methods **111**(2): 75.

http://www.sciencedirect.com/science/article/B6T96-4909KV7-1/2/d0e897df2d49beb9c542361f3efdddae

Adeno-associated virus type 5 (AAV5), which is distinct from the other serotypes of AAV, has attracted considerable interest as a premier gene delivery vector. As do the other serotypes, AAV5 contains its 4.7 kb-sized, single-stranded genome flanked with inverted terminal repeats (ITRs) in a hairpin conformation, which serves frequently as pause and arrest sites for DNA polymerases during PCR. To amplify the full-length of the AAV5 genome in single step, we established a shuttled, long and accurate PCR (LA-PCR) procedure in the present study. Furthermore, helper oligonucleotides, which hybridize with the palindromic sequence elements in ITR, were designed and employed in PCR to prevent the formation of hairpin structures by highly GC-rich ITRs. Consequently, a 4.7 kb-sized PCR product was amplified successfully, and cloned into a pBluescript(R) II KS(+) plasmid. Six plasmids, harboring the full-length AAV5 genome, rescued wild type AAV5 viruses on transfection to HeLa and HEK 293 cells, which were co-infected with helper adenoviruses. Western and Southern blot analyses supported further the fact that the pAAV5 plasmids harbored the full-length AAV5 genome. The PCR method described in this study is applicable for the cloning of genomes containing variable palindromic structures, in addition to AAV genomes of other serotypes.

Legeay, O., S. Bounaix, et al. (1997). "Development of a RT-PCR test coupled with a microplate colorimetric assay for the detection of a swine Arterivirus (PRRSV) in boar semen." Journal of Virological Methods **68**(1): 65.

http://www.sciencedirect.com/science/article/B6T96-3RYCMYM-X/2/6615b6b3f64d2a785fcc20c09bffdfc5

Transmission of porcine reproductive and respiratory syndrome virus (PRRSV) through boar semen has been demonstrated, stressing the need for a reliable semen PRRSV detection test. A diagnostic assay was developed based on amplification of the PRRSV RNA by reverse transcription and polymerase chain reaction (RT-PCR) followed by detection of the amplification

products by hybridization and colorimetric assay in microwell plates. A highly reproducible and efficient method of viral RNA isolation from semen samples was set up. A combined RT-PCR procedure was performed, incorporating the use of uracil-N-glycosylase (UNG) in combination with dUTP instead of dTTP to prevent false positive results due to carry-over contamination. An RNA internal control was added during the RNA isolation procedure to detect false negative results. The colorimetric detection in microwell plates of amplification products from either PRRSV or IC RNA. gave specific and objective results and was automated. A cut-off value of 1000 RNA copies or 10 TCID50 of PRRSV per ml of semen samples could be detected with this assay. Semen samples collected from experimentally-infected boars were tested with this assay and showed PRRSV excretion early after infection and for an extended period.

Levy, L., I.-M. Lee, et al. (1994). "Simple and rapid preparation of infected plant tissue extracts for PCR amplification of virus, viroid, and MLO nucleic acids." Journal of Virological Methods **49**(3): 295.

http://www.sciencedirect.com/science/article/B6T96-476TY1K-5/2/7de6b4c05bc1b67103801eec2e37183f

A rapid, simple method for preparing plant tissues infected with viruses, viroids, or MLOs using a commercial product known as Gene ReleaserTM is described. The Gene ReleaserTM polymeric matrix method produced plant extracts suitable for PCR amplification without the use of organic solvents, ethanol precipitation, or additional nucleic acid purification techniques. Modification of maceration methods and/or extraction buffers resulted in the PCR amplification of potato spindle tuber, apple scar skin, and dapple apple viroids, as well as, genomic segments of plum pox potyvirus, grapevine virus B, grapevine leafroll-associated virus III, and elm yellows MLO. These pathogens were amplified from tissue of woody and herbaceous hosts such as peach, apricot, apple, grapevine, elm, periwinkle and potato. The application of this product for use with intractable tissue avoids lengthy and laborious extraction procedures. In our hands, about 20 samples could be prepared for PCR or RT-PCR in 1-2 h versus 1-3 days.

Lewis, J. G., G.-J. Chang, et al. (1992). "Direct sequencing of large flavivirus PCR products for analysis of genome variation and molecular epidemiological investigations." Journal of Virological Methods **38**(1): 11.

http://www.sciencedirect.com/science/article/B6T96-476RMMH-49/2/1fa0ee383a7171f49574c6fceb90f585

The polymerase chain reaction (PCR) was used to amplify viral DNA from selected regions of dengue genomic RNA by using appropriate 'consensus' primers. DNA amplicons containing the structural genes from all 4 dengue serotypes were prepared and directly sequenced using dengue-virus-specific primers. This method can characterize reliably flavivirus field isolates at the molecular level without extensive virus propagation and molecular cloning, and will be a valuable tool for molecular epidemiological studies.

Lindberg, A. M. and A. Andersson (1999). "Purification of full-length enterovirus cDNA by solid phase hybridization capture facilitates amplification of complete genomes." Journal of Virological Methods **77**(2): 131.

http://www.sciencedirect.com/science/article/B6T96-3VR1B1H-3/2/d12b4eab7656f410aa9b47c4a5375c63

The aim of the study was to develop a method for the selective purification of full-length enterovirus single strand (ss) cDNA for subsequent amplification of complete enterovirus genomes by long distance PCR. As a model system we have used the prototype strain of echovirus 5 (EV5). Due to inefficient first strand cDNA synthesis using EV5 RNA as template, only a few molecules of EV5 sscDNA were completely reverse transcribed and no amplification products were observed when long distance polymerase chain reaction (LD-PCR) was used for amplification of complete EV5 genomes. To purify the complete EV5 cDNA present, an oligonucleotide, derived from the conserved 5' end of an enterovirus genome, was immobilized on paramagnetic beads and complete EV5 sscDNA was captured and purified from the less than full-length cDNAs. LD-PCR using the purified EV5 cDNA resulted in amplification of complete EV5 genomes. Transfection of the EV5 RNA transcribed from these uncloned amplicons resulted in production of replicating viruses. This demonstrates that solid phase hybridization capture of sscDNA is an efficient method that can be used for enrichment and purification of full-length enterovirus sscDNAs.

Lindstrom, A. and J. Albert (2003). "A simple and sensitive 'in-house' method for determining genotypic drug resistance in HIV-1." Journal of Virological Methods **107**(1): 45.

http://www.sciencedirect.com/science/article/B6T96-46YXPVK-1/2/ed6de7176e753ff6aab9650b50d5d8b6

Antiretroviral combination therapy is a major advance in the treatment of HIV infection, but development of antiretroviral resistance is still an important cause of treatment failure. Therefore, resistance testing was recommended recently for follow-up of HIV-1 infected individuals. The aim of this study was to develop a new genotypic resistance assay because simple and affordable assays with sufficient sensitivity for different genetic subtypes and low copy number samples are still lacking. Different methods and primers for RNA extraction from plasma, cDNA synthesis, nested PCR and sequencing on an ABI310 automated sequencer were evaluated and optimised. The PCR was designed to amplify a fragment covering the protease and the first half of the reverse transcriptase (RT), which harbour most known resistance mutations to licensed antiretroviral drugs. Resistance mutations were identified using resistance analysis tools available over the Internet. The optimised assay had a sensitivity of approximately 200 RNA copies per ml. The method was evaluated on plasma samples from treated patients infected with different subtypes of HIV-1 and appeared to have similar sensitivity for all subtypes. Samples that had failed previously in routine testing were analysed successfully with the new assay. The new assay is more sensitive and robust than the current routine method.

Liu, H.-J. and J. J. Giambrone (1997). "Amplification, cloning and sequencing of the [sigma]C-encoded gene of avian reovirus." Journal of Virological Methods **63**(1-2): 203.

http://www.sciencedirect.com/science/article/B6T96-3RHM7T6-1H/2/5790e7b61c10fbfb2ba8900d41488400

The [sigma]C-encoding cDNA of avian reovirus (ARV) 1733 strain was amplified, cloned and sequenced using double nested polymerase chain reaction (PCR). The ARV [sigma]C protein is a minor component of the outer capsid that induces type-specific neutralization antibodies. Four overlapping [sigma]C-encoding cDNA fragments were obtained. Together, the four fragments represented the whole coding sequence. The nucleotide and deduced amino acid sequences of [sigma]C-encoded gene of U.S. (S1133 and 1733) and Australian isolates (RAM-1 and SOM-4) were compared. The U.S. isolates were closely related, but different from Australian isolates. The degree of differences between the U.S. and Australian isolates was over 44.89% at both the nucleotide and deduced amino acid levels and suggested that the virus is evolving separately in

different continents. The deduced amino acid sequences of ARV [sigma]C indicated a heptapeptide repeat in the N-terrninal region of ARV [sigma]C existed in all ARVs. The results suggested that ARV [sigma]C is structurally related to mammalian reovirus (MRV) [sigma]1.

Liu, H.-J., J. J. Giambrone, et al. (1994). "Detection of genetic variations in serotype I isolates of infectious bursal disease virus using polymerase chain reaction and restriction endonuclease analysis." Journal of Virological Methods **48**(2-3): 281.

http://www.sciencedirect.com/science/article/B6T96-476F6FR-F0/2/8d9eb2293f30db9da00d0aadc2bf7aab

Reverse transcription with polymerase chain reaction (PCR) followed by restriction endonuclease analysis detected genetic variations among serotype I isolates of infectious bursal disease virus (IBDV). Using a set of synthetic primers derived from the large genome segment of APHIS-IBDV, the hypervariable region (AccI-SpeI fragment) located in the VP2 gene was amplified. With all strains, a cDNA fragment of approximately 643 bp was amplified, indicating that there were no apparent deletions or insertions in this region among isolates. Fragments amplified from 9 isolates were digested with 14 restriction enzymes. Restriction fragment profiles generated by restriction enzymes NaeI, StuI, TaqI, and SacI, showed genetic variations among isolates. This study provided a simple and sensitive method for detection of genetic variations among isolates that are closely related serologically and could not be differentiated using current serologie methods.

Liu, H. J., J. J. Giambrone, et al. (1997). "Molecular characterization of avian reoviruses using nested PCR and nucleotide sequence analysis." Journal of Virological Methods **65**(2): 159.

http://www.sciencedirect.com/science/article/B6T96-3RJG407-3/2/71d5c699c9ae427595bd1952d9d58c72

A nested polymerase chain reaction (PCR) with subsequent nucleotide sequence analysis identified and differentiated avian reoviruses (ARVs). PCR products amplified from the S1 gene segment of ARV of USA isolates were 738 and 342 bp, respectively. PCR products were conformed by Southern and dot blot hybridizations. The amplified cDNA fragments were cloned into the pUC18 vector and subjected to DNA sequencing. The nucleotide and deduced amino acid sequences of four USA (S1133, 1733, 2408, and CO8) and two Australian isolates (RAM-1 and SOM-4) were compared. Results of paired difference analysis and a predicted dendrogram revealed that USA isolates were closely related, but different from, Australian isolates. The deduced amino acid sequences of the N-terminal region of ARV [sigma]C showed a heptapeptide repeat of hydrophobic residues in all ARV isolates.

Lokensgard, J. R., D. G. Thawley, et al. (1991). "Enzymatic amplification of latent pseudorabies virus nucleic acid sequences." Journal of Virological Methods **34**(1): 45.

http://www.sciencedirect.com/science/article/B6T96-476RM08-6/2/71eb01468a54bc451de3ccf8eb88addf

To investigate various aspects of the latency of pseudorabies virus in swine (PRV, suid herpesvirus 1) we developed in vitro nucleic acid amplification methods based upon the polymerase chain reaction. Primers flanking a 156-bp region of the pseudorabies virus gp II gene

were annealed to purified PRV DNA as well as DNA isolated from the trigeminal ganglia of swine latently infected with PRV and subjected to PCR amplification. Following amplification, 100 fg of PRV DNA was visualizable on stained gels and 1 fg (equivalent to 6 viral genome copies) was detectable when amplification was combined with blot hybridization. PRV-specific DNA sequences which remained undetectable by direct blot hybridization assays were amplified to levels visualizable on ethidium-bromide-stained gels in 5 of 5 experimental latently infected animals. In addition, oligonucleotide primers specific for a 223-bp region of the PRV immediate-early gene (IE 180) were capable of amplifying overlapping latency associated transcripts (LATs), via a cDNA intermediate, in 6 of 6 latently infected swine. These nucleic acid amplification methods should be applicable to the investigation of PRV latency, and gene expression during latency and reactivation, in which few cells harbor latent virus.

Lovatt, A., J. Black, et al. (1999). "High throughput detection of retrovirus-associated reverse transcriptase using an improved fluorescent product enhanced reverse transcriptase assay and its comparison to conventional detection methods." Journal of Virological Methods **82**(2): 185.

http://www.sciencedirect.com/science/article/B6T96-3XT0BN2-B/2/296d10f4df7d1baea340b4627345daf3

The development and application of a novel, sensitive TaqMan fluorescent probe-based product enhanced RT test (F-PERT) for the detection of retrovirus are described. The assay allows discrimination between the amplification signals generated by genuine positive signals that result from retroviral RT activity and the RT-like activity from DNA polymerases. The RT-like activity from DNA polymerases was suppressed by the addition of activated calf-thymus DNA with no reduction in the RT activity. A linear relationship between threshold cycle (CT) and the number of virus particles was demonstrated, allowing quantification of retroviruses in unknown samples. The F-PERT assay was able to detect a wide range of retroviral RT activities, including that from porcine endogenous retrovirus (PoERV), murine leukaemia virus (MLV), simian foamy virus (SFV), simian immunodeficiency virus (SIVmac) and squirrel monkey retrovirus (SMRV). The detection limit of SMRV, MLV and PoERV was approximately 100 virion particles and the test was able to detect at least 102 molecules of purified RT enzyme. RT activity was not detected in cellular lysates and supernatants from MRC-5, BT, VERO, or Raji cells, whereas RT activity was detected in C127I, Mus dunni, K-Balb, BHK-21, CHO-K1, SP2/0-Ag14 and NSO cell supernatants. RT activity was also detected in the Spodoptera cell line Sf9.

M. Shamloul, A., N. A. Abdallah, et al. (2001). "Sensitive detection of the Egyptian species of sugarcane streak virus by PCR-probe capture hybridization (PCR-ELISA) and its complete nucleotide sequence." Journal of Virological Methods **92**(1): 45.

http://www.sciencedirect.com/science/article/B6T96-427JWSG-6/2/bb1896ce52bc9d2c82bef67844bcb837

A rapid and sensitive assay for the specific detection of Sugarcane streak virus (SSV) using PCR-probe capture hybridization (PCR-ELISA) was developed. Nucleic acids suitable for PCR were extracted from SSV-infected tissue using organic solvents or Fast DNA kit. SSV cDNA was amplified using viral specific primers and the amplified SSV cDNA (amplicon) was DIG-labelled during the amplification process. The amplicon was then detected in a colorimetric hybridization system by a microtiter plate using a biotinylated cDNA (22 nt), cDNA (789 nt) or cRNA (789 nt) capture probe. This system combines the specificity of molecular hybridization, the ease of the colorimetric protocol, and is 10-100 fold more sensitive than agarose gel electrophoretic analysis in detecting the amplified product. Long cDNA or cRNA capture probe was 2-7 fold more sensitive than the oligo cDNA probe for the detection. Complete nucleotide sequence of SSV

from Naga Hammady, Egypt, revealed that SSV-EG is a new species of SSV that shares 66% nucleotide identity with the virus species from Natal, South Africa.

Mackie, N., S. Dustan, et al. (2004). "Detection of HIV-1 antiretroviral resistance from patients with persistently low but detectable viraemia." Journal of Virological Methods **119**(2): 73.

http://www.sciencedirect.com/science/article/B6T96-4C76GN5-2/2/f048012db030a1f2adef5984b4777052

We modified the Abbott diagnostics HIV-1 Viroseq version 2 assay(TM) in order to detect the presence of HIV-1 drug resistance mutations in patients with viraemia below 1000 copies/ml of plasma.One hundred and forty-four patients with a detectable HIV-1 plasma viral load below 1000 copies/ml were selected and HIV-1 genetic analysis carried out using a modification of the Abbott Diagnostics Viroseq 2.0 assay(TM). The procedure differs from the standard protocol in that a nested PCR amplification step was introduced. The oligonucleotide primers for the first round of PCR were those supplied in the RT-PCR module of the kit. The nested PCR primers were primers A and H taken from the sequencing module. One hundred and twenty-eight out of 144 (89%) plasma samples with an HIV-1 viral load of less than 1000 copies/ml (ranging from 54 to 992 copies) were successfully sequenced. HIV-1 genotypes were obtained from 68 out of 81 (84%) samples with a viral load of greater than 50 but less than 300 copies/ml and 60/63 (95%) of samples with a viral load of greater than 300 but less than 1000 copies/ml. Serial dilution of a sample with a high viral load did not affect the detection of resistance mutations. Multiple sequencing of samples with low viral load did not result in detection of additional mutations, although, in one sample the K103N mutation was detected in 3/6 replicates while wild-type was detected in 2/6 and a mixture of wild-type/mutant in 1/6. Samples from patients infected with both clade B and non-B clades of HIV-1 could be genotyped at low copy number. Modification of the Abbott Viroseq assay allows reproducible sequencing of the HIV-1 genome from patients with low, but detectable, plasma virus burden.

Malet, I., M. Belnard, et al. (2003). "From RNA to quasispecies: a DNA polymerase with proofreading activity is highly recommended for accurate assessment of viral diversity." Journal of Virological Methods **109**(2): 161.

http://www.sciencedirect.com/science/article/B6T96-4891C2T-1/2/71eda00073c8b9f038aed98b0ec9c9a5

RNA viruses are characterized by their high rates of genetic variation. Their genetic diversity is generally studied by reverse transcription (RT) followed by polymerase chain reaction (PCR) amplification and nucleotide (nt) sequence determination. The misinterpretation of viral diversity due to copy errors introduced by the enzymes used in this two-step protocol has not yet been assessed systematically. In order to investigate the impact of such errors, we sought to bypass the intrinsic viral heterogeneity by starting from a homogeneous cDNA template. With this in mind, the hepatitis C virus (HCV) 5' non-coding region (5'NCR) was amplified either by PCR starting from a homopolymeric cDNA template or by RT-PCR starting from the in vitro RNA transcript derived from the same original cDNA template. Amplicons were cloned and the 17-20 individual clones were sequenced in each assay. Different quasispecies patterns were obtained with various commercially available DNA polymerases, resulting in different computed error rates. The non-proofreading Taq DNA polymerase provided the highest error rate which was seven times higher than that obtained with the most reliable of the proofreading polymerases tested. We, therefore, emphasize that the misleading interpretation of the observed heterogeneity for a given viral sample could be due to ignorance of the fidelity of the polymerase used for viral genome amplification, and thus that proofreading DNA polymerases should be preferred for the

investigation of natural genetic diversity of RNA viruses.

Malmstrom, C. M. and R. Shu (2004). "Multiplexed RT-PCR for streamlined detection and separation of barley and cereal yellow dwarf viruses." Journal of Virological Methods **120**(1): 69.

http://www.sciencedirect.com/science/article/B6T96-4CPM5P6-1/2/ae2085cad0e040878f54f4f6c0a38ab2

Two novel multiplexed RT-PCR assays that can efficiently detect and distinguish among different barley and cereal yellow dwarf viruses (B/CYDVs) are described. The basic multiplex can produce two fragments simultaneously, a ~830-bp fragment indicating the presence of the BYDV-PAV, BYDV-MAV, or BYDV-SGV viruses and a ~372-bp fragment indicating the presence of the CYDV-RPV, BYDV-RMV, or BYDV-GPV viruses. The enhanced multiplex produces two additional fragments, which further differentiate between BYDV-PAV, BYDV-MAV, and BYDV-SGV. These assays fulfill the critical need for a streamlined diagnostic procedure for B/CYDVs that can be cost-effectively applied to large numbers of small samples. The assays are useful not only in the basic diagnosis of B/CYDVs, but also for studies examining the ecological roles of B/CYDVs in natural systems and for longer-term epidemiological studies of grasses and cereals.

Markowski-Grimsrud, C. J., M. M. Miller, et al. (2002). "Development of strain-specific real-time PCR and RT-PCR assays for quantitation of chicken anemia virus." Journal of Virological Methods **101**(1-2): 135.

http://www.sciencedirect.com/science/article/B6T96-44M2HSJ-1/2/d513f8604d65fa9a9d84899d6a894076

Chicken anemia virus (CAV) is a ubiquitous pathogen of poultry. A CAV specific TaqMan(TM)-based PCR and RT-PCR assay for real-time quantitation of viral load and relative quantitation of virus-specific transcript levels was developed. Detection of viral DNA copy number from infected MDCC-CU147 cells was determined by extrapolation from a CAV plasmid-based standard curve. Viral load increased proportionally with increasing cell number harvested, increasing from 4 x 102 copies in 250 cells with 38% virus positive cells in an indirect immunofluorescence assay to 8 x 105 copies in 250,000 cells with 64% infected cells. The estimated average viral copy number per infected cell ranged from 5 to 14. Strain-specific primers were developed to distinguish between the Cux-1 and CIA-1 strains of CAV. These primers exhibited a 3 to 4 log differential in amplification comparing homologous versus heterologous virus-primer combinations. The sensitivity of the real-time assay was found to be comparable to a nested PCR assay using DNA samples from a SPF poultry flock exposed to the SH-1 strain of CAV. The real-time PCR detected from 1.7 to 4.2 target molecules in three out of four samples that were positive by nested PCR using 50% of the DNA used in the nested PCR. Relative viral transcript levels for Cux-1 and CIA-1 infected cell cultures increased proportionally with increasing cell numbers harvested for RNA extraction. This assay will be important for both diagnosis and in understanding the complex pathogenesis of CAV infection.

Marshall, R. L., J. Cockerill, et al. (1998). "Detection of GB virus C by the RT-PCR LCx(R) system." Journal of Virological Methods **73**(1): 99.

http://www.sciencedirect.com/science/article/B6T96-3T2PCND-F/2/a71c64db59aa0681dd9f0a9d2c65d436

The recent publication of representative genomic sequences of GBV-C has permitted the selection of PCR primers for detection of GBV-C in clinical samples by PCR techniques. Traditional amplification methodologies which couple reverse transcription polymerase chain reaction (RT-PCR) and Southern blot detection are slow, cumbersome, and can be technique dependent. This has hampered studies to determine the clinical significance of GBV-C. We report the selection of highly conserved PCR primers and a probe useful for semi-automated RT-PCR using the Abbott LCx(R) system. This adaptation of the LCx(R) system expands its capabilities to include the detection of RNA by RT-PCR, in addition to DNA detection by ligase chain reaction (LCR).

Maudru, T. and K. Peden (1997). "Elimination of background signals in a modified polymerase chain reaction-based reverse transcriptase assay." Journal of Virological Methods **66**(2): 247.

http://www.sciencedirect.com/science/article/B6T96-3RYCMYM-B/2/e6c2f0bca9b23a5dc1daf372cf6bedd1

Three highly sensitive reverse transcriptase (RT) assays were recently published that are at least one million times more sensitive than conventional RT assays. These assays derive their high sensitivities through the ability to amplify the complementary DNA (cDNA) product of the RT reaction by the polymerase chain reaction (PCR). We describe a modified PCR-basecl RT (PBRT) assay that retains the high sensitivities of the original assays while reducing their inherent background signals. The background signal of the PBRT assay was found to be due to an intrinsic RNA-dependent DNA polymerase activity of the Taq DNA polymerase, the enzyme used for the PCR. It could be eliminated by inserting a ribonuclease digestion step prior to amplifying the cDNA product of the RT reaction by PCR and by using a thermostable DNA polymerase identified as having reduced RNA-dependent DNA polymerase activity. Comparable results were obtained using three RNA templates with two purified RT enzymes. This modified assay is capable of detecting reliably between 10 and 100 molecules of RT, which is equivalent to between 1 and 10 retrovirus particles.

Maunula, L., H. Piiparinen, et al. (1999). "Confirmation of Norwalk-like virus amplicons after RT-PCR by microplate hybridization and direct sequencing." Journal of Virological Methods **83**(1-2): 125.

http://www.sciencedirect.com/science/article/B6T96-3XVPG88-G/2/8c72d648973bd6cfe8fc00cef880ed9b

A large number of Norwalk-like viruses (NLVs) have been identified from stool samples by RT-PCR by amplifying part of the polymerase-coding gene. A set of probes were selected based on sequence analysis of the viruses circulating in Finland during the years 1996-97 for confirmation of the findings by hybridization. A microplate hybridization test, which provides a rapid semi-automatic detection for PCR products, was designed and compared with agarose gel electrophoresis. From the material of 210 stool samples, mainly from diarrheal outbreaks during years 1997-1998, three probes, one for NLV genogroup GGI and one for each of the two GGII subgroups (Toronto-like and Lordsdale-like), were sufficient to detect 87.8% (36/41) of GGI and 89.0% (49/55) of GGII samples positive by gel electrophoresis. Amplicon sequencing of the strains not detected by the above probes revealed genetic variability in the sequences. Biotin-streptavidin binding was used both for microplate hybridization assays and for direct sequencing to identify the amplicons. Based on the sequences three more probes for the hybridization panel were added so that all the different NLVs of this study could be recognized.

McCann, S. H. E., J. A. Mumford, et al. (1995). "Development of PCR assays to detect genetic variation amongst equine herpesvirus-1 isolates as an aid to epidemiological investigation." Journal of Virological Methods **52**(1-2): 183.

http://www.sciencedirect.com/science/article/B6T96-3YXC151-14/2/3040affac2accaa52598832be6104e5f

A search for variable restriction sites has been carried out for equine herpesvirus-1 (EHV-1) in an attempt to develop markers which can be used to group epidemiologically related viruses into groups, and to learn more about the dynamics of EHV-1 disease. Crude viral DNA extracts of EHV-1, prepared by Hirt extraction, were digested with AluI, HaeIII, or RsaI, and Southern blotted following electrophoresis. DNA fingerprints, produced by probing the Southern blots with the EHV-1 EcoR1-I fragment, separated 56 isolates into 16 groups. The variable sites within the EcoR1-I fragment were mapped approximately using fragments from within EcoR1-I, and the precise location of the variable sites determined from the DNA sequence of this fragment. Oligonucleotide primers flanking the variable sites were synthesized, and used in PCR assays to detect variable fragments. The AluI variable fragment was found to result from the presence or absence of a single AluI site. In contrast, the variable bands seen with HaeIII and RsaI, resulted from variation in the copy number of two tandemly repeated sequences, one of which had not previously been recognized. In addition, HaeIII digests of EHV-1 isolates probed with the glycoprotein B (gB) gene of EHV-1 also separated isolates into two groups. The variable HaeIII site was mapped towards the 5'-end of the gB gene and a PCR assay established. The distribution of the variable AluI site within the EcoR1-I fragment and the HaeIII site within the gB gene were estimated on a large number of clinical isolates using PCR on unpurified viral tissue culture medium. Both sites had a good distribution and together with additional variable sites should provide the basis for the rapid DNA fingerprinting of EHV-1 isolates.

McElhinney, L. M., R. J. Cooper, et al. (1995). "Multiplex polymerase chain reaction for human herpesvirus-6, human cytomegalovirus, and human [beta]-globin DNA." Journal of Virological Methods **53**(2-3): 223.

http://www.sciencedirect.com/science/article/B6T96-3YYT72B-6/2/4f1f27b6af10e0e75dc01add9b99589d

Human cytomegalovirus and human herpesvirus-6 are closely related viruses which cause similar diseases, have similar cellular repositories of latent infection, and may be detected largely in the same types of clinical specimens. DNA amplification appears likely to play an increasing role in the diagnosis of recent and remote infection with these agents. A sensitive multiplex polymerase chain reaction was therefore developed for the two viruses and for human [beta]-globin DNA. Optimization of parameters such as the primers, primer concentrations, magnesium concentration, and buffer constituents was crucial in achieving a sensitive assay. Preliminary results indicated that the assay could simultaneously monitor DNA extraction from clinical specimens and allow detection of HCMV or HHV-6 in patients with diseases possibly caused by either pathogen.

Merel, P., I. Pellegrin, et al. (2001). "Comparison of capillary electrophoresis sequencing with the new CEQ 2000 DNA Analysis System to conventional gel based systems for HIV drug resistance analysis." Journal of Virological Methods **98**(1): 9.

http://www.sciencedirect.com/science/article/B6T96-43VYKRP-2/2/a74ac886f6d28b6e5d854150025656d1

To date the majority of sequencing technologies have been based on use of gel plates. In this study sequencing by capillary electrophoresis for HIV-1 genotyping on the CEQ 2000 sequencer (Beckman Coulter Inc.) has been investigated and compared to an 'in house' protocol on the Prism-377 sequencer (Applied Biosystems) and to the HIV-1 TruGene kit (Visible Genetics Inc.), two gel plate-based systems. Plasma from 20 HAART-treated patients with virological failure were analyzed for protease (PR) and reverse transcriptase (RT) genes. A total of 520 RT codons (26/patient) and 360 PR codons (18/patient) related to antiretroviral drug resistance were evaluated. The overall agreement between CEQ 2000 and Prism-377 results was 100% for the RT and PR primary and secondary mutations. The overall agreement between CEQ 2000 and TruGene was 100% for primary and>=97% for secondary mutations. Discrepant results would have never led to errors in genotype interpretation. Performances for a 24 patients/week/one technician genotyping throughput were analyzed. For Prism-377, TruGene and CEQ 2000, manual processing required 5, 4 and 2,5 days, sequence data analysis needed additional 3, 1 and 2 days and cost/patient was[ap]49, 214 and 39 $, respectively. The CEQ 2000 sequencer offers a reliable alternative for fast and cost effective HIV drug resistance analysis.

Merzouki, A., T. Mo, et al. (1994). "Accurate and differential quantitation of HIV-1 tat, rev and nef mRNAs by competitive PCR." Journal of Virological Methods **50**(1-3): 115.

http://www.sciencedirect.com/science/article/B6T96-47DKYF8-D/2/61a9db200bf9c6c8d8b4202c283ebd1c

An accurate method is described for measuring the relative abundance of HIV-1 regulatory mRNAs directly in clinical specimens. Specimen RNA is reverse transcribed and coamplified with a common competitor containing tat, rev and nef internal standards using fluorescent primers and a competitive polymerase chain reaction. After amplification, individual products are separated and analyzed on a fluorescent DNA sequencer. Using this approach, it was possible to measure reproducibly two-fold differences in the relative abundance of mRNAs with coding potential for tat, rev and nef from as little as 0.2 ng of total RNA extracted from peripheral blood mononuclear cells of HIV-1 infected persons. The ratio method eliminates the need to account for variability in RNA recovery during sample processing and provides a powerful tool for measuring the differential expression of HIV-1 regulatory genes in vivo.

Meyer, R. F., G. D. Babcock, et al. (1997). "Baculovirus expressed 2C of foot-and-mouth disease virus has the potential for differentiating convalescent from vaccinated animals." Journal of Virological Methods **65**(1): 33.

http://www.sciencedirect.com/science/article/B6T96-3XY8R56-5/2/1ebf5bcac2100792c58b962740a63853

Determining whether animals have been infected with foot-and-mouth disease virus or vaccinated is important because infected animals frequently become carriers of the virus, shed it intermittently and thus may be the source of new outbreaks of the disease. We had shown previously that the sera of convalescent animals contain antibodies to 2C, a highly conserved non-structural protein, whereas the sera of vaccinated animals do not. This is explained by the observation that 2C is retained on the membranes of cells used for growing the virus for vaccine production. In contrast, the non-structural protein 3D, which is released into the medium, is not removed by centrifugation or filtration during vaccine production and therefore stimulates an immune response in both vaccinated and convalescent cattle. In this study we produced 2C and 3D in insect cells infected with recombinant baculoviruses. As demonstrated by serology and electron microscopy, 2C is also retained on the membranes of the insect cells. Both expressed proteins react with sera of convalescent animals, indicating that they are conformationally similar,

but the 2C does not react with sera from vaccinated animals. The baculovirus expressed 2C appears to be a suitable antigen for the development of a reliable diagnostic test.

Michael Lindberg, A., C. Polacek, et al. (1997). "Amplification and cloning of complete enterovirus genomes by long distance PCR." Journal of Virological Methods **65**(2): 191.

http://www.sciencedirect.com/science/article/B6T96-3RJG407-6/2/fdeb3a40c6bc05cca66145b20fac4d7e

A method for amplification and cloning of complete enterovirus cDNA genomes is described. Viral RNA was reverse transcribed using an optimized protocol and a reverse transcriptase with reduced RNase H activity. Amplicons corresponding to complete genomes of 14 prototype strains of group B coxsackieviruses and echoviruses were amplified using oligonucleotide primers derived from the Coxsackievirus B3 genomic sequence of the 5' and 3' ends and a mixture of thermostable DNA polymerases. Coxsackievirus B2 amplicon was then cloned and the terminal sequences of the insert were determined. Lipofection of individual clones resulted in productive Coxsackievirus B2 infection. The method described makes it possible to obtain large amounts of complete enterovirus cDNAs and simplifies the construction of infectious full-length cDNA clones. Successful amplification of all enterovirus prototype strains tested emphasizes the general use of the method described, which provides a rapid and efficient alternative to traditional cloning strategies.

Moen, E. M., J. Sleboda, et al. (2002). "Real-time PCR methods for independent quantitation of TTV and TLMV." Journal of Virological Methods **104**(1): 59.

http://www.sciencedirect.com/science/article/B6T96-45CDM11-3/2/bf814f74647b9e35a57695929170d5be

There is considerable interest in the possible clinical effects of the human circoviruses TT virus (TTV) and TTV-like mini virus (TLMV). Most people appear to have at least one of these viruses replicating actively in their bodies, thus mere correlation of the presence of virus and disease states are probably less informative than a quantitative analysis of viraemia. Real-time PCR based methods, with either SYBR Green or TaqMan probe, designed to quantitate selectively TTV and TLMV are described. The suggested TaqMan-based protocols were suitable for quantitation of viruses in the range of 102-109 copies/ml of sample; and proved, by sequencing of PCR products, to be specific for each of the two viruses.

Molitor, T. W., K. Oraveerakul, et al. (1991). "Polymerase Chain Reaction (PCR) amplification for the detection of porcine parvovirus." Journal of Virological Methods **32**(2-3): 201.

http://www.sciencedirect.com/science/article/B6T96-476KXFR-J7/2/5edd0839b161e30756147ad9d8ea6e68

A polymerase chain reaction (PCR) amplification method was developed and evaluated to detect porcine parvovirus (PPV). A pair of 20-base primers and an oligonucleotide probe were derived from the DNA sequences common to two isolates of PPV, NADL-8 and NADL-2. The primers flanked 118-bp nucleotides within the region coding for the major structural protein VP2. After DNA amplification of PPV replicative form (RF), a 158-bp fragment was detected in agarose gels. This amplified fragment was shown to be specific for PPV DNA after Southern transfer and

hybridization to a 20-base internal probe. The amplified fragment also contained a single EcoRI cleavage site. Various conditions, such as number of cycles and annealing temperature, were examined to optimize the conditions for detecting viral DNAs from infected cell cultures and swine fetal tissues. Four different isolates of PPV, NADL-8, NADL-2, KBSH and Kresse, and two other viruses, canine parvovirus (CPV) and pseudorabies virus (PRV), were included to determine specificity of amplification. Slot blot hybridization with a radiolabeled probe was used to evaluate the sensitivity of PCR amplification. The optimized protocol was specific for PPV detecting equally all four strains of PPV, but failing to amplify CPV or PRV sequences. The PCR method could detect at least 100 fg of viral replicative form (RF) DNA or the equivalent of 1 PFU of infectious virus. The applications of this method include routine detection of PPV in clinical samples and as a contaminant in mammalian cell lines.

Naito, H. and K. Abe (2001). "Genotyping system of GBV-C/HGV type 1 to type 4 by the polymerase chain reaction using type-specific primers and geographical distribution of viral genotypes." Journal of Virological Methods **91**(1): 3.

http://www.sciencedirect.com/science/article/B6T96-426XYK0-J/2/e597903a5704609ffee9fb685bbe6a9b

Based on variation in nucleotide sequence of 5'-untranslated region, GB virus type C (GBV-C) and hepatitis G virus (HGV) can be classified into three major genotypes. In addition to this classification, a novel genotype of GBV-C/HGV was identified and designated type 4. However, genotyping of GBV-C/HGV has been hampered by the lack of suitable assays. In this study, a simple and precise genotyping system based on PCR using the type-specific primers was developed for the determination of genotypes 1, 2, 3, and 4 of GBV-C/HGV. A total of 235 serum samples obtained from 11 different countries were tested by our PCR genotyping system of GBV-C/HGV. The results revealed that type 1 was prevalent mainly in Ghana, type 2 was prevalent in the USA, Spain, Egypt, Nepal and Myanmar, type 3 was prevalent in Japan and Bolivia, and type 4 was prevalent in Vietnam and Myanmar among the countries investigated in the present study. To confirm the specificity of the results of genotyping by PCR, phylogenetic analysis in the 5'-untranslated region of GBV-C/HGV was undertaken in 99 serum samples. By this analysis, the specificity of the genotyping system by PCR was confirmed. This assay system may be useful for rapid typing of GBV-C/HGV RNA when either epidemiological or transmission studies of this agent are carried out.

Najioullah, F., D. Thouvenot, et al. (2001). "Development of a real-time PCR procedure including an internal control for the measurement of HCMV viral load." Journal of Virological Methods **92**(1): 55.

http://www.sciencedirect.com/science/article/B6T96-427JWSG-7/2/1e33f8b1e295ab442ae4f648e13142ed

Human cytomegalovirus (HCMV) infections are frequent in immuno-compromised patients. The recent development of real-time PCR procedures that allow the rapid quantification of genome load will be helpful for accurate monitoring of these infections. Two extraction procedures were evaluated using 30 blood samples that were processed pure and diluted (1/10). Repeatability and reproducibility of the quantitative PCR procedure using an internal control for amplification were analysed, and its sensitivity compared to a qualitative PCR procedure using 50 HCMV culture positive blood samples. The real-time PCR and qualitative PCR procedures were positive in 46 and 48 of the samples tested, respectively. Discrepancies were observed for samples with a low viral load. The sensitivity of the real-time PCR procedure was evaluated at 500 HCMV DNA copies per ml of sera. The use of an internal control concomitantly processed during the HCMV

quantification did not alter the sensitivity of the procedure, and was relevant for the detection of putative PCR inhibitors that may interfere with the amplification process. This procedure was used to measure genome load in two bone marrow transplant patients with HCMV disease, confirming that this new PCR procedure should be used widely for diagnosing and monitoring HCMV infections in transplant patients.

Navas-Castillo, J., J. A. Diaz, et al. (1998). "Improvement of the print-capture polymerase chain reaction procedure for efficient amplification of DNA virus genomes from plants and insect vectors." Journal of Virological Methods **75**(2): 195.

http://www.sciencedirect.com/science/article/B6T96-3V5F5S6-7/2/ed24dcf3583c7e7ab5354f937afafa05

A rapid and simple procedure is described to amplify efficiently geminivirus DNA genomes by improving the print-capture polymerase chain reaction (PCR) procedure reported recently for RNA viruses. This method, termed print-PCR (P-PCR), allows direct amplification of DNA from infected plant or whitefly tissues printed directly on Whatman 3MM paper, without the need of any grinding, incubation, or washing steps previous to the amplification reaction. P-PCR reduces sample manipulation and avoids previous extraction of nucleic acids, thereby diminishing the possibilities of cross-contamination between samples. P-PCR has been successfully applied to whiteflies and various plant species infected by two different tomato yellow leaf curl viruses, TYLCV-Sr and TYLCV-Is, and for the amplification of the full-length genome of TYLCV-Is from infected plants.

Nemchinov, L. and A. Hadidi (1998). "Specific oligonucleotide primers for the direct detection of plum pox potyvirus-cherry subgroup." Journal of Virological Methods **70**(2): 231.

http://www.sciencedirect.com/science/article/B6T96-3SBVX4H-F/2/99c020d48a0c46e673cc680cfae9648c

A specific polymerase chain reaction assay was developed for direct identification of the distinct subgroup of plum pox potyvirus (PPV) isolates from cherry trees (PPV-cherry, PPV-C) and its differentiation from other known subgroups of PPV. The specificity of the assay is based on using a pair of primers whose nucleotide sequences are located on the coat protein gene of PPV-sour cherry (SoC) at regions of high nucleotide divergence between PPV-SoC and other isolates of PPV. The technique will be useful for studying the epidemiology of PPV-C as well as for practical testing in certification and quarantine programs worldwide.

Nie, X. and R. P. Singh (2002). "A new approach for the simultaneous differentiation of biological and geographical strains of Potato virus Y by uniplex and multiplex RT-PCR." Journal of Virological Methods **104**(1): 41.

http://www.sciencedirect.com/science/article/B6T96-45CDM11-1/2/8361a181eef499c15344058b0eeb8ba3

Two strains of Potato virus Y (PVY), the common (PVYO) and the tobacco veinal necrosis (PVYN) have been known for decades. More recently, a tuber ringspot necrosis (PVYNTN), and several recombinants of PVYO and PVYN (designated here as PVYN:O) have been described. Further, the PVYN group of strains have been assigned to two geographical subgroups of

European (EU) PVYN/NTN and the North American (NA) PVYN/NTN. The evolution of new PVYN strains, has complicated the diagnosis, which requires a combination of bioassay, serological and molecular assays. To simplify the identification and differentiation of various PVYN strain groups, a competitive (single antisense and multiple sense primers) reverse transcription-polymerase chain reaction (RT-PCR) was used, making use of minor differences in the variable region part of the PVY genome. Specifically, primers based on small variations in nucleotide stretches of P1 gene permitted a broad range separation of PVYO and PVYN groups and the specific detection of strain subgroups. The primer pairs designed for identifying PVYO, EU-PVYN/NTN, NA-PVYN and NA-PVYNTN are described. Primer pairs can be used in a uniplex (single pair of primer) or multiplex (duplex, tetraplex or pentaplex) competitive RT-PCR, allowing simultaneous testing for any combination of PVYO, EU-PVYN/NTN, NA-PVYN and NA-PVYNTN.

Nie, X. and R. P. Singh (2001). "A novel usage of random primers for multiplex RT-PCR detection of virus and viroid in aphids, leaves, and tubers." Journal of Virological Methods **91**(1): 37.

http://www.sciencedirect.com/science/article/B6T96-426XYK0-P/2/e3a672edc650138234f031d660a2841f

A multiplex reverse transcription polymerase chain reaction (m-RT-PCR) was developed for the simultaneous detection of five potato viruses and a viroid. The synthesis of cDNAs used for amplification was primed by hexanucleotides (random primers, RP). An RNA extraction procedure employing DNase I, is routinely used to isolate potato viruses and viroid (Potato virus S, PVS; Potato leafroll virus, PLRV; Potato virus X, PVX; Potato virus A and Y, PVA, PVY; and Potato spindle tuber viroid, PSTVd) from infected tissues. This extraction method produced deoxy-oligonucleotides, which in turn were used to prime the reverse transcription of RNA templates of all the viruses and the viroid. A time-course study from 15 s to 30 min showed optimal oligonucleotide generation by DNase I occurred at 10 min, an incubation time already incorporated in the extraction protocol. The presence of oligonucleotides capable of priming cDNA synthesis was also demonstrated in RNA preparations from aphids, leaves, and tubers. In order to duplicate the priming of templates by oligonucleotides, commercially available hexanucleotides were used as RP. When fragments were amplified from 5'- and 3'-ends of the random primed cDNA of PVY genome, bands of similar intensity were observed. In contrast, when two fragments (short and long) from the P1 gene region of the PVA genome were amplified, the yield of the short fragment was significantly higher in intensity than that of the long fragment in random primed cDNA. Irrespective of the origin of the primers (generated during extraction vs. commercially purchased), single or multiple viruses or the viroid were detected by amplification of random primed cDNAs present individually in the reaction or in a cDNA pool consisting of five viruses and the viroid. The cDNA produced by RP or virus specific primers (SP) was used to detect PLRV and PVY from infected tubers in a duplex reverse transcription polymerase chain reaction (d-RT-PCR). The RP cDNA gave increased detection. Comparison of RP primed cDNAs from dormant or sprouted tubers and leaves showed that for some cultivars, such as 'Shepody', leaves were more reliable for PVY and PLRV detection than the tubers, in both the d- and m-RT-PCR.

Njouom, R., C. Pasquier, et al. (2003). "Assessment of HIV-1 subtyping for Cameroon strains using phylogenetic analysis of pol gene sequences." Journal of Virological Methods **110**(1): 1.

http://www.sciencedirect.com/science/article/B6T96-488G8YB-3/2/3a9299f22aaf60d094f4d445fdd214ca

Phylogenetic analysis of human immunodeficiency virus type 1 (HIV-1) pol gene is a useful method for subtyping European strains of HIV-1. The suitability of this method for genetically

diverse African strains was evaluated by comparing HIV-1 subtyping of Cameroon strains using a long fragment of the pol gene sequence to the findings obtained using env gene sequences. When the pol gene could not be amplified, the reverse transcriptase (RT) or the protease (PR) genes were used. Phylogenetic analysis of the env C2/V3 gene sequences of 60 HIV-1 isolates showed 52 to be subtype A, 2 subtype G, plus one each of subtypes C, F2 and H, with 3 subtypes not determined. A long fragment of the pol gene was amplified successfully and sequenced in 23% of cases. The RT region was amplified for 42% of the samples that could not be typed by analysing the long fragment, and the PR gene was amplified for 40% of them. Thus, 63% of samples were typable. Env and pol gene subtypings were in agreement in 86% of cases. It is concluded that the phylogenetic analysis of pol gene sequences is not a practical method for HIV-1 subtyping in areas of high subtype diversity, despite the good agreement between the env and pol gene subtypings. However, it can be a useful method for HIV-1 subtyping, provided that the gene is amplifiable.

Nuanualsuwan, S. and D. O. Cliver (2002). "Pretreatment to avoid positive RT-PCR results with inactivated viruses." Journal of Virological Methods **104**(2): 217.

http://www.sciencedirect.com/science/article/B6T96-45WGH3G-2/2/8fd0074a4e4ba9f4f3c80b21156d2545

Enteric viruses that are important causes of human disease must often be detected by reverse transcription-polymerase chain reaction (RT-PCR), a method that commonly yields positive results with samples that contain only inactivated virus. This study was intended to develop a pretreatment for samples, so that inactivated viruses would not be detected by the RT-PCR procedure. Model viruses were human hepatitis A virus, vaccine poliovirus 1 and feline calicivirus as a surrogate for the Norwalk-like viruses. Each virus was inactivated (from an initial titer of [ap]103 PFU/ml) by ultraviolet light, hypochlorite or heating at 72 [deg]C. Inactivated viruses, that were treated with proteinase K and ribonuclease for 30 min at 37 [deg]C before RT-PCR, gave a negative result, which is to say that no amplicon was detected after the reaction was completed. This antecedent to the RT-PCR method may be applicable to other types of viruses, to viruses inactivated in other ways and to other molecular methods of virus detection.

Nunan, L. M. and D. V. Lightner (1997). "Development of a non-radioactive gene probe by PCR for detection of white spot syndrome virus (WSSV)." Journal of Virological Methods **63**(1-2): 193.

http://www.sciencedirect.com/science/article/B6T96-3RHM7T6-1G/2/fcb9d76a3619ac6449cbbb965963b13b

Combining primers created from the sequence information of two baculo-like viruses of penaeid shrimp, Baculovirus penaei (BP) and Monodon baculovirus (MBV), produced a 750 bp band on a 0.8% agarose gel using White Spot Syndrome Virus (WSSV), from Penaeus monodon, as the DNA template. The PCR fragment was ligated to a plasmid vector, (pGEM-T) and transformed, creating a 3.7 Kbp clone. The DNA insert was sequenced, and the original primer pair was located. Using restriction enzymes, the insert was isolated, excised and non-radioactively labeled. This cloned labeled fragment was tested by in situ hybridization for specificity and reactivity with BP, MBV and WSSV-infected shrimp tissues. The major advantage of this novel method of gene probe development is that no DNA sequence information of the targeted infectious agent needed to be known or available. In addition, tedious viral isolation and purification was circumvented. In this study, knowledge of the possible viral strain was important in limiting the PCR primer pairs investigated. The use of arbitrary primers designed for PCR assays from two other possibly related shrimp viruses, increased the likelihood that a generated PCR product would be specific for WSSV.

Nystrom, K., M. Biller, et al. (2004). "Real time PCR for monitoring regulation of host gene expression in herpes simplex virus type 1-infected human diploid cells." Journal of Virological Methods **118**(2): 83.

http://www.sciencedirect.com/science/article/B6T96-4BVP8FY-1/2/ecdaff1c9d755a984c63b7f183a5585f

Herpes simplex virus type 1 (HSV-1) induces prominent shifts in the rates of transcription of host cellular genes of relevance for the outcome of the viral infection. The quantitative analysis of transcription may be obscured by virus-induced alterations in the levels of RNA encoded by cellular housekeeping genes that are used commonly for normalisation of real time reverse transcription PCR (RT-qPCR). In the present study, we analysed [beta]-actin, GAPDH and 18S rRNA for their usefulness in normalisation of RT-qPCR analysis of the transcription of the HSV-1 gamma gB-1 gene and FUT5, a cellular gene induced by viral infection. The transcription of these genes was monitored in a TaqMan-based real time RT-PCR system over a 24 h interval of virus infection of human embryonic lung fibroblasts. The levels of gB-1 and FUT5 RNA were normalised via difference in the threshold cycle ([Delta]Ct) values relative to each and one of the housekeeping genes or calculated in relation to the number of infected cells without any further normalisation. The levels of RNA encoded by [beta]-actin or GAPDH were found to vary by several orders of magnitude during HSV-1 infection, introducing large errors in the estimation of the gB-1 and FUT5 RNA levels. In contrast, the variation of Ct values for 18S rRNA was less than one cycle during 24 h period of HSV-1 infection. The FUT5 and gB-1 RNA figures obtained by [Delta]Ct normalisation relative 18S rRNA were identical to those calculated in relation to the number of infected cells. These data recommend 18S rRNA for normalisation in HSV-1-infected human cells but discourage the use of [beta]-actin and GAPDH RNA for this purpose. By applying these procedures, it was shown that the transcription of FUT5 was increased by 50-fold 5-24 h after HSV-1 infection and 200-fold by the inhibition of viral DNA replication in HSV-infected cells.

O'Shea, M. K. and P. A. Cane (2004). "Development of a highly sensitive semi-quantitative real-time PCR and molecular beacon probe assay for the detection of respiratory syncytial virus." Journal of Virological Methods **118**(2): 101.

http://www.sciencedirect.com/science/article/B6T96-4BY42V3-1/2/72cdebe02966a90458710dc3c2a18c4c

Molecular beacons are a novel class of oligonucleotide probe capable of reporting the accumulation of target amplicon during real-time PCR by the emission of a fluorescent signal. A novel assay for the detection and estimation of respiratory syncytial virus (RSV) nucleic acid in clinical specimens based on real-time PCR utilising such a probe was developed. The probe consisted of two short arm sequences and a central loop sequence complementary to a region of the N gene (the target amplicon). The probe was characterised and a semi-quantitative nested real-time PCR using a LightCycler instrument was optimised. Standard curves were generated using cycle threshold (Ct) values calculated from several assays over a range of logarithmic RSV titres. Linear coefficient correlations were close to one (r2=0.998) and the detection limit of the optimised assay was reproducibly shown to be 1 x 10-4 pfu/ml. The intra-assay coefficient of variation (CV) of Ct values of the optimal assay was 0.8% and the CV of quantification data was 6.6%. The interassay CV of Ct values was 2.0% and the quantification CV was 6.7%. The validity of the assay for the detection of RSV in clinical specimens was assessed by analysing ten specimens previously assayed in a different laboratory. Real-time PCR analysis was completely consistent with the results of prior analysis. The rapidity, sensitivity and specificity of the assay should greatly facilitate epidemiological studies, particularly in adults as existing methods perform better on clinical specimens from children.

Okamoto, H., S. Kobata, et al. (1996). "A second-generation method of genotyping hepatitis C virus by the polymerase chain reaction with sense and antisense primers deduced from the core gene." Journal of Virological Methods **57**(1): 31.

http://www.sciencedirect.com/science/article/B6T96-3VXJ8FF-4/2/4f83ebd4df7935cae0f18c24b6941bdb

A second-generation method of genotyping hepatitis C virus (HCV) was developed by the polymerase chain reaction (PCR) with sense as well as antisense primers deduced from the core gene. HCV RNA specimens extracted from sera were reverse-transcribed and amplified with universal primers in the first round of PCR to obtain fragments of 433 base pairs representing nucleotides 319-751. In the second round of PCR, portions of PCR products were amplified separately with sense and antisense primers specific for each of the five common genotypes prevailing across the world i.e., I/1a, II/1b, III/2a, IV/2b and V/3a. The specificity of the method was verified by a panel of 177 HCV isolates of various genotypes in the genetic groups 1-9. It allowed clear differentiation of genotype I/1a from II/1b which was not always accomplished by the previous method. When 501 sera from blood donors and hepatitis patients with HCV viremia from various countries were genotyped by the second-generation method, 478 (95.4%) were classified into the five genotypes. HCV RNA samples from 23 (4.6%) sera were not classifiable into any of the five common genotypes and, by sequence analysis, 22 were found to be of four genotypes in group 4 and one of genotype 1c in Simmond's classification.

Okuda, M. and K. Hanada (2001). "RT-PCR for detecting five distinct Tospovirus species using degenerate primers and dsRNA template." Journal of Virological Methods **96**(2): 149.

http://www.sciencedirect.com/science/article/B6T96-43DDGXG-5/2/9f7f4a76d586d0086922e829e8a244b5

RT-PCR procedures for detection of multiple species of tospovirus from plant tissues were investigated. Downstream primers were designated from the 3' untranslated sequences of the S RNA. An upstream primer was designated from the degenerated sequences of the nucleocapsid protein. Approximately 450 bp DNA fragments were detected when Tomato spotted wilt virus (TSWV)- or Impatiens necrotic spot virus (INSV)- infected tissues were examined. Approximately 350 bp DNA fragments were detected when Watermelon silver mottle virus (WSMoV)- or Melon yellow spot virus (MYSV)-infected tissues were examined. Template RNA was extracted using CF 11 cellulose powder, and nonspecific amplification became unnoticeable when double-stranded RNA was used. The amplified fragments of WSMoV were differentiated from those of MYSV by AluI or TaqI digestion. The amplified fragments of TSWV were differentiated from those of INSV by DraI or HindIII digestion. An alstroemeria plant that was infected with an unidentified tospovirus was also examined, and a 350 bp fragment that was 97% identical to Iris yellow spot virus was detected. This method, therefore, detected at least five distinct Tospovirus species.

Olvera, A., M. Sibila, et al. (2004). "Comparison of porcine circovirus type 2 load in serum quantified by a real time PCR in postweaning multisystemic wasting syndrome and porcine dermatitis and nephropathy syndrome naturally affected pigs." Journal of Virological Methods **117**(1): 75.

http://www.sciencedirect.com/science/article/B6T96-4BMK1TM-1/2/6aad5437203cfcf9072aff5468feebfa

Postweaning multisystemic wasting syndrome (PMWS) diagnosis is based on the presence of characteristic histopathological lymphoid lesions and porcine circovirus type 2 (PCV2) within these lesions. Previous studies indicate that PCV2 load is higher in PMWS affected than in PCV2 infected, healthy pigs. On the other hand, PCV2 has been suggested to play a role in porcine dermatitis and nephropathy syndrome (PDNS) pathogenesis. This study describes a new TaqMan(c) real time PCR assay and its use to quantify viral load in serum samples. Serum viral loads were related with different degrees of PMWS characteristic lesions and PDNS cases. DNA extracted from serum samples from 75 animals with mild, moderate and severe PMWS lesions and 12 animals with PDNS was used as template. PCV2 DNA was quantified in 69 of 75 PMWS cases and in 11 of 12 PDNS cases. Significant differences in PCV2 load were observed between animals with severe, moderate and mild PMWS lesions, although variability within each group was high, probably due to heterogeneity in disease progression. These results suggest that high viral load is a major feature of PMWS affected pigs. PDNS affected animals had lower PCV2 loads. No significant differences in viral load were found between animals suffering from PDNS and animals with mild PMWS characteristic lesions, which were unaffected clinically.

Oviedo, J. M., F. Rodriguez, et al. (1997). "High level expression of the major antigenic African swine fever virus proteins p54 and p30 in baculovirus and their potential use as diagnostic reagents." Journal of Virological Methods **64**(1): 27.

http://www.sciencedirect.com/science/article/B6T96-3RHM7T6-1X/2/7af5f3d9c9d003c6323ebe27c7e37be9

At present, the eradication of African swine fever (ASF) in affected countries is based only on an efficient diagnosis program because of the absence of an available vaccine. The highly antigenic ASF virus proteins p54 and p30, encoded by genes E183L and CP204L respectively, were expressed in baculovirus for diagnostic purposes. A sequence comparison analysis of these genes from different field virus strains which are geographically diverse and isolated in different years, revealed that both genes are completely conserved among the isolates. Partially purified baculovirus-expressed proteins were used in ELISA and Western blot for ASF antibody detection in sera from experimentally inoculated pigs and field sera from ASF innaparent carriers. These comparative analyses showed that p54 presents better reactivity than p30 in Western blot. However, recombinant p30 was more efficient for antibody detection by ELISA, improving the discrimination between positive and negative sera by this technique. These data suggest the convenience of using p30 as ELISA antigen, while p54 should be the selected antigen for ASF virus antibody detection by Western blot. The combined use of both antigens for serodiagnosis of ASF disease will improve the sensitivity of innaparent carriers detection, facilitating also the interpretation of the tests, and eliminating the use of ASF virus in antigen production.

Paragas, J. and J. A. Blaho (1998). "Cosolvents facilitate DNA synthesis in the herpes simplex virus 1 unique short (Us) inverted repeat." Journal of Virological Methods **73**(1): 53.

http://www.sciencedirect.com/science/article/B6T96-3T2PCND-6/2/250e8b0381b3b0894cc9aac7c4a6a214

DNA synthesis under standard conditions is not successful within a portion of the Us1 gene of HSV-1 which is juxtaposed to an 86% G+C-containing tract in the Us inverted repeat sequence. We report that the independent addition of specific amounts of at least three different types of cosolvents is capable of facilitating DNA synthesis within this G+C-rich region. In addition, this strategy was used to successfully place a specific site-directed mutation in the Us1 gene. Consideration of these observations should enable future site-specific mutational analyses of portions of the HSV-1 genome which have traditionally been unamenable to genetic

manipulations.

Parshionikar, S. U., J. Cashdollar, et al. (2004). "Development of homologous viral internal controls for use in RT-PCR assays of waterborne enteric viruses." Journal of Virological Methods **121**(1): 39.

http://www.sciencedirect.com/science/article/B6T96-4CVR4D3-1/2/9bd36ffdffc5ee6c642d648e20707746

Enteric viruses often contaminate water sources causing frequent outbreaks of gastroenteritis. Reverse transcription-polymerase chain reaction (RT-PCR) assays are commonly used for detection of human enteric viruses in environmental and drinking water samples. RT-PCR provides a means to rapidly detect low levels of these viruses, but it is sensitive to inhibitors that are present in water samples. Inhibitors of RT-PCR are concentrated along with viruses during sample processing. While procedures have been developed to remove inhibitors, none of them completely remove all inhibitors from all types of water matrices. This problem requires that adequate controls be used to distinguish true from potentially false-negative results. To address this problem, we have developed homologous viral internal controls for hepatitis A virus (HAV), poliovirus, Norwalk virus and rotavirus. These internal controls can be used in RT-PCR assays for the detection of the above viruses by competitive amplification, thereby allowing the detection of false negatives in processed water samples. The internal controls developed in this study were successfully tested with virus-seeded environmental water sample concentrates.

Pasquier, C., N. Millot, et al. (2001). "HIV-1 subtyping using phylogenetic analysis of pol gene sequences." Journal of Virological Methods **94**(1-2): 45.

http://www.sciencedirect.com/science/article/B6T96-42Y12XF-6/2/d80f24b7001e51dc6faacfbf8b23f572

HIV-1 pol gene sequencing is now used routinely in France to identify mutations associated with resistance to reverse transcriptase (RT) or protease (PR) inhibitors. These sequences may also provide other information, such as the HIV-1 subtype. HIV-1 subtyping was compared using the RT and PR gene sequences to heteroduplex mobility assay (HMA) of the envelope gene. The RT and PR genes of 51 samples that had been subtyped earlier by HMA were sequenced. Sequences were aligned and subtypes were determined by phylogenetic analysis with reference HIV sequences. HMA gave the following subtypes: A (20), B (19), C (1), D (3), F (1), G (3) and CRF01-AE (4). Phylogenetic analysis of the RT gene gave: A (5), B (19), C (2), D (3), F (1), G (6), J (2), CRF01_AE (4), CFR02_AG (7) and undetermined (2). PR gene analysis did not infer subtypes with sufficient confidence. HMA and RT subtyping was not in agreement in nine cases. RT subtyping can identify CFR02_AG and CRF01_AE variants from A subtype RT. It was shown that phylogenetic analysis of the RT gene could provide a useful method for HIV-1 subtyping. The length of the amplicon and the relative performance of each primer pair used in this study favoured RT sequences as a subtyping tool. One potential advantage over env subtyping HMA is the ability to identify some circulating recombinant forms (CRFs).

Petrik, J., G. J. M. Pearson, et al. (1997). "High throughput PCR detection of HCV based on semiautomated multisample RNA capture." Journal of Virological Methods **64**(2): 147.

http://www.sciencedirect.com/science/article/B6T96-3RJ9BMF-5/2/9cff2ed2ded22d32025f3dcb5ae1436b

An amplification procedure based on a semiautomated 60-sample RNA capture, including combined reverse transcription/polymerase chain reaction (RT-PCR) and nested PCR/Tagman amplicon detection, is described. It can be completed within a working day and is suitable for the development of a fully automated system. HCV RNA-specific capture is independent of the sequence variations as it targets the poly(U) tract commonly present at the 3'-end of the HCV genome (U-capture). The type specificity of the assay determined in a panel of 56 confirmed HCV antibody-positive samples (genotypes 1-6) was slightly better when compared to a commercial assay. The sensitivity evaluated on serial dilutions of representative samples was equal for genotypes 1, 2, 5, 6, or increased for genotypes 3 and 4 with the U-capture assay.

Poon, H., E. Jimenez, et al. (1993). "Detection of bovine leukemia virus RNA in serum using the polymerase chain reaction." Journal of Virological Methods **41**(1): 101.

http://www.sciencedirect.com/science/article/B6T96-476RMYJ-6K/2/5fef2abb18e0a655d107030563c44935

A method was developed for detecting bovine leukemia virus (BLV) RNA in serum samples using a pair of primers from the BLV polymerase gene in the polymerase chain reaction (PCR). The PCR was able to detect 3800-7600 molecules of BLV RNA. At this level of sensitivity eleven pools of adult and one fetal bovine serum appeared free from BLV contamination.

Pratelli, A., V. Martella, et al. (2001). "Genomic characterization of pestiviruses isolated from lambs and kids in southern Italy." Journal of Virological Methods **94**(1-2): 81.

http://www.sciencedirect.com/science/article/B6T96-42Y12XF-9/2/65624063a12db9b5ada050304765320d

A nested polymerase chain reaction was used to identify 13 pestivirus strains isolated from small ruminants in several mixed (sheep and goats) flocks of Southern Italy, and for classification as bovine viral diarrhoea virus (BVDV) type 1, BVDV type 2, and Border disease virus (BDV) genotypes. Of the nine ovine isolates, two were characterized as BVDV type 1, and seven as BVDV type 2. The four pestiviruses isolated from kids belong to BVDV type 1. None of the pestivirus strains tested could be classified as 'true' BDV (genotype 3). Although BVDV type 2 has been described in Europe rarely, the characterization of BD/90-1M strain as BVDV type 2, isolated in Italy in 1990, demonstrates that this genotype has been circulating in Italy since the 1990s.

Pratelli, A., V. Martella, et al. (2003). "Genetic diversity of a canine coronavirus detected in pups with diarrhoea in Italy." Journal of Virological Methods **110**(1): 9.

http://www.sciencedirect.com/science/article/B6T96-488G8YB-1/2/97c330073d15a9fb1cf3545d25dd2edf

The sequence of the S gene of a field canine coronavirus (CCoV), strain Elmo/02, revealed low nucleotide (61%) and amino acid (54%) identity to reference CCoV strains. The highest correlation (77% nt and 81.7% aa) was found with feline coronavirus type I. A PCR assay for the S gene of strain Elmo/02 detected analogous CCoVs of different geographic origin, all which exhibited at least 92-96% nucleotide identity to each other and to strain Elmo/02. The evident genetic divergence between the reference CCoV strains and the newly identified Elmo/02-like

CCoVs strongly suggests that a novel genotype of CCoV is widespread in the dog population.

Pratelli, A., V. Martella, et al. (2003). "Identification of coronaviruses in dogs that segregate separately from the canine coronavirus genotype." Journal of Virological Methods **107**(2): 213.

http://www.sciencedirect.com/science/article/B6T96-47DTDTF-N/2/66948c55e9362947f2496c0e4bc5eafd

The genetic diversity of 16 canine coronavirus (CCoV) samples is described. Samples were obtained from pups infected naturally living in different areas. Sequence data were obtained from the M gene and pol1a and pol1b regions. The phylogenetic relationships among these sequences and sequences published previously were determined. The canine samples segregated in two separate clusters. Samples of the first cluster were intermingled with reference strains of CCoV genotype and therefore could be assigned to this genotype. The second cluster segregated separately from CCoV and feline coronavirus genotypes and therefore these samples may represent genetic outliers. The reliability of the classification results was confirmed by repeating the phylogenetic analysis with nucleotide and amino acid sequences from multiple genomic regions.

Pratelli, A., M. Tempesta, et al. (1999). "Development of a nested PCR assay for the detection of canine coronavirus." Journal of Virological Methods **80**(1): 11.

http://www.sciencedirect.com/science/article/B6T96-3WM5CT3-2/2/d5d4acb70659bd6b1089e8b5c3c06679

A diagnostic test for canine coronavirus (CCV) infection based on a nested polymerase chain reaction (n-PCR) assay was developed and tested using the following coronavirus strains: CCV (USDA strain), CCV (45/93, field strain), feline infectious peritonitis virus (FIPV, field strain), trasmissible gastroenteritis virus (TGEV, Purdue strain), bovine coronavirus (BCV, 9WBL-77 strain), infectious bronchitis virus (IBV, M-41 strain) and fecal samples of dogs with CCV enteritis. A 230-bp segment of the gene encoding for transmembrane protein M of CCV is the target sequence of the primer. The test described in the present study was able to amplify both CCV and TGEV strains and also gave positive results on fecal samples from CCV infected dogs. n-PCR has a sensitivity as high as isolation on cell cultures, and can therefore be used for the diagnosis of CCV infection in dogs.

Pratelli, A., A. Tinelli, et al. (2002). "PCR assay for the detection and the identification of atypical canine coronavirus in dogs." Journal of Virological Methods **106**(2): 209.

http://www.sciencedirect.com/science/article/B6T96-46WSX8S-6/2/dbd1d38351561ef0cb23980d01cd8c23

Comparative sequence analysis of the PCR products of the M gene and fragments of the pol1a and pol1b genes of canine coronavirus (CCoV) have demonstrated that two separate clusters of CCoV are present in dogs. This note describes a PCR assay to identify atypical CCoV strains with nucleotide substitutions in the M gene. A total of 177 faecal samples from dogs CCoV positive previously with the PCR assay were analysed. Sixty-two of the 177 samples were amplified with the PCR described in the present study and were thus considered atypical CCoVs. The specificity of the PCR typing assay was confirmed by sequence analysis of the PCR

products.

Puig, M., S. Pina, et al. (2000). "Description of a DNA amplification procedure for the detection of bacteriophages of Bacteroides fragilis HSP40 in environmental samples." Journal of Virological Methods **89**(1-2): 159.

http://www.sciencedirect.com/science/article/B6T96-429RNYH-J/2/0a8d63b24e9477e01fb6c69bcd067de4

A molecular test based on DNA amplification by PCR was developed for the detection of bacteriophages of Bacteroides fragilis strain HSP40 in the environment. These specific phages are associated with faecal contamination of human origin. A homologous DNA region of 1.5 kb, identified previously by hybridisation, was used to design primers for the detection of B. fragilis HSP40 phages. A nested-PCR procedure for the DNA amplification of those phages was developed. The sensitivity of the nested-PCR was between 10-1 and 10-2 PFU for purified HSP40 phage solutions, sewage and seawater samples, and between 1 and 10 PFU for river water samples. Specific amplification of HSP40 phages was observed when viral suspensions of 103 PFU/ml or lower were used. Common levels of B. fragilis phages found in sewage are 101-102 PFU/ml. A total of 24 water samples (sewage, river water and seawater) were tested both by PCR and by plaque assay, to evaluate the efficiency of the molecular method in field samples. The data obtained by PCR in environmental samples showed good concordance with the PFU counts and a higher sensitivity.

Quan, C. M., M. Krajden, et al. (1993). "High-performance liquid chromatography to assess the effect of serum storage conditions on the detection of hepatitis C virus by the polymerase chain reaction." Journal of Virological Methods **43**(3): 299.

http://www.sciencedirect.com/science/article/B6T96-476RMR1-59/2/839f0ee3725ce81e60bbf457cd804527

The effect of various serum storage conditions on the detection of hepatitis C virus (HCV) by the polymerase chain reaction (PCR) was assessed. 50 [mu]l aliquots of serum from four HCV PCR positive patients were subjected, in triplicate, to: (a) storage at -20[deg]C for 1, 5, 10 days; (b) storage at room temperature (RT) for 1, 2, 7 days; (c) 1, 3, 5, and 10 successive freeze-thaw cycles; (d) incubation at 37[deg]C, and 56[deg]C for 1, 3, 24 h; and (e) storage in guanidium-thiocyanate extraction buffer at RT, and 4[deg]C for 1, 5, 10 days. PCR products were detected by agarose gel electrophoresis (AGE) and quantitatively by high-performance liquid chromatography (HPLC). Only storage in extraction buffer at RT for 5-10 days and incubation at 56[deg]C for 24 h appeared to result in a loss of [ges]50% of detectable HCV PCR product. Up to 10 successive freeze-thaw cycles, storage at -20[deg]C for up to 10 days or at RT for up to 7 days, storage in extraction buffer at RT for 1 day or at 4[deg]C for up to 10 days, and incubation at 37[deg]C for up to 24 h resulted in minimal PCR signal loss. HPLC was a reproducible method of detecting and quantitating HCV PCR products, and was more sensitive than AGE.

Ray, R., P. J. Cooper, et al. (1996). "Direct in situ reverse transcriptase polymerase chain reaction for detection of measles virus." Journal of Virological Methods **60**(1): 1.

http://www.sciencedirect.com/science/article/B6T96-3VYV029-1/2/5c11b5c2bcbc450ea425e8689d6c9641

New methods are described for combined intracellular reverse transcription (RT) and polymerase chain reaction (PCR) using single primer pairs, with direct incorporation of digoxigenin-11-dUTP into amplificants (direct in situ RT/PCR). Routinely used fixatives and minimal pre-treatments were employed. Target sequences of measles virus nucleocapsid (N) and phosphoprotein genes were detected within measles virus infected Vero cells, both in suspension and in formalin-fixed sections, that had been treated by in situ reverse transcription and 30 cycles of direct in situ PCR. Uninfected cells, omission of Taq polymerase, and irrelevant primers were used as controls. Distribution of measles virus within infected cells was determined by in situ hybridisation and immunocytochemistry for measles virus N gene and protein, respectively. Confirmation of amplification within sections was by gel electrophoresis, Southern blotting and sequencing of extracted amplicons. In the majority of cases, measles-infected cells exhibited intense cytoplasmic signal after direct in situ PCR; this was not seen in uninfected cells or infected cells reacted either with irrelevant primers or without Taq polymerase. Unfixed cells in suspension required nested reaction. Measles-specific in situ hybridisation and immunocytochemistry gave an identical signal distribution in sections. Nuclear artifact occurred in some sections and was unpredictable, although it was greatest either in areas of cellular damage, following DNase predigestion, or with vigorous protease pre-treatment. In situ RT-PCR is feasible for measles virus in acutely infected cells both in sections and in suspension. Further work is required to improve the procedure and to eliminate artefactual nuclear signal.

Reetoo, K. N., S. A. Osman, et al. (1999). "Development and evaluation of quantitative-competitive PCR for quantitation of coxsackievirus B3 RNA in experimentally infected murine tissues." Journal of Virological Methods **82**(2): 145.

http://www.sciencedirect.com/science/article/B6T96-3XT0BN2-6/2/603d7118f2bb100f7c234314488bba18

A method is described for quantitation of enterovirus RNA in experimentally infected murine tissues. Viral RNA was extracted from tissue samples and amplified by reverse transcriptase PCR in the presence of an internal standard RNA. The ratio of PCR product derived from viral RNA and internal standard RNA was then determined using specific probes in a post-PCR electrochemiluminescent hybridization assay. This provided an estimate of the viral RNA copy number in the original sample, and detection of PCR product derived from internal standard RNA validated sample processing and amplification procedures. RNA copy number correlated with viral infectivity of cell culture-derived virus, and one tissue culture infective dose was found to contain approximately 103 genome equivalents. The ratio of RNA copy number to infectivity in myocardial tissue taken from mice during the acute phase of coxsackievirus B3 myocarditis was more variable ranging from 104-107, and was dependent on the stage of infection, reflecting differential rates of clearance for viral RNA and viral infectivity. The assay is rapid, and could facilitate investigations which currently rely upon enterovirus quantitation by titration in cell culture. This would be useful for experimental studies of viral pathogenesis, prophylaxis and antiviral therapy.

Revilla-Fernandez, S., B. Wallner, et al. "The use of endogenous and exogenous reference RNAs for qualitative and quantitative detection of PRRSV in porcine semen." Journal of Virological Methods **In Press, Corrected Proof** http://www.sciencedirect.com/science/article/B6T96-4FJGW2B-3/2/4b6e018f1b0d1f46b449b1acfc7a000f

Semen is known to be a route of porcine reproductive and respiratory syndrome virus (PRRSV) transmission. A method was developed for qualitative and quantitative detection of the seminal cell-associated PRRSV RNA in relation to endogenous and exogenous reference RNAs. As endogenous control for one-step real-time reverse transcription (RT)-PCR UBE2D2 mRNA was

selected. Particularly for the analysis of persistent infections associated with low copy numbers of PRRSV RNA, UBE2D2 mRNA is an ideal control due to its low expression in seminal cells and its detection in all samples analysed (n = 36). However, the amount of UBE2D2 mRNA in porcine semen varied (up to 106-fold), thus its use is limited to qualitative detection of PRRSV RNA. For quantitation, a synthetic, non-metazoan RNA was added to the RNA isolation reaction at an exact copy number. The photosynthesis gene ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) from Arabidopsis thaliana was used as an exogenous spike. Unexpectedly, PRRSV RNA was detected in a herd of specific pathogen-free (SPF) boars which were tested ELISA-negative for anti-PRRSV antibodies. Therefore, RT-PCR for seminal cell-associated PRRSV is a powerful tool for managing the SPF status during quarantine programs and for routine outbreak investigations.

Rijsewijk, F., S. Pritz-Verschuren, et al. (2005). "Development of a polymerase chain reaction for the detection of Anguillid herpesvirus DNA in eels based on the herpesvirus DNA polymerase gene." Journal of Virological Methods **124**(1-2): 87.

http://www.sciencedirect.com/science/article/B6T96-4F1503P-2/2/9df0f56d5ef0c68078e3d12a1633c505

Anguillid herpesvirus (AnHV, also known as Herpesvirus anguillae or HVA) is found in both Japanese and European eels. Based on restriction enzyme analysis a small number of differences were found between AnHV isolated from Japanese eels and from European eels. The total genome size of both is about 245 kb, which is confirmed by alternating-field electrophoresis. Using a set of degenerate primers based on conserved regions within DNA-directed DNA polymerase coding regions, a 463 base pair fragment was isolated from both Japanese and European AnHV. Nucleotide sequence analysis showed that the cloned regions of both viruses have identical sequences. Based on this part of the DNA-polymerase sequence, primers were selected and used to develop a sensitive PCR to detect AnHV DNA in eel tissue samples. To avoid false negative results and to estimate the number of AnHV genome copies found in tissues, 100 copies of an internal control plasmid were added to the tissue samples. This semi-quantitative AnHV PCR can be used for both the European and Japanese isolates of AnHV, detects as few as 10 genome copies and is 100 times more sensitive than standard virus isolation.

Rimstad, E. and K. Ueland (1992). "Detection of feline immunodeficiency virus by a nested polymerase chain reaction." Journal of Virological Methods **36**(3): 239.

http://www.sciencedirect.com/science/article/B6T96-476RM6B-1S/2/1fd9de23b89049a52b4dc338f65dfe2b

A specific and sensitive polymerase chain reaction (PCR) procedure for the detection of feline immunodeficiency virus (FIV) in peripheral blood mononuclear cells (PBMC) was developed. PBMC from both blood samples and cultures were digested by proteinase K in a lysis buffer, and after heat inactivation of the proteinase, the resultant material was used in a two step amplification protocol using nested sets of primers. Two independent amplifications, from the gag and pol genes respectively, were performed in each tube. The PCR was positive for six of 14 samples from FIV seropositive adult cats, while all 36 samples from seronegative cats were negative. In comparison with an antigen-capturing ELISA procedure, the PCR detected FIV infection in PBMC cultures on average two days earlier.

Roberts, C. A., R. G. Dietzgen, et al. (2000). "Real-time RT-PCR fluorescent detection of tomato spotted wilt virus." Journal of Virological Methods **88**(1): 1.

http://www.sciencedirect.com/science/article/B6T96-40TY33T-1/2/d294efce2cdf87866a0c40d592f87db1

A real-time reverse transcription-polymerase chain reaction assay based on TaqMan(TM) chemistry was developed for the detection and quantification of tomato spotted wilt virus (TSWV). This method enabled sensitive, reproducible and specific detection of TSWV in 'leaf soak' and total RNA extracts from infected plants. TaqMan reliably detected TSWV in as little as 500 fg total RNA. The assay was 10-fold more sensitive than visualisation of ethidium bromide-stained bands following agarose gel electrophoresis. TSWV isolates from various crops and locations were detected with a cycle threshold of 20-26 in 1 ng total RNA extracted from fresh or freeze-dried leaves. A dilution series of in vitro transcripts from a cloned 628 base pair fragment of TSWV S RNA served as standard for quantification of viral template in infected leaf samples. The TaqMan assay detected reproducibly 1000 molecules of the target transcript.

Rohayem, J., S. Berger, et al. (2004). "A simple and rapid single-step multiplex RT-PCR to detect Norovirus, Astrovirus and Adenovirus in clinical stool samples." Journal of Virological Methods **118**(1): 49.

http://www.sciencedirect.com/science/article/B6T96-4BT7JK6-1/2/c783ae5d862a1aa5da9d4aa1aa5ea7e1

A single-step multiplex reverse transcription-polymerase chain reaction (RT-PCR) assay that detects and identifies Norovirus, Astrovirus and Adenovirus in clinical stool samples is described. Four hundred sixty stool samples were tested from patients with non-rotavirus acute gastroenteritis, that were either stored at -80 [deg]C and tested retrospectively, or tested immediately after viral nucleic acid extraction in a prospective manner, including outbreaks of gastroenteritis that occurred in Germany during the winter of 2003. The multiplex RT-PCR was validated against simplex RT-PCR with published primers for Norovirus (JV12/JV13 and p289/p290) and Astrovirus (Mon340/348), and against simplex PCR for Adenovirus. In both retrospective and prospective settings, the multiplex RT-PCR was equally sensitive and specific in detecting non-rotavirus infections compared with simplex RT-PCR/PCR. The specificity of the multiplex RT-PCR was assessed by sequencing of the amplicons that showed high nucleotide identities to Norovirus genogroup I/1, I/4, II/2, or II/4 clades, as well as to Astrovirus serotypes 1, 2, 4, or 8. The multiplex RT-PCR was also more sensitive than Astrovirus and Norovirus antigen enzyme immunoassays (IDEIA, Dako), as well as Astrovirus isolation in cell culture. This novel multiplex RT-PCR is an attractive technique for the rapid, specific, and cost-effective laboratory diagnosis of non-rotavirus acute gastroenteritis.

Ros, C., M. E. Riquelme, et al. (1999). "Improved detection of five closely related ruminant alphaherpesviruses by specific amplification of viral genomic sequences." Journal of Virological Methods **83**(1-2): 55.

http://www.sciencedirect.com/science/article/B6T96-3XVPG88-7/2/d5c0abe062eee652b1ff5ac334d67da2

The detection and discrimination of five closely related ruminant alphaherpesviruses, bovine herpesvirus 1 (BHV-1), bovine herpesvirus 5 (BHV-5), caprine herpesvirus 1 (CapHV-1), cervine herpesvirus 1 (CerHV-1), and rangiferine herpesvirus 1 (RanHV-1), were achieved by the

development of specific PCR systems. The highly variable N-terminal of the glycoprotein C was chosen to select the diagnostic primers, except for the CerHV-1 primers, which targeted the glycoprotein D region. All the assays proved specific since no heterologous virus was amplified. BHV-1 and BHV-5 were detected by using the same PCR assay and the different sizes of the amplification products allowed their identification on agarose gels. The practical diagnostic applicability of the novel PCR assays, with special regard to the BHV-1 system, has been evaluated on clinical samples from experimentally infected animals.

Sagazio, P., M. Tempesta, et al. (1998). "Antigenic characterization of canine parvovirus strains isolated in Italy." Journal of Virological Methods **73**(2): 197.

http://www.sciencedirect.com/science/article/B6T96-3TGVK28-7/2/95394439de447d154ef4dd58b6eac5ee

28 isolates of canine parvovirus type-2 (CPV-2) were obtained from dogs with hemorrhagic gastroenteritis in Italy. The antigenic structure of CPV-2 isolates was characterized, using four discriminating monoclonal antibodies. In addition, four vaccinal strains were examined. Similar to reports from Australia and the United Kingdom, a much higher prevalence of CPV-2a (25/28 isolates) was observed than the other variant type, CPV-2b (3/28 isolates). DNA fragments (2.2 kbp) of representative strains of CPV-2, CPV-2a and CPV-2b were amplified by the polymerase chain reaction (PCR) and the products were digested by the restriction enzymes (RE) RsaI, HpaII, HindIII and PvuII. The RsaI enzyme allows the differentiation of CPV-2 from CPV-2a and CPV-2b.

Saito, T., S. Matsumoto, et al. (1998). "Multicyclic reverse transcription-polymerase chain reaction assay system for quantitation of GB virus-C/hepatitis G virus RNA in serum." Journal of Virological Methods **74**(2): 185.

http://www.sciencedirect.com/science/article/B6T96-3V5082S-7/2/99c3cccab7fe3b48c34d9ef79a26f4d5

A new quantitative reverse transcription-polymerase chain reaction (RT-PCR) method is described for analyzing the amount of GB virus-C (GBV-C)/hepatitis G virus (HGV) RNA in serum. This multicyclic RT-PCR (MRT-PCR) method used oligonucleotide primers deduced from the 3' noncoding region (3'NCR) that is highly conserved among GBV-C/HGV isolates. Quantitation of GBV-C/HGV RNA using MRT-PCR ranged between 102 and 1010 copies/ml when PCR cycle number was regulated at exponential amplification of the products. Competitive RT-PCR (CRT-PCR) was carried out with mutant RNA and sample that had been measured by MRT-PCR. Quantitation of GBV-C/HGV RNA using both methods agreed. MRT-PCR detected viral RNA in a single step PCR, and demonstrated a high degree of sensitivity that was equal to that of the RT-PCR procedure, which used nested primers deduced from the non-structural (NS) 3 region. The MRT-PCR method for quantitation of GBV-C/HGV RNA in serum may prove useful for diagnosis.

Saito, T., Y. Munakata, et al. (2003). "Evaluation of anti-parvovirus B19 activity in sera by assay using quantitative polymerase chain reaction." Journal of Virological Methods **107**(1): 81.

http://www.sciencedirect.com/science/article/B6T96-46YXPVK-6/2/6624b5ec4f802415c0141af6f7bced2b

Human parvovirus B19 (B19) infects cells of erythroid lineage. Production of neutralizing antibodies (Abs) is indispensable for recovery from B19-related disease state. In this study, we used a convenient method to measure neutralizing activities in human sera by using a real-time quantitative PCR based assay. Erythroid cell line KU812Ep6 was incubated with test sera before infection with B19 virus. The copy number of B19-DNA in cultures was decreased in the presence of the sera from patients who recovered from acute B19 infection, whereas no decrease in B19-DNA was in cultures incubated with sera from healthy volunteers who had no B19 infection. The decrease in B19-DNA copy number was calculated and the inhibition percentage was expressed as neutralizing activity to B19. A clinical study showed that the levels of neutralizing ability were high in patients who recovered soon after acute B19 infection, but were low in some patients with a prolonged clinical course for recovery from B19 infection. This method is simple and convenient compared with methods described previously, showing its usefulness to evaluate the neutralizing activity to B19.

Saiz, M., S. Castro, et al. (1994). "Serotype-specific detection of bean common mosaic potyvirus in bean leaf and seed tissue by enzymatic amplification." Journal of Virological Methods **50**(1-3): 145.

http://www.sciencedirect.com/science/article/B6T96-47DKYF8-H/2/87f59a02642bd2455bef08fc0ec16279

An assay involving reverse transcription and polymerase chain reaction (PCR) is described for specific detection of serotypes A and B BCMV isolates in bean leaf and seed tissues. Three oligonucleotide primers designed according to the sequence data available allow, in appropriate combination, serotype-specific detection of BCMV. The sensitivity of the method was sufficient to detect BCMV in as little as 100 fg and 50 pg of infected leaf and seed tissues, respectively.

Sanchez-Seco, M. P., D. Rosario, et al. "Generic RT-nested-PCR for detection of flaviviruses using degenerated primers and internal control followed by sequencing for specific identification." Journal of Virological Methods **In Press, Corrected Proof**
http://www.sciencedirect.com/science/article/B6T96-4FKYDM9-1/2/578f3dab73d77e4adfd7444b2f611d7a

Flaviviruses are a widespread and numerous group of arboviruses that can cause serious illness in humans. The continuous and slow spread of certain flaviviruses, such as Dengue viruses, and the recent entry and spread of West Nile virus to the American continent, point to the need to control these infections. This control requires the use of suitable techniques for diagnostic and surveillance programmes. A generic RT-nested-PCR that is, theoretically, able to detect each member of the group has been designed. The identification of the detected virus is carried out by sequencing. The introduction of an internal control would reduce the number of false negative results and could be used to quantify the viral load in clinical samples where the method works well.

Sanchez-Seco, M. P., D. Rosario, et al. (2001). "A generic nested-RT-PCR followed by sequencing for detection and identification of members of the alphavirus genus." Journal of Virological Methods **95**(1-2): 153.

http://www.sciencedirect.com/science/article/B6T96-433NRB5-H/2/3f2609203a01d4d108621be750cc519d

A specific and sensitive nested RT-PCR method was developed for the detection of members of the alphavirus genus. Based on available sequences, degenerated primers were selected in the nsP4 gene. Reaction components and thermal cycling parameters were investigated and standardised, and optimal ones were selected. As few as 25 pfu/tube could be detected. The identities of the amplified fragments were confirmed by sequencing, and phylogenetic analysis was carried out. The resulting phylogenetic tree could be applied to classify every alphavirus according to its serogroup. This technique is suitable for rapid, sensitive and reliable detection of these viruses and may be very valuable for diagnostic applications and surveillance.

Saulnier, P., M. Vidaud, et al. (2003). "Development and validation of a real-time PCR assay for the detection and quantitation of p53 recombinant adenovirus in clinical samples from patients treated with Ad5CMV-p53 (INGN 201)." Journal of Virological Methods **114**(1): 55.

http://www.sciencedirect.com/science/article/B6T96-49SNFD4-1/2/83c3e27a4029aea7fba1d9122574acf1

The purpose of this study was to assess the usefulness of real-time PCR as a quantitative, highly reproducible, and sensitive method, for detecting and quantifying p53 recombinant adenovirus in biological samples from cancer patients receiving injections of Ad5CMV-p53. The dynamic range of this real-time PCR-based assay was wide (at least five orders of magnitude). Our assay used an internal positive control in the same PCR tube that is capable of detecting residual PCR inhibitors. Serial spiked samples in plasma with known quantities of Ad5CMV-p53 were evaluated. The minimum detection limit was 2 pfu per PCR (~50 pfu per ml of plasma) and the quantification values were reproducible. A total of 2069 controls tested with 1780 plasma samples from 286 patients enrolled in gene therapy trials using Ad5CMV-p53 were investigated. Using calibrators to adjust the quantitation value, the results confirmed the good performance of the assay. In conclusion, the high sensitivity, simplicity and reproducibility of the real-time Ad5CMV-p53 assay, allowing screening of large numbers of samples, combined with its wide dynamic range, make this method particularly suitable for monitoring gene therapy trials.

Sears, J. F. and A. S. Khan (2003). "Single-tube fluorescent product-enhanced reverse transcriptase assay with Ampliwax(TM) (STF-PERT) for retrovirus quantitation." Journal of Virological Methods **108**(1): 139.

http://www.sciencedirect.com/science/article/B6T96-47T8W17-4/2/d01195ea63d2951ed22610babe264af9

A TaqMan fluorescent probe-based product enhanced reverse transcriptase (RT) assay is described in which the RT and polymerase chain reaction (PCR) steps are set-up in a single tube, in two compartments separated by Ampliwax(TM) (designated as single-tube fluorescent product-enhanced reverse transcriptase assay (STF-PERT)). This simplification of the two-step method resulted in increased assay reproducibility and handling efficiency while maintaining the sensitivity of the PERT assay (<10 virions). The STF-PERT assay can be used to quantitate low amounts of retrovirus in clinical and research materials and to evaluate retrovirus contamination in cell substrates and biological products in human use.

Shafer, R. W., A. Warford, et al. (2000). "Reproducibility of human immunodeficiency virus type 1 (HIV-1) protease and reverse transcriptase sequencing of plasma samples from heavily treated HIV-1-infected individuals." Journal of Virological Methods **86**(2): 143.

http://www.sciencedirect.com/science/article/B6T96-403W2YC-5/2/211fd2658611ede185f47b60f768fccd

The reproducibility of population-based human immunodeficiency virus type 1 (HIV-1) protease and reverse transcriptase (RT) sequencing was assessed using replicate aliquots of cryopreserved plasma samples obtained from seven heavily treated HIV-1-infected individuals. The sequence of each sample replicate was compared with the consensus sequence for that sample and 99.4% of 35128 amino acids were found to be concordant with the sample consensus. Partial discordances were present at 0.5% of positions and complete discordances were present at <0.1% of positions. To assess the reproducibility at detecting mutations (defined here as differences from the subtype B consensus sequence), the proportion of sequences having a mutation when at least two sequences from that sample had the same mutation were examined. There was a median of 13 protease and 18 RT mutations per sample for a total of 3126 mutations; 95% of these mutations were detected. However, sequencing of multiple clones from two samples demonstrated that those mutations present in a minority of clones were often not detected by population-based sequencing. These results suggest that HIV-1 protease and RT sequencing of circulating plasma virus is highly reproducible but that the sensitivity at detecting mutations may be low if those mutations are present as minor variants.

Shamloul, A. M., F. Faggioli, et al. (2002). "A novel multiplex RT-PCR probe capture hybridization (RT-PCR-ELISA) for simultaneous detection of six viroids in four genera: Apscaviroid, Hostuviroid, Pelamoviroid, and Pospiviroid." Journal of Virological Methods **105**(1): 115.

http://www.sciencedirect.com/science/article/B6T96-46041YT-1/2/4e56918384176482201d2cdf6a26b133

A rapid and sensitive assay was developed for the detection and identification of viroids by standard or multiplex reverse transcription-polymerase chain reaction (RT-PCR)-probe capture hybridization (RT-PCR-ELISA). The assay was applied successfully for the detection and identification of the following six viroid species from infected tissues: Potato spindle tuber viroid (Pospiviroid), Peach latent mosaic viroid (Pelamoviroid), Apple scar skin viroid (Apscaviroid), Apple dimple fruit viroid (Apscaviroid), Pear blister canker viroid (Apscaviroid), and Hop stunt viroid (Hostuviroid). Total RNA was obtained from infected tissue by the Qiagen RNeasy kit and, then viroid cDNA was synthesized using viroid specific complementary DNA primer. To identify and differentiate the amplicons of the six viroids, each amplicon was digoxigenin (DIG)-labelled during the amplification process, and then detected by a colorimetric system using a biotinylated cDNA capture probe specific for each viroid. The results revealed that each capture probe hybridized only to its complementary DIG-labelled amplicon. Thus the six viroids can be detected and differentiated in a multiplex RT-PCR-ELISA assay. In the multiplex assay, cDNAs of six viroids were synthesized simultaneously in one tube, DIG-labelled during amplification, then a portion of the DIG-labelled amplified products was hybridized with selected capture probe. All the six viroid capture probes hybridized to their respective complementary DIG-labelled RT-PCR-amplified product. These findings are important for viroid detection and identification for studying host-viroid interactions and for management and control viroid diseases.

Shamloul, A. M. and A. Hadidi (1999). "Sensitive detection of potato spindle tuber and temperate fruit tree viroids by reverse transcription-polymerase chain reaction-probe capture hybridization." Journal of Virological Methods **80**(2): 145.

http://www.sciencedirect.com/science/article/B6T96-3X29D70-5/2/e3613f2ae2220c4fea31c8809811e72f

A rapid and sensitive assay for the specific detection of plant viroids using reverse transcription-polymerase chain reaction (RT-PCR) -probe capture hybridization (RT-PCR-enzyme-linked immunosorbent assay (ELISA)) was developed. The assay was applied successfully for the detection of potato spindle tuber viroid, peach latent mosaic viroid, or apple scar skin viroid from viroid infected leaf tissue. Clarified sap extract from infected leaf tissue was treated first with GeneReleaser(TM) polymeric matrix to remove inhibitors of RT-PCR reactions. Viroid cDNA was then synthesized and amplified using viroid specific primers in RT-PCR assays and the amplified viroid cDNA (amplicon) was digoxigenin (DIG) -labelled during the amplification process. The amplicon was then detected in a colorimetric hybridization system in a microtiter plate using a biotinylated cDNA capture probe. This system combines the specificity of molecular hybridization, the ease of the colorimetric protocol, and is at least 100-fold more sensitive than gel electrophoretic analysis in detecting the amplified product. Viroid cRNA may replace viroid cDNA as the capture probe. The cRNA probe was several fold more sensitive than the cDNA probe for viroid detection. Six to seven hours are needed to complete the RT-PCR-ELISA for viroid detection from infected leaf tissue.

Shin, J., E. M. Bautista, et al. (1998). "Quantitation of porcine reproductive and respiratory syndrome virus RNA in semen by single-tube reverse transcription-nested polymerase chain reaction." Journal of Virological Methods **72**(1): 67.

http://www.sciencedirect.com/science/article/B6T96-3SXDXRR-8/2/03cca086219a7ca36f7b08cbf32d7738

Porcine reproductive and respiratory syndrome virus (PRRSV) is in boar semen for extended periods of time as determined by reverse transcription-nested polymerase chain reaction (RT-nPCR) assay. The concentration of PRRSV RNA in semen and the biological significance of the detection level, however, remain to be resolved. In order to determine the concentration of PRRSV VR-2332 (a prototypic strain of North American isolates) in semen following infection, we established a 'standard curve'-quantitative competitive (SC-QC)-RT-nPCR assay as well as an equimolar QC-RT-nPCR assay. A deletion-type competitor RNA derived from the Lelystad virus, a European strain of PRRSV, ORF-7 gene standard which shares the nested sets of primer recognition sequences with the VR-2332 ORF-7 gene was used as an internal standard. The equimolar QC-RT-nPCR assay results revealed that the number of copies of PRRSV RNA in 1 TCID50/ml of virus derived from CL-2621 cell culture supernatants varied depending upon the type of samples in which virus was added; 143+/-24.0 and 266.5+/-48.5 copies in serum and semen samples spiked with PRRSV VR-2332, respectively. For the establishment of SC-QC-RT-nPCR assay, a standard curve was generated from band intensity ratios versus a series of known initial numbers of wild-type RNA copies which were quantified by the equimolar QC-RT-nPCR assay. Various initial numbers of copies of wild-type PRRSV RNA and each band intensity ratio with 1000 copies of competitor RNA were well correlated within the range of 100 to 200000 copies (R2=0.947). A 'standard curve' quantitation assay using competitive single-tube RT-nPCR will offer a rapid and reliable way to quantify low concentrations of PRRSV RNA in semen.

Singh, M. and R. P. Singh (1996). "Factors affecting detection of PVY in dormant tubers by reverse transcription polymerase chain reaction and nucleic acid spot hybridization." Journal of Virological Methods **60**(1): 47.

http://www.sciencedirect.com/science/article/B6T96-3VYV029-5/2/30e07e74fe28d8beeae6c23e901c5df2

A reverse transcription polymerase chain reaction (RT-PCR) protocol was developed using two 20-mer primers located in nuclear inclusion genes NIa and NIb of potato virus Y (PVY). A 1017

bp PCR-product was detected in dormant potato tubers, infected with PVYO, but not in tubers from healthy plants. The PCR product was specific to PVY, as determined by Southern blot detection by hybridization with a PVYO-specific probe. As little as 1 pg of purified PVYO-RNA can be detected after RT-PCR amplification. The presence of phenolics or polysaccharides in tuber nucleic acids inhibited PVYO amplification, which was eliminated by diluting nucleic acid preparations prior to cDNA synthesis, modifying the nucleic acid extraction procedure by isopropanol precipitation and using phosphate-buffered saline-Tween in the cDNA mix. Potato cultivars differed in PVYO concentration in tubers as much as 128-fold. Tuber parts used for nucleic acid extractions were important in potato cultivars with low virus titres and combining of saps from bud and stem ends improved the PVYO detection. Storage of tubers up to 90 days at 4 [deg]C did not result in reduced detection of PVYO by both nucleic acid spot hybridization and RT-PCR, but RT-PCR band intensity was lower at longer storage periods. The primer pair developed in this study exhibited broad specificities with field isolates from Peru, Scotland and North America.

Singh, R. P. (1999). "A solvent-free, rapid and simple virus RNA-release method for potato leafroll virus detection in aphids and plants by reverse transcription polymerase chain reaction." Journal of Virological Methods **83**(1-2): 27.

http://www.sciencedirect.com/science/article/B6T96-3XVPG88-4/2/977b58dd229d4b6f7912a88e1a7867d2

A one-step, rapid and economical method for potato leafroll virus (PLRV) RNA release that is applicable to the use on a microcentrifuge scale is described. Discs (3-6 mm diameter) from leaves, petioles, stems, and tubers of potato plants were incubated in microcentrifuge tubes with detergent solution. The supernatants were used directly for reverse transcription (RT) and polymerase chain reaction (PCR). Of the seven nonionic detergents of the TritonX series evaluated, Triton X405R was the most effective, although X-405 and X-100R were also effective in releasing PLRV RNA. Application of the detergent method for detecting PLRV in greenhouse-grown potato organs (leaves, petioles, stems, tubers) and in field-grown tubers was demonstrated and compared to the multi-step phenol method. When individual aphids, Myzus persicae, were ground in 20 [mu]l of detergent solution and supernatants were used for RT-PCR, virus was detected in single aphids in undiluted solutions and up to a dilution of 1:4. The concentration of PLRV RNA released by the detergent method was substantially lower than that released by the phenol method. However, the detergent method was sensitive enough to detect PLRV from potato leaves, petioles, and stems 2 weeks after graft inoculation. The detergent method was rapid and economical, and has potential for large-scale application. The extracts survived over 37 days at room temperature, thus making it possible to mail extracts from remote areas lacking specialised RT-PCR facilities to a central laboratory for PLRV testing.

Singh, R. P., J. Kurz, et al. (1995). "Detection of potato leafroll virus in single aphids by the reverse transcription polymerase chain reaction and its potential epidemiological application." Journal of Virological Methods **55**(1): 133.

http://www.sciencedirect.com/science/article/B6T96-3YYTF0W-1D/2/01e92bca3148bbe3d53dafaec5171d9c

A reverse transcription and polymerase chain reaction (RT-PCR) system was developed using two 20-mer primers located in the potato leafroll virus (PLRV) capsid gene. A 336-bp PCR product was detected from aphids (Myzus persicae) which had been fed on PLRV-infected plants. The PCR band was specific to PLRV as determined by Southern blots and detection by a PLRV-specific probe. As little as 5 min exposure of aphids to PLRV-infected leaves resulted in the

presence of PLRV-specific bands in 13% of aphids. However, the percentage of PLRV-positive aphids increased with longer exposure to infected sources and reached 90% after 3-4 days of feeding. PLRV can be detected from a single viruliferous aphid or a single viruliferous aphid combined with up to 29 non-viruliferous aphids. PLRV can be detected from freshly collected aphids, those stored at -70 [deg]C, or those stored in 70% ethanol at room temperature for extended periods. This method is applicable to assess the viruliferous nature of aphids caught in yellow-pan traps during the growing season or stored for over a year.

Singh, R. P., X. Nie, et al. (2000). "Duplex RT-PCR: reagent concentrations at reverse transcription stage affect the PCR performance." Journal of Virological Methods **86**(2): 121.

http://www.sciencedirect.com/science/article/B6T96-403W2YC-3/2/c4001009ce2ed15163382fd0658efd00

Test conditions for the simultaneous detection of potato leafroll virus (PLRV) and potato virus Y (PVY) in dormant tubers and leaves by reverse transcription-polymerase chain reaction (RT-PCR) were optimized. Various factors optimized at the reverse transcription (RT) stage rather than at the amplification (PCR) stage affected the outcome. In the simplex RT-PCR a onefold dNTPs concentration (0.5 mM) was sufficient in yielding a PLRV or PVY band. In contrast, the duplex RT-PCR required a minimum twofold dNTPs concentration (1.0 mM) during RT to produce distinct bands in PCR. Similarly, various proportions of antisense primers of PLRV and PVY used during RT affected subsequent duplex RT-PCR. Optimal amplification of both viruses were obtained at a ratio of 0.90:0.49 [mu]M of PLRV:PVY antisense primers. An interaction of dNTPs and RNA template concentration was observed. A higher concentration of RNA was required at onefold dNTPs concentration than at twofold dNTPs. Dilutions down to 1:300 of RNA template yielded distinct bands of both viruses at twofold dNTPs concentration. At optimized conditions of the duplex RT-PCR both viruses were reliably detected in composite samples at a ratio of one part infected sap mixed with 399 parts of sap from healthy tubers. Application of optimized conditions to singly- and doubly-infected tubers detected both viruses from naturally infected field-grown tubers. A nearly perfect correlation (r2=0.99) was observed between visible plant symptoms and the virus detection from leaves and tubers by the duplex RT-PCR.

Singh, R. P., X. Nie, et al. (2002). "Sodium sulphite inhibition of potato and cherry polyphenolics in nucleic acid extraction for virus detection by RT-PCR." Journal of Virological Methods **99**(1-2): 123.

http://www.sciencedirect.com/science/article/B6T96-448BG21-F/2/e2ba6de7b19d265c3bcb9cec6271bd11

Phenolic compounds from plant tissues inhibit reverse transcription-polymerase chain reaction (RT-PCR). Multiple-step protocols using several additives to inhibit polyphenolic compounds during nucleic acid extraction are common, but time consuming and laborious. The current research highlights that the inclusion of 0.65 to 0.70% of sodium sulphite in the extraction buffer minimizes the pigmentation of nucleic acid extracts and improves the RT-PCR detection of Potato virus Y (PVY) and Potato leafroll virus (PLRV) in potato (Solanum tuberosum) tubers and Prune dwarf virus (PDV) and Prunus necrotic ringspot virus (PNRSV) in leaves and bark in the sweet cherry (Prunus avium) tree. Substituting sodium sulphite in the nucleic acid extraction buffer eliminated the use of proteinase K during extraction. Reagents phosphate buffered saline (PBS)-Tween 20 and polyvinylpyrrolidone (PVP) were also no longer required during RT or PCR phase. The resultant nucleic acid extracts were suitable for both duplex and multiplex RT-PCR. This simple and less expensive nucleic acid extraction protocol has proved very effective for potato cv. Russet Norkotah, which contains a high amount of polyphenolics. Comparing commercially available RNA extraction kits (Catrimox(TM) and RNeasy(TM)), the sodium sulphite based

extraction protocol yielded two to three times higher amounts of RNA, while maintaining comparable virus detection by RT-PCR. The sodium sulphite based extraction protocol was equally effective in potato tubers, and in leaves and bark from the cherry tree.

Singh, R. P., M. Singh, et al. (1998). "Use of citric acid for neutralizing polymerase chain reaction inhibition by chlorogenic acid in potato extracts." Journal of Virological Methods **74**(2): 231.

http://www.sciencedirect.com/science/article/B6T96-3V5082S-F/2/70fd6d5044a0673a2a80a8866266f339

Polyphenolics are a widely distributed class of plant constituents implicated in the inhibition of the polymerase chain reaction (PCR). Since chlorogenic acid forms the bulk of phenolics in the potato tuber, a range of chlorogenic acid concentrations were tested for inhibition of reverse transcription (RT) and PCR steps. The RT step was inhibited at chlorogenic acid concentrations of 6.0 to 7.5 [mu]g, while the PCR step was inhibited at much lower concentrations of 1.2 to 1.8 [mu]g. Incorporation of 1.2% citric acid at the nucleic acid extraction step prevented darkening of the extract and neutralized the inhibitory effect of chlorogenic acid.

Singh, S., V. T. K. Chow, et al. (2000). "RT-PCR, nucleotide, amino acid and phylogenetic analyses of enterovirus type 71 strains from Asia." Journal of Virological Methods **88**(2): 193.

http://www.sciencedirect.com/science/article/B6T96-411PGRW-9/2/a1b8376344b1295742393fd5e48e326c

A specific and sensitive method based on RT-PCR was developed to detect enterovirus 71 (EV71) from patients with hand, foot and mouth disease, myocarditis, aseptic meningitis and acute flaccid paralysis. RT-PCR primers from conserved parts of the VP1 capsid gene were designed on the basis of good correlation with sequences of EV71 strains. These primers successfully amplified 44 strains of EV71 including 34 strains isolated from Singapore in 1997 and 1998, eight strains from Malaysia isolated in 1997 and 1998, one Japanese strain and the neurovirulent strain EV71/7423/MS/87. RT-PCR of 30 strains of other enteroviruses including coxsackievirus A and B, and echoviruses failed to give any positive amplicons. Hence, RT-PCR with these primers showed 100% correlation with serotyping. Direct sequencing of the RT-PCR products of 20 EV71 strains revealed a distinct cluster with two major subgroups, thus enabling genetic typing of the viruses. The genetic heterogeneity of these strains culminated in amino acid substitutions within the VP1, VP2 and VP3 regions. The sequencing of a 2.9 kb fragment comprising the capsid region and the major part of 5' UTR of two Singapore strains revealed that they belonged to a group distinct from the prototype EV71/BrCr strain and the EV71/7423/MS/87 strain. The dendrogram generated from 341 bp fragments within the VP1 region revealed that the strains of Singapore, Malaysia and Taiwan belong to two entirely different EV71 genogroups, distinct from the three genogroups identified in another recent study.

Smith, G. R., R. Van de Velde, et al. (1992). "PCR amplification of a specific double-stranded RNA region of Fiji disease virus from diseased sugarcane." Journal of Virological Methods **39**(3): 237.

http://www.sciencedirect.com/science/article/B6T96-476F61S-67/2/e61cf83990890fba99d5dcd116139156

A 450-bp region from one species of the segmented dsRNA genome of Fiji disease virus (FDV)

was amplified from total nucleic acid extracts of diseased plants by reverse transcription with MMLV, followed by amplification with Taq DNA polymerase (RT-PCR). Other FDV-specific regions (c 150 bp and c 270 bp) were also amplified from the dsRNA template. FDV cDNA was only synthesised when the viral dsRNA template was boiled and quenched with FDV-specific or random hexamer primers. The reverse transcriptase/DNA polymerase enzyme rTth appeared to yield only the 150 bp fragment from the dsRNA template under the conditions used. The level of sensitivity of RT-PCR for purified FDV dsRNA was 100 ag, approximately 104-fold more sensitive than detection with biotinylated DNA probe.

Smith, J., L. M. McElhinney, et al. (2000). "Assessment of template quality by the incorporation of an internal control into a RT-PCR for the detection of rabies and rabies-related viruses." Journal of Virological Methods **84**(2): 107.

http://www.sciencedirect.com/science/article/B6T96-3YDG9HS-1/2/e00ff628ace2e3a0272d32f5b1bf6c98

A method is described to assess RNA template quality by the incorporation of a ribosomal RNA (rRNA) internal (in tube) control into a standard rabies and rabies-related virus specific RT-PCR. Specific virus and rRNA templates were co-amplified in a duplex reaction from RNA extracts derived from 60 isolates representing all six of the established lyssavirus genotypes. To ensure a wide species applicability of this technique we demonstrated that the rRNA assay was capable of functioning using the cells or tissues of 14 different mammals. Parallel studies between the duplex and the unlinked lyssavirus assay demonstrated only a minor reduction in the sensitivity of the former test. The ribosomal and viral targets (unlike [beta]-actin RNA) were shown to have similar degradation kinetics making rRNA amplification a good control for viral target integrity. As a consequence, the use of this system would reduce the likelihood of obtaining false negative RT-PCR results from lyssavirus infected material.

Stamey, F. R., M. DeLeon-Carnes, et al. (2003). "Comparison of a microtiter plate system to Southern blot for detection of human herpesvirus 8 DNA amplified from blood and saliva." Journal of Virological Methods **108**(2): 189.

http://www.sciencedirect.com/science/article/B6T96-47PGC4N-6/2/68d06de6865414884ae2c743798ae715

The recent discovery of human herpesvirus 8 (HHV-8) as the etiologic agent of Kaposi's sarcoma (KS) has led to the interest in the development of PCR for this virus that is accurate, rapid, and convenient. We developed a sensitive PCR assay for HHV-8 with microtiter plate detection of amplimers. DNA was purified from white blood cells and saliva from HIV-infected men with and without Kaposi's sarcoma and one-step PCR was undertaken with primer sets specific for the N-terminal region of the glycoprotein B gene and open reading frame (orf) 26 of HHV-8. PCR was performed on 40 clinical specimens, followed by Southern blot and microtiter plate detection of amplimers. Results from the two methods of detection were nearly identical. Sensitivity for both methods based on serial dilution of a known standard was five to ten copies of HHV-8 per 400 ng of cellular DNA. In conclusion, microtiter plate detection of HHV-8 PCR amplimers is as sensitive and specific as Southern blot with much faster turnaround time at comparable cost, and utilizes common laboratory equipment.

Stocher, M., V. Leb, et al. (2003). "A convenient approach to the generation of multiple internal control

DNA for a panel of real-time PCR assays." Journal of Virological Methods **108**(1): 1.

http://www.sciencedirect.com/science/article/B6T96-47PGC4N-4/2/ff45cc19f684bd3f3af8a09818fdd0b3

Real-time polymerase chain reaction (PCR) assays allow convenient detection and quantitation of virus-derived nucleic acids in clinical specimens. When specimens are assayed for the presence of virus-derived nucleic acids against external standards, sample adequacy is not monitored. This can be achieved by using internal controls that are co-amplified with the virus-specific DNA in competitive PCR. Each of the various real-time PCR assays in a routine clinical laboratory requires its specific internal control. In order to complement a panel of virus-specific real-time PCR assays with internal controls, a convenient approach is described to generate the several internal controls within single DNA fragment. By applying composite primer technology, PCR primer sequences used in real-time PCR assays were added in 5' and 3' of a stretch of heterologous DNA during consecutive preparative PCRs. The heterologous DNA was used for internal control specific detection by e.g. FRET-hybridisation probes. The presented example of such a multiple internal control DNA contained five internal controls for five competitive LightCycler-PCR assays. All five PCR products derived from the multiple internal control DNA were detected with a single pair of specific FRET-hybridisation probes. The example described proved useful in real-time PCR assays specific for the detection of EBV-, CMV-, VZV- HSV-, and HBV-DNA on the LightCycler instrument. This methodology should enable laboratories to conveniently complement their panel of existing real-time PCR assays with a single multiple internal control DNA.

Strappe, P. M., T. H. Wang, et al. (1998). "In situ polymerase chain reaction amplification of HIV-1 DNA in brain tissue." Journal of Virological Methods **70**(2): 119.

http://www.sciencedirect.com/science/article/B6T96-3SBVX4H-1/2/110f9577f665297b8bc637b2348039f5

A direct in situ polymerase chain reaction (IS-PCR) assay is described for the detection of HIV-1 proviral DNA in formalin fixed paraffin embedded brain tissue. Biotin-16-dUTP is incorporated during the PCR process and microwave pretreatment of tissue sections ensures that no non-specific incorporation into damaged or nicked genomic DNA occurs. Two methods are compared to detect the biotinylated amplified product, the use of an avidin-biotin-alkaline phosphatase complex (ABC) and the application of tyramide signal amplification (TSA) which allows both chromogenic and fluorescence detection. TSA detection enhances the sensitivity of IS-PCR, permitting fewer PCR cycles and preserving tissue morphology.

Syrjanen, S., B. Andersson, et al. (1991). "The use of polymerase chain reaction in generation of biotinylated human papillomavirus DNA probes for in situ hybridization." Journal of Virological Methods **31**(2-3): 147.

http://www.sciencedirect.com/science/article/B6T96-47DM18P-H/2/5a673ca2c3dc4bbbf3f5895972b7eca0

The polymerase chain reaction (PCR) was used to produce biotin-labelled human papillomavirus (HPV) 16- and 18-specific DNA probes for in situ hybridization (ISH). PCR was performed by using AmplitaqTM DNA amplification reagent kit according to the manufacturer's instructions, except that dTTP was substituted by different concentrations of biotinylated dUTP (bio-11-UTP). As template DNA, DNA extracted either from CaSki or HeLa cells was used. The reaction mixture

was taken through up to 40 cycles of amplification in a Perkin-Elmer Cetus Thermal Cycler. The highest yield was achieved when the concentrations of dTTP and biotinylated dUTP were 150 and 50 [mu]M, respectively. ISH results compatible with those obtained with biotinylated whole genomic HPV DNA probes were demonstrated when primers from E7 and E6 ORF of the HPV-18 genome were used to produce the biotinylated probe by PCR. With HPV-16, several areas of the genome had to be amplified to generate a PCR probe with equal sensitivity as the whole genomic probe. The background staining was always stronger with the PCR probes than with the whole genomic probes. The sensitivity of the PCR probes does not seem to bear a clear-cut correlation with the size or nucleotide content of the probe, but it might rather depend on the three dimensional structure of the probe and the availability of biotin for the detection system by ISH.

Szemes, M., M. M. Klerks, et al. (2002). "Development of a multiplex AmpliDet RNA assay for simultaneous detection and typing of potato virus Y isolates." Journal of Virological Methods **100**(1-2): 83.

http://www.sciencedirect.com/science/article/B6T96-44M1FHH-8/2/469ee9271f622a5bae98781123d31802

A multiplex AmpliDet RNA assay was developed for the specific detection of potato virus Y (PVY), and for the differentiation of the PVYN, PVYO/C strains and the tuber necrotic isolates (PVYNTN). The assay is based on the generic amplification of a region within the coat protein coding region of all known PVY isolates by nucleic acid sequence-based amplification (NASBA(TM)) and strain-specific detection by molecular beacons. PVYNTN-specific diagnosis is achieved by detecting PVYN and PVYO-specific sequences flanking a recombination site that is associated with the tuber necrotic pathotype. The assay exhibited good specificity toward the various PVY strains in both single and mixed infections. The technique was validated by the use of 47 PVY isolates originating from six countries. The results of the AmpliDet RNA assay were identical or consistent with those of biological characterisation in the decisive majority of cases.

Tai, J. H., M. S. Ewert, et al. (2003). "Development of a rapid method using nucleic acid sequence-based amplification for the detection of astrovirus." Journal of Virological Methods **110**(2): 119.

http://www.sciencedirect.com/science/article/B6T96-48R3KGG-1/2/da81470d0bd99e7695e005ccec634d79

We have developed a rapid method to detect astrovirus in fecal specimens utilizing nucleic acid sequence-based amplification (NASBA) and several detection methodologies, including a sandwich hybridization assay based on DNA-tagged liposomes (liposome-strip detection assay). RNA was extracted from 65 stool specimens that were positive for astrovirus by enzyme immunoassay and was amplified by both NASBA and reverse transcriptase PCR (RT-PCR). Also extracted and amplified were 19 specimens containing rotavirus, 20 specimens containing norovirus, five specimens containing adenovirus, 15 water negative control specimens, and eight specimens containing astrovirus reference strains. NASBA products were detected by electrochemiluminescence detection (ECL) and by liposome-strip detection; RT-PCR products were detected by ethidium bromide staining following gel electrophoresis and by liquid hybridization assay (LHA). There was no significant difference in the detection rates of NASBA- and RT-PCR-based assays, with one exception in which the NASBA/ECL assay detected astrovirus in eight specimens that tested negative by the RT-PCR/LHA assay. These results suggest that these NASBA-based detection methods have detection rates that are as good as or better than those of RT-PCR-based methods. Both NASBA and liposome-strip detection may be useful for field studies and environmental testing because these methods are rapid and do not require specialized equipment.

Tasca, S. I., R. J. Hoffman, et al. (1993). "Detection of Moloney murine sarcoma virus in tissues and cultured cells by the polymerase chain reaction." Journal of Virological Methods **41**(3): 255.

http://www.sciencedirect.com/science/article/B6T96-476F65D-84/2/de74827c1b229f85cf7653d7be953ecb

The polymerase chain reaction was used for Moloney murine sarcoma virus (MoMuSV) detection in frozen and formalin-fixed, paraffin-embedded tissue sections and cultured cells isolated from MoMuSV-induced tumors. Rapid DNA extraction by proteinase K digestion, followed by CHROMA SPIN + TE-100 column purification proved to be satisfactory. Two pairs of overlapping primers, flanking 1026 base pair (bp) to 221 bp, allowed to choose among four different length of DNA-amplified segments. Although net amplification was obtained for frozen tissue and tumor cultured cells in all combinations of primers, the maximum specificity and sensitivity resulted with 602 bp fragment. This product was fully and adequately digestible using Apa I and Sau3A I restriction endonucleases. DNA extracted from paraffinembedded sections yielded an amplification product only when the primer pair which delineated a 221-bp segment was used. This reproducible method could be useful for diagnostic and for pathogenetic investigations of MoMuSV infections.

Telenti, A., P. Imboden, et al. (1992). "Competitive polymerase chain reaction using an internal standard: Application to the quantitation of viral DNA." Journal of Virological Methods **39**(3): 259.

http://www.sciencedirect.com/science/article/B6T96-476F61S-69/2/ecc5c7eaa4887b555bee09b652206856

A general strategy for the construction of an internal standard for the polymerase chain reaction (PCR) is described together with its application in the evaluation of clinical samples. This internal standard is a plasmid containing a modified target sequence that is co-amplified with the native target using the same set of primers. The co-amplification reaction will generate two fragments of different size that are readily separated without the need for restriction enzyme digestion. Thereafter, they are detected and quantitated by hybridization to the same probe. Detection of HIV proviral DNA was chosen as a model for this competitive PCR. The assay proved to be a sensitive tool for the detection of PCR inhibitors and allowed quantitation of HIV with a 20-30% variation coefficient.Despite limitations that appear inherent to the amplification process, internal standards appear to be useful tools for quantitative analysis by PCR.

Thomson, D. and G. Smith (1999). "Herpesvirus genome mapping: a rapid generic approach." Journal of Virological Methods **82**(1): 1.

http://www.sciencedirect.com/science/article/B6T96-3XBV608-1/2/84aeedf0b4d0df6502118200de3091e8

A protocol for mapping the genome of the alphaherpesvirus macropodid herpesvirus 1 is described. This protocol greatly simplifies a similar protocol that was used to map the genome of the poxvirus molluscum contagiosum virus. A single restriction digestion is carried out on the viral DNA, and the fragments cloned into a plasmid vector. The ends of each cloned fragment are sequenced, translated, and used to search peptide sequence databases. Putative genomic maps are constructed by assembling contiguous fragments identified by the sharing of common open reading frames and through the demonstrated colinearity of herpesvirus genomes belonging to

the same subfamily. Oligonucleotide primers designed from the nucleotide sequence at the ends of each cloned fragment enable confirmation of putative contiguous fragments by PCR. Fragments not identified by searches of peptide databases are subcloned using a rapid subcloning method. This approach involves restriction digestion of the cloned fragment with restriction enzymes present in both the multiple cloning site of the vector, and within the fragment. Digested fragments larger than the vector are recircularised and transformed into bacteria to generate subclones for sequence analysis. This subcloning method can also be used to order rapidly genes within large clones.

Thomson, D. and G. Smith (2001). "PCR-based plasmid vector construction for generation of recombinant viruses." Journal of Virological Methods **94**(1-2): 7.

http://www.sciencedirect.com/science/article/B6T96-42Y12XF-2/2/e09addc730d9a9b95ea8228568f29b40

A totally polymerase chain reaction (PCR)-based protocol for construction of plasmids for production of recombinant macropodid herpesvirus 1 (MaHV-1) is described. This protocol greatly simplifies traditional methods that use restriction enzyme-based cloning or a combination of restriction enzyme cloning and the PCR. PCR is used to amplify the vector backbone containing an origin of replication and selectable marker, and the inserts to be cloned (5' and 3' viral homologous recombination regions and the reporter gene green fluorescent protein (GFP)). The inserts are cloned in a sequential manner with the intermediate vectors then amplified to produce the next vector. At its most basic, this involves, after the initial PCR amplification of vector and inserts, two additional PCR amplifications and three ligation events. This protocol is however totally generic, and can be used not only for construction of plasmids for production of recombinant viruses, but also for any general cloning applications.

Trottier, M., B. P. Schlitt, et al. (2002). "Enhanced detection of Theiler's virus RNA copy equivalents in the mouse central nervous system by real-time RT-PCR." Journal of Virological Methods **103**(1): 89.

http://www.sciencedirect.com/science/article/B6T96-453NT2F-4/2/0b435e59016aaecd22d4db3a142d416b

Infection of mice by low-neurovirulence Theiler's murine encephalomyelitis virus (TMEV), such as BeAn and DA viruses, provides a relevant experimental animal model for multiple sclerosis (MS). As a step toward determining the kinetics of a persistent central nervous system (CNS) infection that leads to chronic demyelination, we adapted a rapid, accurate and highly specific real-time reverse transcriptase-polymerase chain reaction (RT-PCR) assay for detection and quantitation of BeAn virus RNA copy equivalents in mouse tissues. The assay enabled detection of as few as 20-30 copies of BeAn virus RNA per [mu]g of total RNA from infected mouse tissues and results for spinal cord revealed the same high levels of BeAn RNA as detected by Northern hybridization during the first 4 months of the persistent infection, but also was able to detect virus RNA copies as late as 1 year post-infection. Real-time RT-PCR analysis of BeAn virus RNA copy equivalents in different parts of the CNS, analyses not possible by Northern hybridization, revealed the following cline of virus persistence: spinal cord>brainstem/cerebellum>cerebrospinal fluid (CSF)>cerebral hemispheres. Systemic organs, including heart, intestine and mesenteric lymph nodes of infected mice, showed no evidence of viral persistence at 4 months post-infection.

Tsuda, F., H. Okamoto, et al. (1999). "Determination of antibodies to TT virus (TTV) and application to

blood donors and patients with post-transfusion non-A to G hepatitis in Japan." Journal of Virological Methods **77**(2): 199.

http://www.sciencedirect.com/science/article/B6T96-3VR1B1H-9/2/e8194397a661b193f8b3f8313875d1a7

Recently, a nonenveloped single-stranded DNA virus named TT virus (TTV) has been reported in association with non-A to G post-transfusion as well as sporadic acute and chronic liver disease. A method was developed for the detection of antibody to TTV (anti-TTV) by means of immune precipitation and detection of TTV DNA by the polymerase chain reaction. The test serum was incubated with TTV, recovered from feces of a carrier, and after incubation, the formed immune complexes were precipitated with goat antiserum to human IgG. TTV DNA was sought for by the polymerase chain reaction in both precipitate and supernatant. The detection of TTV DNA in the precipitate, but not in the supernatant, was considered to represent anti-TTV in the test serum. Of the 44 healthy blood donors in Japan, anti-TTV was detected in one of the six (17%) with TTV DNA and 11 of the 38 (29%) without TTV DNA. In the two patients with post-transfusion non-A to G hepatitis, free anti-TTV developed as they cleared TTV in serum. Anti-TTV complexed with TTV in serum, detectable by precipitating sera with goat anti-human IgG and testing for TTV DNA, elicited while the patients had elevated alanine transaminase levels. The determination of anti-TTV would be useful for detecting resolved infection in surveys for exposure to TTV in the general population, and for establishing the mechanism of liver injury associated with TTV infection.

Van Laethem, K., K. Beuselinck, et al. (1998). "Diagnosis of human immunodeficiency virus infection by a polymerase chain reaction assay evaluated in patients harbouring strains of diverse geographical origin." Journal of Virological Methods **70**(2): 153.

http://www.sciencedirect.com/science/article/B6T96-3SBVX4H-4/2/a6e16bfe458047f13dfdf93acfaa1415

Since the development of the highly sensitive polymerase chain reaction, PCR has been increasingly used for the diagnosis of viral infections, including the detection of human immunodeficiency virus (HIV), the causative agent of AIDS. In our laboratory a diagnostic PCR is carried out on proviral HIV-1 DNA using a standardised algorithm based on three HIV-1 primer sets. The three primer sets, amplifying a fragment in the LTR-gag gene, in the pol gene and in the env gene, are situated within conserved regions of the HIV-1 genome. These primers allow us to detect not only HIV strains from Belgian patients but also from African patients, who are, for historical reasons, a substantial part of the HIV-positive patients in Belgium. We are able to detect 1-5 copies of proviral HIV-1 DNA with each of the three nested primer sets. A sensitivity and specificity of 92 and 100%, respectively, were achieved when testing 24 Belgian and African HIV-1 seropositive samples. In our lab, the same PCRs are also used for the detection of viral RNA in cases of a doubtful undetectable viral load when using a commercial HIV-1 viral load assay. This is because present-day commercial assays are not entirely reliable with divergent strains. Both our 'in-house' diagnostic DNA and RNA-PCR can also be used semiquantitatively with limiting dilutions.

Vandamme, A.-M., S. Van Dooren, et al. (1995). "Detection of HIV-1 RNA in plasma and serum samples using the NASBA amplification system compared to RNA-PCR." Journal of Virological Methods **52**(1-2): 121.

http://www.sciencedirect.com/science/article/B6T96-3YXC151-

X/2/a3afbcd918ae8929dc7f40d7b7f89270

The presence of HIV-1 RNA in the plasma and serum of European and African patients was monitored using RNA-polymerase chain reaction (RNA-PCR) and the new isothermal NASBA nucleic acid amplification system encompassing a gel-based detection assay (ELGA). Identical RNA extraction procedures, provided by the NASBA amplification system, were used for both methods. The detection limit for HIV-1 RNA, measured on a 10-fold dilution series of spiked HIVIIIB in negative plasma, was about 0.05 CCID50 per test for both methods. Both NASBA and RNA-PCR were more sensitive than a p24 assay for the detection of circulating HIV-1 virus in blood: 17 of the 34 (50%) p24 antigen-tested seropositives were p24-positive while 32 (94%) were positive by NASBA and 30 (88%) by RNA-PCR. Among the 45 seropositives, 34 of which were tested for p24 antigen, 43 (96%) were positive by NASBA and 41 (91%) by RNA-PCR. Almost all seropositives had a detectable viral load in 100 [mu]l plasma. Lower viral loads were only encountered in some healthy seropositives with a higher CD4 count. There was no cross-reactivity with HIV-2 or HTLV-I with both the RNA-PCR and NASBA. The extraction method used permitted the detection of HIV-1 RNA equally well in serum and in plasma with heparin or EDTA.

Vanderhallen, H. and F. Koenen (1997). "Rapid diagnosis of encephalomyocarditis virus infections in pigs using a reverse transcription-polymerase chain reaction." Journal of Virological Methods **66**(1): 83.

http://www.sciencedirect.com/science/article/B6T96-3RYCMYM-1D/2/85242a8bdd7275411681e02274e3e176

Encephalomyocarditis virus (EMCV) is widespread and the economic losses caused by an EMCV outbreak in pig holdings and the similarity between a foot-and-mouth disease virus (FMDV) and an EMCV infection in young piglets stress the need for a rapid, specific and broad diagnostic assay. An alternative to the time-consuming seroneutralisation assay, currently used for the characterisation of EMCV, is described. An EMCV specific reverse transcription-polymerase chain reaction (RT-PCR), using primers located in a conserved region of the 3D gene of the viral genome, was developed and tested on 114 different EMCV isolates. The identity of the respective amplicons was confirmed by sequencing. The potential of this assay for future diagnostic purposes was demonstrated by applying the RT-PCR on tissue samples collected from an experimentally infected piglet.

Venter, M., G. Napier, et al. (2000). "Cloning, sequencing and expression of the gene that encodes the major neutralisation-specific antigen of African horsesickness virus serotype 9." Journal of Virological Methods **86**(1): 41.

http://www.sciencedirect.com/science/article/B6T96-3YS34HX-6/2/e135748986aec5916949874fdc8c4b72

A marked improvement in the efficiency of cloning the large double stranded RNA (dsRNA) genome segments of African horsesickness virus (AHSV) was achieved when the dsRNA polyadenylation step was carried out with undenatured rather than strand-separated dsRNA. It is a prerequisite to use dsRNA of very high purity because in the presence of even trace amounts of single stranded RNA, the dsRNA appears to be poorly polyadenylated as judged by its effectiveness as a template for oligo-dT-primed cDNA synthesis. The full-length VP2 gene of AHSV-9, cloned by this approach, was sequenced and it was found to show the highest percentage identity (60%) to VP2 of AHSV-6, providing an explanation of why these two serotypes show some cross protection. The VP2 protein was also expressed in Spodoptera

frugiperda (Sf9) cells by means of a baculovirus recombinant. The yield of the expressed VP2 was high, but the protein was found to be largely insoluble. Nine smaller, truncated VP2 peptides were subsequently expressed in insect cells, but no significant improvement in solubility of the peptides, as compared to that of the full-sized protein, was observed. A western immunoblot analysis of the overlapping peptides indicated the presence of a strong linear epitope located within a large hydrophilic domain between amino acids 369 and 403.

Verhofstedea, C., K. Fransen, et al. (1996). "Isolation of HIV-1 RNA from plasma: evaluation of eight different extraction methods." Journal of Virological Methods **60**(2): 155.

http://www.sciencedirect.com/science/article/B6T96-3W0NTKY-6/2/a3a12d1506e4ca8f8f19793c34cc7317

The efficacy of eight different methods for the extraction of HIV-1 RNA from plasma was compared. The RNA preparation method that gave the best results by RT-PCR was the one described by Chomczynski and Sacchi (1987, Anal. Biochem. 162, 156-159). This method consists of a guanidine thiocyanate treatment followed by three phenol-chloroform-isoamylalcohol extractions and an ethanol precipitation. The disadvantage of this method is that it is time consuming and less suitable for the extraction of large series of samples. Moreover, due to the large number of procedural steps, there is a greater risk of sample mix-up or contamination. Of the single-step RNA purification methods, good results were obtained with the TRIzol method (Gibco Life Technologies, Paisley, UK) and with the extraction method offered by the NASBA kit (Organon Teknika, Turnhout, Belgium). The above single-step methods are recommended since both are sensitive enough to detect low copy numbers of HIV-RNA in the plasma of asymptomatic patients, and require only 2 h for completion.For most of the methods evaluated the inter-test variablity was acceptable (mean variation coefficient between duplicate extraction varied between 17.3 and 47.3%). Inter-laboratory reproducibility was evaluated only for the TRIzol-method and found to be low (mean variation coefficient 63.4).

Vesanen, M., H. Piiparinen, et al. (1996). "Detection of herpes simplex virus DNA in cerebrospinal fluid samples using the polymerase chain reaction and microplate hybridization." Journal of Virological Methods **59**(1-2): 1.

http://www.sciencedirect.com/science/article/B6T96-3VYV049-D/2/81db02d5cb631156ed287cb194b19db9

As conventional polymerase chain reaction (PCR) procedures are time-consuming and laborious, we developed and evaluated a rapid semi-automatic microplate method to detect the amplified PCR products. The use of PCR, with subsequent hybridization in microplates, is described for the detection of herpes simplex virus (HSV) DNA in cerebrospinal fluid samples. The principle of the method is based on two phases. Firstly, the amplification of the viral DNA in the sample is undertaken using a pair of primers of which one is biotinylated. Secondly, the amplified viral genomic sequences are bound to the wells of streptavidin-coated microplates and hybridized with digoxigenin-labeled oligonucleotide probes which are then detected using anti-digoxigenin antibody enzyme conjugates and either a photometric, fluorometric or luminometric substrate and microplate reader. The method is highly sensitive allowing the detection of as few as five purified DNA molecules. Compared to conventional gel electrophoresis followed by Southern blotting the established microplate hybridization is also much less time-consuming and involves less manual work. The applicability of the method is described for use as a routine diagnostic procedure for detection of early central nervous system infections caused by HSV-1 and HSV-2.

Vorndam, V., G. Kuno, et al. (1994). "A PCR-restriction enzyme technique for determining dengue virus subgroups within serotypes." Journal of Virological Methods **48**(2-3): 237.

http://www.sciencedirect.com/science/article/B6T96-476F6FR-DT/2/6fe56633fe4d60a49583d278a9193c34

The polymerase chain reaction (PCR) and restriction enzyme analysis were used to develop a rapid and simple procedure for identifying geographic subgroups of dengue virus within serotypes for epidemiologic investigations. The entire structural protein region of dengue viruses was amplified and the products were digested with the endonucleases AluI or DdeI. By comparing the restriction fragment length polymorphisms (RFLPs), we recognized dengue-2 and dengue-3 subgroups that corresponded to those previously determined by oligonucleotide fingerprinting or genomic sequencing. This procedure can be performed in 2 days without the use of radioisotopes, and results can be interpreted without computer analysis. For those analyses which require only subgroup affiliations, this is a useful tool for rapidly screening multiple virus isolates.

Weilguny, H. and R. P. Singh (1998). "Separation of Slovenian isolates of PVYNTN from the North American isolates of PVYN by a 3-primer PCR." Journal of Virological Methods **71**(1): 57.

http://www.sciencedirect.com/science/article/B6T96-3SY3H5D-8/2/064363d0928e36dcee94b5a9422cbb11

The potato tuber ringspot necrosis isolate of potato virus Y (PVYNTN) is a recently recognized and highly aggressive isolate of the PVYN group of strains. In order to screen specifically sources of resistance to PVYNTN a method to separate PVYNTN from PVYN is needed. To achieve this, 61 isolates from 13 imported and locally developed potato cultivars in Slovenia were studied. On the basis of the reactions in indicator plants Nicotiana tabacum cv. Samsun and Solanum brachycarpum and with a PVYN specific monoclonal antibody (4E7), all Slovenian isolates (Sl-NTN) were identified as PVYN. Using two primer pairs from the P1 gene of a Hungarian isolate of PVYNTN by a conventional single primer pair, reverse transcription polymerase chain reaction (RT-PCR) both PVYNTN and PVYN were amplified similarly. However, specific amplification of PVYNTN was achieved by a nested-PCR at an annealing temperature of 63[deg]C. A simplified form of the nested-PCR, termed 3-primer PCR was developed, which is applicable for large-scale testing of samples. Using the 3-primer PCR at annealing temperature of 63[deg]C, known mixtures of PVYNTN and PVYN were correctly separated. PVYNTN was detected in dormant tubers and leaves from all Sl-NTN isolates. The 3-primer PCR was specific to PVYNTN and did not react with nine isolates of PVYN, 13 isolates of PVYo, one isolate of PVYC, six commonly occurring potato viruses and a viroid.

Weinberger, K. M., E. Wiedenmann, et al. (2000). "Sensitive and accurate quantitation of hepatitis B virus DNA using a kinetic fluorescence detection system (TaqMan PCR)." Journal of Virological Methods **85**(1-2): 75.

http://www.sciencedirect.com/science/article/B6T96-3YGDCNX-8/2/ad601a0499d0fc4e745f78d7b4d9cd97

The laboratory diagnosis of hepatitis B virus (HBV) infection is based mainly on serological assays. Yet the detection and quantitation of viral DNA is necessary when addressing directly the

question of infectivity or when monitoring the viral load during therapy. Standard hybridization assays allow for exact quantitation, but their sensitivity is limited to 105-106 viral genomes per ml of serum. The most sensitive tests for HBV DNA are nested PCR systems, which recognize virtually one molecule of the target DNA per reaction. However, these assays only provide very coarse quantitative statements. To take advantage of both methods, a new assay for HBV DNA is described based on the commercial TaqMan(c) system. This assay is capable of quantifying HBV DNA from the theoretical lower limit up to 1010 genome equivalents per ml of serum and, thus, covers the complete range of naturally occurring states of infections. The method was calibrated on the basis of serial plasmid dilutions and compared with a well-established nested PCR system. More than 100 HBV positive sera and serial dilutions of the Eurohep standard for both ad and ay subtypes were analyzed. The assay reliably detected all HBV positive samples. It shows minimal run-to-run deviations, allows for quantitation that covers eight orders of magnitude, and finally, completely avoids the risk of cross-contamination by PCR products. Thus, this technique combines the sensitivity of PCR amplification and the quantitation potential of hybridization tests and it is time efficient and safer.

Whitby, J. E., P. R. Heaton, et al. (1997). "Rapid detection of rabies and rabies-related viruses by RT-PCR and enzyme-linked immunosorbent assay." Journal of Virological Methods **69**(1-2): 63.

http://www.sciencedirect.com/science/article/B6T96-3S2BMBF-8/2/f1836ca8f6b43d0ddfefd1f8ae781af9

A rapid detection method for the six established genotypes of rabies and rabies-related viral RNA using RT-PCR-ELISA is described. The detection of digoxigenin-labelled amplified products is performed by solution hybridization to two specific, biotin-labelled, capture probes, which are complementary to the inner region of the amplification products. The capture probe and amplified product hybrid are then immobilised on a streptavidin-coated microtitre plate, bound products are detected by an anti-DIG Fab fragment conjugated to peroxidase, and colorimetric reaction automatically measured. This method was up to 100-fold more sensitive than Southern blot hybridization, detecting 0.00002 TCID50/ml of a genotype 1, classical rabies virus strain. The complete detection methodology from RT-PCR to PCR-ELISA detection could be completed within 10 h. Using this procedure, we were 100% successful in detecting 60 isolates from a representative selection of the six established genotypes from all over the world. This test is a useful additional tool for the detection of the rabies and rabies-related viruses, which is easy to perform, rapid and highly sensitive.

Wilborn, F., T. Binder, et al. (1998). "Human herpesvirus type 6 variants identified by single-strand conformation polymorphism analysis." Journal of Virological Methods **73**(1): 21.

http://www.sciencedirect.com/science/article/B6T96-3T2PCND-3/2/fd50b7c2d5c603a4b2e7bb4e2e164683

Six human herpesvirus 6 (HHV-6) variants were analyzed for heterogeneity using the polymerase chain reaction (PCR) and single-strand conformation polymorphism (SSCP). Two independent DNA regions were selected: a fragment of the gene U11 (position 18966-21578) coding for a basic phosphoprotein, the major antigenic structural protein pp100; and a fragment from an open reading frame (ORF) area of the gene U67, previously referred to as 13R (position 102458-103519), coding for a product of unknown function. The two PCR systems based on the above DNA sequences yielded products of 187 bp and 223 bp, respectively. DNA obtained from three laboratory reference strains (U1102, R104 and St.W.) and from HHV-6 infected peripheral white blood cells of bone marrow transplant patients and blood donors was used to test the applicability of two different SSCP analysis systems for the identification of HHV-6 variants using amplicons

derived by PCR from the two genomic regions described above (U11 [pp100], U67 [13R-ORF]). The generation of characteristic SSCP patterns enables the rapid differentiation of HHV-6 A and B strains for the classification of variants derived from clinical samples, reducing the need for expensive and time-consuming direct sequencing analyses.

Williamson, A.-L. and S. J. Dennis (1991). "The use of the polymerase chain reaction for the detection of human papillomavirus type 13." Journal of Virological Methods **31**(1): 57.

http://www.sciencedirect.com/science/article/B6T96-47DM172-6/2/06e1159bac2c9ff344416aed504f309b

Human papillomavirus type 13 (HPV-13) is associated with oral focal epithelial hyperplasia (FEH). The purpose of this study was to establish conditions for the application of polymerase chain reaction (PCR) to the specific detection and amplification of HPV-13 DNA. To design primers for HPV-13 a part of the HPV-13 genome was sequenced first: the smallest BamHI fragment (597 bp) of HPV-13 was subcloned and sequenced. The sequence was found to be part of a large open reading frame and had significant homology with the L1 gene of other HPVs. HPV-13 specific primers were designed to amplify a 240 bp fragment from the L1 gene by PCR. Conditions for PCR were standardized for this set of primers.

Yamaguchi, Y., T. Hironaka, et al. (1992). "Increased sensitivity for detection of human cytomegalovirus in urine by removal of inhibitors for the polymerase chain reaction." Journal of Virological Methods **37**(2): 209.

http://www.sciencedirect.com/science/article/B6T96-476F5YM-5P/2/ba31f393d5b9280ecefaea1201d709a8

The presence of inhibitors in urine interferes with the enzymatic reaction of the polymerase chain reaction (PCR) for detection of human cytomegalovirus (HCMV). To remove inhibitors, HCMV virions in urine were precipitated with polyethylene glycol, or DNA was extracted from urine by the use of glass powder and subjected to PCR followed by Southern blot hybridization with alkaline phosphatase-linked oligonucleotide probes. These simple, rapid methods increased significantly the sensitivity of PCR for detection of HCMV in urine.

Yang, J.-H., J.-P. Lai, et al. (2002). "Real-time RT-PCR for quantitation of hepatitis C virus RNA." Journal of Virological Methods **102**(1-2): 119.

http://www.sciencedirect.com/science/article/B6T96-450HHM7-1/2/a9ecfe7916725d98c498b1dece24ac36

A newly developed real-time RT-polymerase chain reaction assay for quantitation of hepatitis C virus (HCV) RNA in human plasma and serum was applied. A pair of primers and a probe (molecular beacon) were designed that are specific for the recognition of a highly conservative 5'-non-coding region (5'-NCR) in HCV genome. HCV real-time RT-PCR assay had a sensitivity of 1000 RNA copies per reaction, with a dynamic range of detection between 103 and 107 RNA copies. The coefficient variation of threshold cycle (Ct) values in intra- and inter-runs were less than 1.37 and 4.66%, respectively. The real-time RT-PCR assay on the HCV sero-positive samples yielded reproducible data, with less than 2.09% of the inter-assay variation. In order to determine its potential for clinical diagnosis, real-time RT-PCR was used to examine the HCV

RNA levels in plasma from sero-positive and negative subjects, showing that the assay is highly sensitive and has specificity of 100%. It was demonstrated that the real-time RT-PCR was able to amplify HCV RNA in reference sera with seven genotypes (1A, 1B, 2B, 3A, 4, 5A and 6A) that include six major HCV genotypes circulated in the world. Since HCV is a major pathogen of post-transfusion and community-transmitted non-A, non-B hepatitis, this assay has a broad application for basic and clinical investigations.

Ying, C., J. Van Pelt, et al. (1999). "Use of digoxigenin-labelled probes for the quantitation of HBV-DNA in antiviral drug evaluation." Journal of Virological Methods **81**(1-2): 155.

http://www.sciencedirect.com/science/article/B6T96-3XMPJNX-N/2/09b8fc47c5efc19fda48eb13900d78f5

The use of digoxigenin-labelled probes was studied for quantitation of HBV-DNA during antiviral drug evaluation. Digoxigenin (dig)-labelled probes were generated either via incorporation of dig-dUTP in a polymerase chain reaction (PCR) or a random priming reaction. Using the PCR-labelled probe (delineating a 523 bp fragment in the core gene of the HBV) as little as 1 pg of immobilized HBV-DNA could be detected following an 8 h exposure of the hybridized membrane. A close correlation (r=0.95) was found between the amount of HBV-DNA (range 2.5-200 pg) and the signal generated by the probe hybridized to its target DNA. By using a probe that was labelled with digoxigenin via random priming, the minimal quantity of immobilized HBV plasmid DNA that could be detected following an 8 h exposure was 4 pg, whereas a 32P-labelled probe, generated in parallel by random priming, allowed the detection of 16 pg of HBV plasmid DNA following a 4-day exposure. The PCR-generated digoxigenin-labelled probe proved to be useful for antiviral drug evaluation, i.e. to detect HBV-DNA in total cellular DNA from HBV-positive hepatoma cells (HepG2.2.15) that had either been treated with reference antiviral agents or left untreated. The 50% effective concentrations (EC50) that were calculated for inhibition of HBV-DNA production by lamivudine (3TC), penciclovir (PCV), lobucavir (LBV), adefovir (PMEA) and tenofovir (PMPA) were comparable to those reported in the literature. The use of digoxigenin-labelled probes thus appears to be a simple, convenient, rapid, reliable and non-radioactive method for use for anti-HBV screening. In addition, and in contrast to 32P-labelled probes, digoxigenin-labelled probes can be stored for >1 year without loss of specific activity, which makes these probes particularly attractive for large-scale antiviral drug evaluation purposes.

Yu, Q., N. Hu, et al. (2001). "Rapid acquisition of entire DNA polymerase gene of a novel herpesvirus from green turtle fibropapilloma by a genomic walking technique." Journal of Virological Methods **91**(2): 183.

http://www.sciencedirect.com/science/article/B6T96-426XYG8-B/2/4d5ace1b6ced9aff356ac5778285f744

A 4837-bp sequence of a newfound green turtle herpesvirus (GTHV), implicated in the etiology of green turtle fibropapilloma, was obtained from tumor tissues of a green turtle with fibropapilloma using a genomic walking method based on restriction enzyme digestion, self-ligation and inverse polymerase chain reaction (IPCR). The 4837-bp sequence was 56.23% G/C rich and contained three nonoverlapping open reading frames (ORF). The largest ORF (3507-bp) encoded the DNA polymerase gene (pol gene), which exhibited a high degree of homology at both amino acid and nucleotide levels with the DNA pol genes of human and animal herpesviruses, with a predicted protein of 1169 amino acids and molecular weight of 132.6 kilodaltons. The ATG at 518 to 520 was the first initiation codon in the ORF and was presumed to be the first methionine codon of the pol gene. Phylogenetic analysis, based on the amino acid sequence of the GTHV DNA pol gene and the corresponding regions of other known human and animal herpesviruses, indicated that

GTHV belonged to the Alphaherpesvirinae subfamily. The upstream ORF of the pol gene encoded the N-terminal region of the GTHV homologue of the DNA-binding protein gene, whereas the downstream ORF was the C-terminal region of a gene which was homologous to ORFs conserved in human and animal herpesviruses, i.e., herpes simplex virus 1 (HSV1) gene UL31, Epstein-Barr virus (EBV) gene BFLF2, equine herpesvirus 1 (EHV1) gene 29, and alcelaphine herpesvirus 1 (AHV1) hypothetical protein 69 gene. The arrangement of these three genes in GTHV genome was identical to that seen in other alphaherpesviruses. The sequence and location of the DNA pol gene in the GTHV genome should greatly facilitate future studies of the viral life cycle.

Zhang, H., B. Soteriou, et al. (1997). "Characterisation of genomic RNA of Coxsackievirus B3 in murine myocarditis: reliability of direct sequencing of reverse transcription-nested polymerase chain reaction products." Journal of Virological Methods **69**(1-2): 7.

http://www.sciencedirect.com/science/article/B6T96-3S2BMBF-2/2/386b5d4dd1c82c240be7445ad7a5aba5

SWR mice develop viral myocarditis histologically similar to the human disease following inoculation with a cardiovirulent Coxsackievirus B3 (CVB3), reactivated from a sequenced cDNA clone of Nancy strain. A sequence of 215 nucleotides, or 628 nucleotides in representative cases, of the 5'non-translated region (5'NTR) of CVB3 genome was amplified from myocardial samples of the infected mice by reverse transcription-nested polymerase chain reaction (RT-NPCR). In order to verify the viral nucleotide sequence and detect the mutation frequency of the viral RNA, the nucleotide sequence of NPCR products were determined by direct sequencing in both orientations. The amplified products from mouse heart on day 1-13 post-inoculation were sequenced and, in each case, the consensus sequence was identical to the published sequence of CVB3 (Nancy strain). To evaluate further the reproducibility of these techniques, three tissue samples from the same infected mouse heart were processed independently. Sequences of their RT-NPCR products were identical to each other as well as to the published sequence. When two attenuated CVB3 mutants were amplified and sequenced, single mutations were detected. To evaluate the overall fidelity of these two combined techniques, genomic RNA of a different CVB3 Nancy strain stock, Coxsackievirus A9 or poliovirus sabin 1 was amplified and the NPCR products sequenced. Each product showed 100% homology with its published sequence. These results demonstrate that the coupled technique of the enterovirus RT-NPCR with direct sequencing of NPCR products generates accurate consensus sequence data and this technique proved to be useful in verification of enteroviral amplicons and in detection of nucleotide mutations. In addition, a low mutation frequency was found in the 5'NTR of CVB3 detected in myocardial samples of immunocompetent mice up to 13 days.

Zimmermann, W., R. Durrwald, et al. (1994). "Detection of Borna disease virus RNA in naturally infected animals by a nested polymerase chain reaction." Journal of Virological Methods **46**(2): 133.

http://www.sciencedirect.com/science/article/B6T96-476F69S-BC/2/b2c6ea7294f02a31ba9e8a947b4de423

Borna disease virus in naturally infected horses, a donkey and sheep was detected for the first time by amplification of viral RNA using PCR. In contrast to a control group of healthy horses, brain tissue was positive by this assay in all animals with neurological symptoms. The use of a second round of PCR with nested primers following Southern hybridization confirmed the specificity and increased the sensitivity of the test. Comparison with conventional methods recommends this technique for monitoring of BDV infections at a molecular level.

Zingg, W., W. Bossart, et al. (1999). "Detection and quantification of cell-free Epstein-Barr virus by polymerase chain reaction and subsequent DNA enzyme immunoassay." Journal of Virological Methods **79**(2): 141.

http://www.sciencedirect.com/science/article/B6T96-3WJDTS6-3/2/20d346e54823d0c479dde2d2deef0326

Amplification by polymerase chain reaction and subsequent DNA enzyme immunoassay (DEIA) were employed to determine the number of genome equivalents of cell-free Epstein-Barr virus (EBV) DNA in peripheral blood. The assay detected cell-free EBV DNA in the serum of 14 out of 18 patients with primary, productive EBV infection (sensitivity 77.7%) but not in healthy EBV carriers with latent infection (specificity 100%). Our assay has the potential for a clinical diagnostic tool to monitor patients at risk for EBV reactivation and productive infection with subsequent EBV-induced lymphoproliferative diseases.

Life Sciences (31)

Bogdarina, I., H. C. Murphy, et al. (2004). "Investigation of the role of epigenetic modification of the rat glucokinase gene in fetal programming." Life Sciences **74**(11): 1407.

http://www.sciencedirect.com/science/article/B6T99-4B42FPW-4/2/a9308159457ab5a29ef64848d6018514

Fetal malnutrition is associated with development of impaired glucose tolerance, diabetes and hypertension in later life in humans and several mammalian species. The mechanisms that underlie this phenomenon of fetal programming are unknown. We hypothesize that adverse effects in utero and early life may influence the basal expression levels of certain genes such that they are re-set with long-term consequences for the organism. An excellent candidate mechanism for this re-setting process is DNA methylation, since post-natal methylation patterns are largely established in utero. We have sought to test this hypothesis by investigating the glucokinase gene (Gck) in rat offspring programmed using a maternal low protein diet model (MLP). Northern blot reveals that fasting levels of Gck expression are reduced after programming, although this distinction disappears after feeding. Bisulphite sequencing of the hepatic Gck promoter indicates a complete absence of methylation at the 12 CpG sites studied in controls and MLP animals. Non-expressing cardiac tissue also showed no DNA methylation in this region, whereas brain and all fetal tissues were fully methylated. These findings are not consistent with the hypothesis that programming results from differential methylation of Gck. However, it remains possible that programming may influence methylation patterns in Gck at a distance from the promoter, or in genes encoding factors that regulate basal Gck expression.

Caronti, B., C. Calderaro, et al. (1998). "Dopamine receptor mRNAs in the rat lymphocytes." Life Sciences **62**(21): 1919.

http://www.sciencedirect.com/science/article/B6T99-3T0TY5N-2/2/20f3c6d6de1728310fc1466e4fcd090e

It has been suggested that dopamine might play a role in the regulation of the immune system. In this study, reverse transcriptase-polymerase chain reaction (RT-PCR) was used to investigate the expression of mRNA for the different subtypes of dopamine receptors in the rat lymphocytes. D1, D3 and D5 receptor mRNAs were identified. These results provide further evidence for the interaction of dopamine systems and the immune system, and suggest to further investigate whether the immunosuppressive actions of dopamine and dopaminergic drugs might depend on a direct interaction with dopamine receptors on the lymphocyte membrane. Moreover, they suggest the suitability of this animal species to further investigate the correlation between changes in the expression of central and peripheral dopamine receptors produced by manipulations of the dopamine systems.

Chang, S. L., J. Bersig, et al. (2000). "Chronic cocaine alters hemodynamics and leukocyte-endothelial interactions in rat mesenteric venules." Life Sciences **66**(24): 2357.

http://www.sciencedirect.com/science/article/B6T99-40B7XB5-5/2/0e750bce2094b175774c2b5a74ca74aa

We investigated the effects of chronic cocaine exposure on the microcirculation in the rat mesenteric venules under both non-inflammatory and FMLP-induced inflammatory conditions. Chronic cocaine significantly increased WBC rolling flux in both conditions, and potentiated FMLP-induced leukocyte-endothelial cell adhesion (LEA). In cocaine-treated animals, total WBC number increased by 91%, and the ratio of white blood cell to red blood cell velocity was significantly lower, while vessel diameter was unchanged. Chronic cocaine decreased serum levels of tumor necrosis factor alpha (TNF-[alpha]) and interleukin-6 (IL-6), but had no effect on interleukin-1 beta (IL-1[beta]). Expression of intercellular adhesion molecule-1 (ICAM-1) was increased in mesenteric venules following chronic cocaine exposure, and may be one of the mechanisms underlying enhancement of FMLP-induced LEA. The increase in WBC count, WBC flux and LEA, and the change in cell velocity seen in the cocaine-treated animals could cause a decrease in effective vessel diameter and a change in intravascular resistance, and may underlie the progressive vascular damage seen in chronic cocaine-abusing individuals.

Cushing, D. J., M. Baez, et al. (1994). "Serotonin-induced contraction in canine coronary artery and saphenous vein: Role of a 5-HT1D-like receptor." Life Sciences **54**(22): 1671.

http://www.sciencedirect.com/science/article/B6T99-4751TMH-2NR/2/e8c4e22c9af4e5021bce604d3757f5d3

The identity of the serotonin (5-HT) receptor(s) that mediate(s) contraction in canine coronary artery and saphenous vein remains controversial. Ring segments of endothelium-denuded coronary artery and helical strips of saphenous vein were suspended in organ chambers for measurement of isometric force. 5-HT, [alpha]Me-5-HT and sumatriptan contracted both coronary artery and saphenous vein and the non-selective 5-HT receptor antagonist 1-naphthylpiperazine (100nM) blocked 5-HT-and sumatriptan-induced contraction in both tissues. The agonist rank order potency for contraction (5-HT>sumatriptan>[alpha]Me5-HT>5-MeOT>5-MeT) was similar in both tissues and was consistent with that for a 5-HT1D receptor. Oligonucleotide primers specific for the 5-HT1D receptor sequence were designed for use in a polymerase chain reaction (PCR). cDNA derived from total RNA or mRNA from canine tissues was used in the PCR. PCR resulted in the amplification of a 632 base pair sequence in both canine coronary artery and saphenous vein; consistent with that expected for the 5-HT1D receptor. Southern blot analysis, with an oligonucleotide probe internal to the sequence amplified by the PCR primers, confirmed that the sequence amplified by PCR was the 5-HT1D receptor. Thus, the 5-HT1D receptor is expressed in canine coronary artery and saphenous vein and taken together with the pharmacological data,

supports the possibility that a 5-HT1D-like receptor mediates contraction in these two tissues.

Farooqui, S. M. (1994). "Induction of adenylate cyclase sensitive dopamine D2-receptors in retinoic acid induced differentiated human neuroblastoma SHSY-5Y cells." Life Sciences **55**(24): 1887.

http://www.sciencedirect.com/science/article/B6T99-4754KHC-2/2/cdd8ff78ab5e53087ad8ea0f96dcfc3d

Dopamine D2 receptor (D2-receptor) expression and its coupling to Gi sensitive adenylate cyclase was investigated in human neuroblastoma SHSY-5Y cells. Incubation of SHSY-5Y cells in the presence of 100 nM retinoic acid (RA) for 24 hours resulted in phenotypic differentiation accompanied by a 47% increase in D2-receptor mRNA and a significant increase in the specific binding of a D2-receptor antagonist, [3H]YM09151-2. Stimulation of D2-receptors in differentiated cells by LY171-555, a D2-agonist, attenuated cellular cAMP levels by 30%. The effect of LY171-555 on cAMP levels was blocked by the D2-antagonist, (-)sulpride. Application of these drugs to control undifferentiated cells or differentiated cells incubated with vehicle only had no effect on cellular cAMP levels. These studies suggest that differentiated SHSY-5Y cells express functional D2-receptors and will provide a useful model for future studies on the regulation of expression and function of D2-receptors in cellular differentiation of neuronal cells.

Fujii, T., T. Okuda, et al. (2003). "Detection of the high-affinity choline transporter in the MOLT-3 human leukemic T-cell line." Life Sciences **72**(18-19): 2131.

http://www.sciencedirect.com/science/article/B6T99-47X0WMJ-1/2/bd4a23563cf1c232fb845e187c9bc057

We previously showed that lymphocytes possess the necessary components to constitute an independent, non-neuronal cholinergic system; these include acetylcholine (ACh) itself, choline acetyltransferase (the ACh-synthesizing enzyme), and both muscarinic and nicotinic ACh receptors (AChRs). In addition, we showed that stimulation of AChRs with their respective agonists elicits a variety of biochemical and functional effects, suggesting that lymphocytic cholinergic system is involved in the regulation of immune function. In nerve terminals, choline taken up via the high-affinity choline transporter (CHT1) is exclusively utilized for ACh synthesis. In the present study, therefore, we investigated the expression of CHT1 in T-lymphocytes. Reverse transcription-polymerase chain reaction analysis revealed that MOLT-3 cells, a human leukemic T-cell line used as a T-lymphocyte model, expressed CHT1 mRNA, but that the CEM and Jurkat T-cell lines did not. Consistent with that finding, specific binding of [3H]hemicholinium-3 (HC-3), an inhibitor of CHT1, and HC-3-sensitive [3H]choline uptake were also detected in MOLT-3 cells. These results suggest that CHT1 plays a role in mediating choline uptake in T-lymphocytes and provides further evidence for the presence of an independent lymphocytic cholinergic system.

Funaki, N., J. Tanaka, et al. (1995). "Highly-sensitive identification of [alpha]-fetoprotein mRNA in circulating peripheral blood of hepatocellular carcinoma patients." Life Sciences **57**(17): 1621.

http://www.sciencedirect.com/science/article/B6T99-3YYTH72-41/2/8c8e01ec8bc3030febce7238e62e5249

In order to capture hepatocellular carcinoma (HCC) cells in circulating peripheral blood, we made

analysis to see if a -fetoprotein (AFP) mRNA exists in the peripheral blood obtained from patients with HCC and also, as a control, from hepatitis-viral-marker-positive patients without HCC and a healthy volunteer. As the number of HCC cells in peripheral blood and the quantity of AFP mRNA are expected to be very small, the analysis was performed by the reverse transcription followed by an original three-step polymerase chain reaction. By this highlysensitive method, 5 of 7 HCC patients were positive for AFP mRNA. These 5 positive patients consisted of three with clinically apparent recurrence, one preoperative patient with tumor thrombus in the portal vein and one recurrence-free patient who developed clinically detectable recurrence three months after this analysis. Neither 4 patients with positive viral markers nor a healthy volunteer was positive. The results suggest that detection of AFP mRNA from HCC patients' peripheral blood by our highly-sensitive RT-PCR may be a practical and powerful tool to diagnose the preoperative spreading of HCC and to monitor its recurrence.

Funaki, N. O., J. Tanaka, et al. (1998). "Quantitative analysis of alpha-fetoprotein mRNA in circulating peripheral blood of patients with hepatocellular and alpha-fetoprotein-producing gastric carcinomas." Life Sciences **62**(21): 1973.

http://www.sciencedirect.com/science/article/B6T99-3T0TY5N-9/2/b812097676b1bdb7537297815312ae12

In conjunction with strategies introduced in recent years to identify cancer micrometastasis through amplification of cancer-associated mRNA, we developed a highly sensitive system to detect [alpha] -fetoprotein mRNA in circulating peripheral blood of hepatocellular carcinoma patients. The aim of the present study was to make our original system quantitative. Peripheral venous blood from patients with hepatocellular carcinoma and [alpha] -fetoprotein-producing gastric carcinoma was subjected to reverse transcription followed by our original three-step polymerase chain reaction co-amplifying both the original sequence and our synthetic competitor. We succeeded in modifying our system for quantitative analysis, and investigated the perioperative change, the postoperative change and the change after chemotherapy in order to illustrate the possible application of this method. The quantitative analysis of [alpha]-fetoprotein mRNA present in the peripheral blood represents a useful tool for analyzing the relationship of surgery to recurrence, the effect of chemotherapy, and to predict impending recurrence in patients with hepatocellular and [alpha]-fetoprotein-producing gastric carcinomas.

Funaki, N. O., J. Tanaka, et al. (1997). "Detection of colorectal carcinoma cells in circulating peripheral blood by reverse transcription-polymerase chain reaction targeting cytokeratin-20 mRNA." Life Sciences **60**(9): 643.

http://www.sciencedirect.com/science/article/B6T99-3RHNJK5-N/2/02703f4bc1c5fff59b100070ea40087f

For the detection of circulating colorectal carcinoma cells, we investigated the presence of cytokeratin 20 (CK 20) mRNA in the peripheral blood of colorectal carcinoma patients. Application of our published technique resulted in analysis by reverse transcription followed by three-step nested polymerase chain reaction. This analysis could detect a single Colo 205 colon cancer cell mixed with 1 ml of blood. Our system also successfully detected the presence of CK 20 mRNA in actual patients' peripheral blood samples. Our highly sensitive and specific system for the detection of CK-20 mRNA from patients' peripheral blood thus seems to be useful for screening for circulating colorectal carcinoma cells.

Hattori, M.-a., T. Fujioka, et al. (2002). "Preferential expression of ADP-ribosylation factor gene in the chick embryonic gonads." Life Sciences **70**(17): 2031.

http://www.sciencedirect.com/science/article/B6T99-459BCHR-1/2/5c86818754f4e6794f8644e39de94a3d

cDNA cloning from chick embryonic gonads subtracted from tissues of the brain, heart, liver, gizzard, mesonephros and skeletal muscle was performed to identify genes with expression unique to embryonic gonads. Several cDNA clones encoding characterized as well as many uncharacterized genes were obtained. ADP-ribosylation factor (ARF) of these identified genes was preferentially expressed in the chick embryonic ovary and testis as revealed by reverse transcription-polymerase chain reaction analysis. Expression of the ARF was evaluated through embryonic development, but no difference in the transcript (relative to glyceraldehyde-3-phosphate dehydrogenase transcript) was observed between the left and right ovaries, and between the ovary and testis. In addition, the ARF transcript was detected in the gonads on embryonic days 5 to 21. These findings indicate that the ARF is constantly, but preferentially expressed in the embryonic gonads during development.

Hou, M., M. Malmsjo, et al. (1999). "Increase in cardiac P2X1- and P2Y2-receptor mRNA levels in congestive heart failure." Life Sciences **65**(11): 1195.

http://www.sciencedirect.com/science/article/B6T99-3X9YSFN-C/2/3370cafd4258c51d9079609e7f1413b8

We wanted to study the expression of P2-receptors at the mRNA-level in the heart and if it is affected by congestive heart failure (CHF). To quantify the P2 receptor mRNA-expression we used a competitive RT-PCR protocol which is based on an internal RNA standard. The P2 receptor mRNA-expression was quantified in hearts from CHF rats and compared to sham-operated rats. Furthermore, the presence of receptor mRNA was studied in the myocardium from patients with heart failure. In the sham operated rats the G-protein coupled P2Y-receptors were expressed at a higher level than the ligand gated ion-channel receptor (P2X1). Among the P2Y-receptors the P2Y6-receptor was most abundantly expressed (P2Y6 > P2Y1 > P2Y2 = P2Y4 > P2X1). A prominent change was seen for the P2X1- and P2Y2-receptor mRNA levels which were increased 2.7-fold and 4.7-fold respectively in the myocardium from the left ventricle of CHF-rats. In contrast, the P2Y1-, P2Y4- and P2Y6-receptor mRNA levels were not significantly altered in CHF rats. In human myocard the P2X1-, P2Y1-, P2Y2-, P2Y6- and P2Y11-receptors were detected by RT-PCR in both right and left atria and ventricles, while the P2Y4-receptor band was weak or absent. In conclusion, most of the studied P2-receptors were expressed in both rat and human hearts. Furthermore, the P2X1- and P2Y2-receptor mRNA were upregulated in CHF, suggesting a pathophysiological role for these receptors in the development of heart failure.

Inoue, K., S. Koizumi, et al. (2003). "Signaling of ATP receptors in glia-neuron interaction and pain." Life Sciences **74**(2-3): 189.

http://www.sciencedirect.com/science/article/B6T99-49W2118-2/2/24d745003441ab7ebfb44ddabb096dbb

ATP causes the activation of p38 or ERK1/2, mitogen activated protein kinases (MAPKs) resulting in the release of tumor necrosis factor-[alpha] (TNF) and Interleukin-6 (IL-6) from microglia. We examined the effect of TNF and IL-6 on the protection from PC12 cell death by serum deprivation. When PC12 cells were incubated with serum-free medium for 32 hr, their

viability decreased to 30 %. IL-6 alone slightly protected the death of PC12 cells, whereas TNF alone did not show any protective effect. In the meanwhile, when PC12 cells were pretreated with TNF for 6 hr and then incubated with IL-6 under the condition of serum-free, the viability of PC12 cells dramatically increased. TNF induced an increase of IL-6 receptor (IL-6R) expression in PC12 cells at 4-6 hr. These data suggested that 6 hr pretreatment with TNF increased IL-6R expression in PC12 cells, leading to an enhancement of IL-6-induced neuroprotective action.To elucidate the role of p38 in pathological pain, we investigated whether p38 is activated in the spinal cord of the neuropathic pain model. In the rats displaying a marked allodynia, the level of phospho-p38 was increased in the microglia of injury side in the dorsal horn. Intraspinal administration of p38 inhibitor suppressed the allodynia. These results demonstrate that neuropathic pain hypersensitivity depends upon the activation of p38 signaling pathway in microglia in the dorsal horn following peripheral nerve injury.

Kageyama, H., T. Osaka, et al. (2003). "Fasting increases gene expressions of uncoupling proteins and peroxisome proliferator-activated receptor-[gamma] in brown adipose tissue of ventromedial hypothalamus-lesioned rats." Life Sciences **72**(26): 3035.

http://www.sciencedirect.com/science/article/B6T99-487F0PJ-2/2/4938700a27ee12f04f24566883c266c9

Uncoupling proteins (UCPs) are supposed to be involved in diet-induced thermogenesis. Their activities are usually elevated by feeding and reduced by fasting in normal animals. To investigate whether fasting affects the expression of UCPs mRNA in brown adipose tissue (BAT) of bilateral ventromedial hypothalamus (VMH)-lesioned rats, we determined the gene expression of UCP1, UCP2 or UCP3 in BAT of VMH-lesioned rats and examined oxygen consumption in these rats under fed or 48-h fasted conditions. Northern blotting revealed no difference in the expression of UCPs mRNA in BAT between VMH-lesioned and sham-operated rats under the fed condition, however, expressions were increased markedly in BAT of VMH-lesioned rats under the fasted condition. Under the fed condition, no difference in oxygen consumption was observed between VMH-lesioned and sham-operated rats. Under the fasted condition, oxygen consumption decreased in both rats, however, it decreased in VMH-lesioned less than in sham operated rats. To explore the mechanism that fasting elevated BAT UCPs mRNA in VMH-lesioned rats, we measured peroxisome proliferator-activated receptor (PPAR)-[gamma] mRNA and protein in BAT, because PPAR-[gamma] agonist can elevate UCPs mRNA levels in BAT. Under the fed condition, no differences in the expression of PPAR-[gamma] mRNA and protein content were observed between in BAT of VMH-lesioned and sham-operated rats. Under the fasted condition, however, both increased in BAT of VMH-lesioned rats. These results suggest that VMH-lesions enhance the gene expression of UCPs in BAT under long-term fasting as a defensive reaction to inhibit the reduction of body temperature through an increase in PPAR-[gamma] activity.

Katavic, V., D. Grcevic, et al. (2003). "Non-functional Fas ligand increases the formation of cartilage early in the endochondral bone induction by rhBMP-2." Life Sciences **74**(1): 13.

http://www.sciencedirect.com/science/article/B6T99-49STN7H-3/2/6e893ea6358ff4848b9dad50ce6aacb0

It has previously been shown that mice with a defect in Fas ligand-mediated apoptosis have an enhancement of ectopic bone formation. We investigated the expression of bone-related markers - alkaline phosphatase, collagen, bone sialoprotein, osteocalcin, osteopontin, and bone morphogenetic proteins (BMP) -2, -4, and -7; and cytokines interleukin-1[alpha] (IL-1), IL-1[beta], and tumor necrosis factor-[alpha] (TNF-[alpha]) in ectopic new bone induced by recombinant human (rh) BMP-2 in mice without functional Fas-ligand (gld mice). At day 6 after rhBMP-2

implantation, gld mice formed more cartilage and mesenchyme compared with their wild type littermates. At later stages, gld mice did not differ from the control mice in the volume of newly formed tissue, expressing higher level of BMP genes and lower levels of genes involved in osteoblast maturation - bone sialoprotein and osteopontin. Differences in the levels of expression of IL-1[alpha] and TNF-[alpha] were observed only at day 12 after rhBMP-2 implantation. These results suggest that gld mice have an increased recruitment of cells of mesenchymal origin and an abnormal pattern of differentiation and maturation of the newly formed mesenchymal tissues.

Kishimoto, Y., K. Wada, et al. (1997). "Quantitative analysis of cyclooxygenase-2 gene expression on acute gastric injury induced by ischemia-reperfusion in rats." Life Sciences **60**(8): PL127.

http://www.sciencedirect.com/science/article/B6T99-3RHNJK5-15/2/8852d99180513dfe8666b1461fa88f5b

The rat model of acute gastric damage induced by ischemia-reperfusion (I-R) has been used to evaluate the protective effect of various drugs on gastric injury. However, the quantitative expression state of cyclooxygenase-2 (COX-2), a protein which induces cytoprotective prostaglandins during inflammation, is still unknown in acute gastric injuriy induced by I-R. Therefore, we have quantitatively investigated the level of expression of COX-2 mRNA in injured gastric tissue of this model using the reverse transcription-competitive polymerase chain reaction method. The mRNA for COX-2 was expressed at low or undetectable levels in the normal gastric tissues in control rats, which were fasted for 18 hrs without I-R. The mRNA levels of COX-2 in injured gastric tissues were higher than those of control tissues between 6 hrs and 48 hrs after I-R. The maximum expression of COX-2 mRNA was recorded at 24 hrs (approximately a 200-fold increase). The expression state of COX-2, which has been ascertained in this study, should be useful in evaluating the effect of various drugs on the expression of COX-2 in acute gastric damage.

Liu, Z.-J., M. Maekawa, et al. (2003). "The multiple promoter methylation profile of PR gene and ER[alpha] gene in tumor cell lines." Life Sciences **73**(15): 1963.

http://www.sciencedirect.com/science/article/B6T99-490H4NG-1/2/792c1e218beb6e76ecf29256ce844f35

The changes of methylation status of various gene promoters are a common feature of malignant cells and these changes can occur early in the progression process. Therefore, abnormal methylation can be used as cancer marker. Such studies will first require the development of a panel of methylated markers that are methylated in cancer tissues but unmethylated in normal tissues or methylated status is different between cancer tissues and normal tissues. By using methylation-specific PCR (MSP) assay method, we observed alterations in DNA methylation at the double promoter regions of the progesterone receptor (PR) gene and estrogen receptor (ER[alpha]) gene in various tumor cell lines. Compared with normal white blood cell, the methylation status of PRA promoter in various cancer cell lines changed from unmethylation pattern to methylation pattern. That of PRB promoter changed from both unmethylated and methylated alleles to only methylated allele. The methylation status of ER[alpha]-A and ER[alpha]-B promoter in various cancer cell lines are cell -specific. This study indicates that PR promoter methylation may be a molecular marker in various cancer detections. And the methylation status of ER[alpha]-A and ER[alpha]-B is cell-specific.

Mao, T. K., J. Powell, et al. (2000). "The effect of cocoa procyanidins on the transcription and secretion of interleukin 1[beta] in peripheral blood mononuclear cells." Life Sciences **66**(15): 1377.

http://www.sciencedirect.com/science/article/B6T99-3YXB9SD-1/2/71a41c13f5ebaf4156571a6df0ac2a89

Recent data has demonstrated that cacao liquor polyphenols (procyanidins) have antioxidant activity, inhibit mRNA expression of interleukin-2 and are potent inhibitors of acute inflammation. Given the widespread ingestion of cocoa in many cultures, we investigated whether cocoa, in its isolated procyanidin fractions (monomer through decamer), would modulate synthesis of the pro-inflammatory cytokine, interleukin-1[beta]. Both resting and phytohemagglutinin (PHA)-stimulated peripheral blood mononuclear cells (PBMC) were investigated at the levels of transcription and protein secretion. Individual cocoa fractions were shown to augment constitutive IL-1[beta] gene expression, although values varied between subjects. Interestingly, the smaller fractions of cocoa (monomer-tetramer) consistently reduced IL-1[beta] expression of PHA-stimulated cells by 1-15%, while the larger oligomers (pentamer-decamer) increased expression by 4-52%. These data, observed at the transcription level, were reflected in protein levels in PHA-induced PBMC. The presence or absence of PHA did not alter the effects of the cocoa procyanidins with the exception of the pentamer. This study offers additional data for the consideration of the health-benefits of dietary polyphenols from a wide variety of foods, including those benefits associated specifically with cocoa and chocolate consumption.

Mengheri, E., L. Ciapponi, et al. (1996). "Cytokine gene expression in intestine of rat during the postnatal developmental period: Increased IL-1 expression at weaning." Life Sciences **59**(15): 1227.

http://www.sciencedirect.com/science/article/B6T99-3W2T831-6/2/2f8bf146ca53c502e0d6302462c6e5ac

In the present study we have investigate whether cytokines are constitutively and differently expressed in intestine during the differentiative processes that take place at weaning. We have analyzed the expression of IL-1[beta], IL-2, IL-4 and IFN[gamma] by polymerase chain reaction in Peyer's patches (PP) and in intestine deprived of PP (I-PP) of rats from 16 to 30 days of age. The results showed a constitutive and marked expression of the cytokines already before weaning, with the exception of IL-2 in PP and IFN[gamma] in I-PP. IL-[beta] was the only cytokine to show a different expression at various ages with an initial increase at 19 days and a further elevation at 21 days when intestinal epithelium passes through major differentiative stages, suggesting an involvement of this cytokine in intestinal development.We have also tested whether treatment of rats with the immunosuppressor cyclosporin A (CsA) could affect intestinal differentiation. The results showed that only some markers of differentiation were affected (proliferation of staminal crypt cells and length of crypts). This was probably due to a direct effect rather than an immunomediated effect of CsA, since treatment of three intestinal cell lines (Caco-2, HT-29, FRIC) with CsA indicated that this drug can exert a cytostatic activity on intestinal cells.

Miyamoto, A., Y. Yamazaki, et al. (2003). "Enhancement of endotoxin-induced vascular hyporeactivity to phenylephrine in the thoracic aortas of Mg-deficient rats ex vivo." Life Sciences **73**(21): 2713.

http://www.sciencedirect.com/science/article/B6T99-49H6BGJ-3/2/731d1f2fc76fbed391d32945c0e9ae05

Since endotoxin lethality is enhanced by Mg deficiency in animals, we determined whether endotoxin-induced vascular hyporeactivity to phenylephrine (PE) is enhanced in Mg-deficient rats.

Normal and Mg-deficient adult male Wistar rats were injected with Escherichia coli 011: B4 lipopolysaccharide (1 or 5 mg/kg, i.p.). Six h later, rings prepared from their thoracic aortas showed severe hyporeactivity to PE. This was more pronounced in the Mg-deficient rats, and was reversed by in vitro treatment with a highly selective inducible nitric oxide (NO) synthase inhibitor, 1400 W, or a highly selective soluble guanylyl cyclase inhibitor, ODQ. However, reversal required high doses of both inhibitors in Mg-deficient rats. Endotoxemia for 6 h was associated with elevated serum interleukin (IL)-1[beta] and tumor necrosis factor (TNF)-[alpha] levels, and strong TNF receptor mRNA expression in the abdominal aortas, which were significantly greater in the Mg-deficient rats. Treatment of the thoracic aortas, isolated from control and Mg-deficient rats before endotoxic challenge, with IL-1[beta] or TNF-[alpha] for 6 h in vitro caused hyporeactivity to PE, but its severity did not differ significantly between the two groups. These results suggest that high serum IL-1[beta] and TNF-[alpha] levels, and increased TNF receptor production in the vascular tissue, contribute to vascular hyporeactivity to PE in endotoxemia, and to its enhancement in Mg-deficient rats, via NO/cGMP signaling.

Nara, M., T. Kanda, et al. (1999). "Running exercise increases tumor necrosis factor-[alpha] secreting from mesenteric fat in insulin-resistant rats." Life Sciences **65**(3): 237.

http://www.sciencedirect.com/science/article/B6T99-3X05HTX-11/2/e914656134de1d8140d842a2cce532c8

Tumor necrosis factor-[alpha] (TNF-[alpha]) is an important mediator of insulin resistance in obese subjects, through its overexpression in fat tissue. However, how exercise can modify the expression of TNF-[alpha] is controversial. We examined TNF-[alpha] in adipose tissue using an animal model of insulin resistance that was produced by feeding rats a diet high in sucrose. The rats were allocated to one of three groups: those receiving a starch-based diet (control group): those fed a high-sucrose diet (sucrose-fed group): and those fed a high-sucrose diet and given wheel exercise (exercised group). The animals were allowed to eat and drink ad lib for 4 or 12 weeks (4 wk: control N = 7, sucrose-fed N = 7, exercised N = 10; 12 wk: control N = 5, sucrose-fed N = 5, exercised N = 9). The voluntary wheel exercise was initiated with the feeding of the high-sucrose diet. The rats in the exercise groups ran 15 +/- 3 km/week. We showed that 12-week voluntary running exercise significantly (P Eq/L vs 141 +/- 11 Eq/L) and free fatty acid (0.98 +/- 0.07 Eq/L vs 1.4 +/- 0.05 mEq/L) concentrating in portal vein blood were reduced compared to sucrose-fed group. The amounts of fatty tissue both in mesenteric and subcutaneous tissues were significantly ($P < 0.05$) decreased through running exercise. We consider that up-regulation of TNF-[alpha] in mesenteric fat may be a compensatory mechanism for the reduction of fatty acid in adipose tissues and this change could control metabolic homeostasis during exercise to modulate a hyperinsulinemic state.

Ohashi, S.-i., H. Kaji, et al. (1995). "Effect of fasting and growth hormone (GH) administration on gh receptor (GHR) messenger ribonucleic acid (mRNA) and GH-binding protein (GHBP) mRNA levels in male rats." Life Sciences **57**(18): 1655.

http://www.sciencedirect.com/science/article/B6T99-3YYTH6C-3F/2/edc23366ae0c793fbaa6989260c5f20c

To elucidate whether GHR and GHBP are coordinately regulated or not, we studied the effect of fasting with or without GH administration on the GHR and GHBP mRNAs in the liver as well as in extrahepatic tissues in rats. Tissues were collected from 7-week-old male rats by decapitation 1, 3, and 7 days after the start of fasting. Liver GHR mRNA levels were not affected 1 day after the start of fasting but progressively decreased for the subsequent 3 and 7 days of fasting as compared with those in control rats fed ad libitum. In contrast, liver GHBP mRNA levels

significantly rose after 1 day fasting, returned to the control level after 3 days and further reduced after 7 days of fasting. Changes in GHBP mRNA level after fasting were different among the tissues. A transient increase in GHBP mRNA levels was observed in muscle and heart as well as liver, while the GHBP mRNA levels in fat tissues did not change throughout 7 days of fasting. Next, bovine GH(bGH) was administered ip to the fasted rats and control fed rats for either 1 day(100mg, tid) or 5 days(150mg, daily). In fed rats, liver GHR mRNA level was significantly increased by 1 day bGH treatment, but after 5 days treatment with bGH it was not different from the level in saline-injected control. Accordingly, net increment of plasma IGF-I was 296.0 ng/ml with 1 day bGH treatment and 234.2 rig/ml with bGH administration for 5 days. In fasted rats, liver GHR mRNA level did not changed after 1 day treatment with bGH, but markedly decreased 5 days after bGH administration. Net increment of plasma IGF-I was slightly reduced to 284 ng/ml with 1 day treatment with bGH, and markedly decreased to 37.0 with bGH administration for 5 days. The effect of GH administration on liver GHBP mRNA level was virtually absent in either fasting or fed state. These findings suggest that GHR and GHBP mRNAs in the liver are expressed in different ways and that expression of GHBP mRNA is differently regulated among tissues.

Pietruck, C., G.-X. Xie, et al. (1999). "Alternative exon splicing of cyclic AMP response element-binding protein in peripheral sensory and sympathetic ganglia of the rat." Life Sciences **65**(21): 2205.

http://www.sciencedirect.com/science/article/B6T99-3XX6KNH-5/2/e72d01f6881ea6e50ce27b975671c1d8

Alternative splicing patterns of cyclic AMP response element-binding protein (CREB) in dorsal root ganglia, lumbar sympathetic ganglia and several peripheral tissues of the rat have been investigated by an exon-flanking polymerase chain reaction strategy. A series of RT-PCR with primer pairs flanking all possible alternative splicing sites (corresponding to a genomic region with at least one full exon and two flanking introns) has revealed multiple tissue specific splice variants. These include some novel transcripts that lack the phosphorylation site and part of the leucine zipper region which is crucial for dimerization and DNA binding. Some isoforms previously reported as testis-specific were also detected in rat peripheral ganglia and other tissues. Notably, splicing patterns are specific for some regions. Some of the splice variants indicate inhibitory functions due to lacking phosphorylation sites or partially missing DNA-binding or leucine zipper domains. These findings suggest a complex expression and functional regulation of CREB in peripheral tissues including dorsal root and sympathetic ganglia.

Sango, K., H. Horie, et al. (2002). "Diabetes is not a potent inducer of neuronal cell death in mouse sensory ganglia, but it enhances neurite regeneration in vitro." Life Sciences **71**(20): 2351.

http://www.sciencedirect.com/science/article/B6T99-46R0BWM-4/2/f467496d5906f8373aff8b0e50f81e08

We examined the effects of diabetes on the morphological features and regenerative capabilities of adult mouse nodose ganglia (NG) and dorsal root ganglia (DRG). By light and electron microscopy, no apoptotic cell death was detected in the ganglia obtained from either streptozotocin (STZ)-induced diabetic or normal C57BL/6J mice in vivo. Neurite regeneration from transected nerve terminals of NG and DRG explants in culture at normal (10 mM) and high (30 mM) glucose concentrations was significantly enhanced in the diabetic mice. Chromatolytic changes (i.e. swelling and migration of the nucleus to an eccentric position in the neurons, and a loss of Nissl substance in the neuronal perikarya) and apoptotic cell death (less than one-fifth of the neurons) in the cultured ganglia were present, but neither hyperglycemia in vivo nor high glucose conditions in vitro altered the morphological features of the ganglia or the ratios of

apoptotic cells at 3 days in culture. By semiquantitative RT-PCR analysis, the mRNA expressions of ciliary neurotrophic factor (CNTF) in DRG from both mice were down-regulated at 1 day in culture. The expression in diabetic DRG, but not in control DRG, was significantly up-regulated at later stages (3 and 7 days) in culture. In summary, hyperglycemia is unlikely to induce cell death in the sensory ganglia, but enhances the regenerative capability of vagal and spinal sensory nerves in vitro. The up-regulation of CNTF mRNA expression during the culture of diabetic DRG may play a role in the enhanced neurite regeneration.

Takahata, T., N. Yasui-Furukori, et al. (2003). "Nucleotide changes in the translated region of SCN5A from Japanese patients with Brugada syndrome and control subjects." Life Sciences **72**(21): 2391.

http://www.sciencedirect.com/science/article/B6T99-481N39K-4/2/4e19138efc777137fcbd5aa218911e2c

The mutations of the SCN5A gene have been implicated to play a pathogenetic role in Brugada syndrome, which causes ventricular fibrillation. To determine the Brugada-associated mutations in Japanese patients, facilitate pre-symptomatic diagnosis, and allow genotype-phenotype studies, we screened unrelated patients with Brugada syndrome for mutations. DNAs from 6 Japanese patients were obtained and the sequence in the translated region of SCN5A was determined. We could not find the mutations reported previously, but found 17 sites of nucleotide change, consisting of 7 synonymous and 10 non-synonymous nucleotide changes in our patients. Among them, two non-synonymous nucleotide changes (G1663A and G5227A) are specific to our patients and these changes were not found in 53 healthy controls. In 4 patients out of 6, no specific nucleotide change for Brugada syndrome could be detected. Our findings demonstrating no patient-specific change in the translated region of the SCN5A gene among two thirds of the small number of patients examined here imply that another gene other than the SCN5A may be associated with this disease, supporting previous investigations in Japan and other countries.

Takase, S., K. Suruga, et al. (1995). "Relationship between perinatal appearance of cellular retinol-binding protein, type II and retinal reductase activity in chick liver." Life Sciences **58**(2): 135.

http://www.sciencedirect.com/science/article/B6T99-3VX9XS9-J/2/9ab373902d2a0d9bb11ee3e3eb6587ee

To explore a role of the transiently appearing cellular retinol-binding protein, type II (CRBP(II)) in perinatal chick liver, we have examined whether the relationships exist among the perinatal changes in hepatic CRBP(II) protein and mRNA levels, retinal reductase activity and [beta]-carotene levels in liver and serum. Northern blot analysis for hepatic CRBP(II) revealed a transient expression of CRBP(II) mRNA around hatching. The protein of CRBP(II) was also expressed transiently and the highest levels of CRBP(II) were found in the livers 1-3 days after birth. The retinal reductase activity was very low at embryonic age, but its activity rapidly rose at hatching, peaking at 1 day after birth, followed by a gradual decrease to a lower level in 7-day-old chicks. This perinatal pattern of the retinal reductase activities was similar to the pattern of transient appearance of the hepatic CRBP(II), and was also paralleled to the developmental changes in serum and liver [beta]-carotene concentrations. These findings suggest that hepatic CRBP(II) transiently appearing during the perinatal period may involve in metabolizing hepatic [beta]-carotene, directing the retinal to the retinal reductase and leading further to the subsequent esterification of the converted retinol.

Tsukiji, J., K. Sango, et al. (2004). "Long-term induction of [beta]-CGRP mRNA in rat lungs by allergic inflammation." Life Sciences **76**(2): 163.

http://www.sciencedirect.com/science/article/B6T99-4DN8G64-2/2/01b21ab2616ec51174cf28114fcd3aef

Calcitonin gene-related peptide (CGRP) is one of the major neuropeptides released from sensory nerve endings and neuroendocrine cells of the lung. Two CGRP isoforms, [alpha]-and [beta]-CGRP, have been identified in rats and humans, but no studies have attempted to reveal direct evidence of differences in action or location of these isoforms in allergic inflammation (AI). We investigated mRNA expressions of [alpha]-and [beta]-CGRP in lungs, nodose ganglia (NG), and dorsal root ganglia (DRG) of an animal model for AI of the airways, utilizing a model created by sensitizing Brown Norway (BN) rats with ovalbumin (OVA). By semiquantitative RT-PCR analysis, long-lasting enhanced expression of the [beta]-CGRP mRNA was shown in the lungs of the AI rats (14.5-fold enhancement at 6 hr, 8.1-fold at 24 hr, and 3.7-fold at 120 hr after OVA-challenge compared to the level in the lungs of phosphate-buffered saline (PBS)-challenged control rats). In contrast, the mRNA expression of the [alpha]-CGRP in AI lungs showed only a transient increase after OVA-challenge (2.7-fold at 6 hr) followed by a lower level of expression (0.5-fold at 48 hr and 0.6-fold at 120 hr). The mRNA expressions of both isoforms in NG, but not in DRG, were transiently up-regulated at 6 hr after antigen challenge. In situ RT-PCR in combination with immunohistochemical analysis revealed that [beta]-CGRP was expressed in neuroendocrine cells in clusters (termed neuroepithelial bodies [NEBs]) in AI lungs. These results indicate that the long-term induction of [beta]-CGRP in NEBs may play an important role in pulmonary AI such as bronchial asthma.

Vrana, K. E., P. J. Rucker, et al. (1994). "Recombinant rabbit tryptophan hydroxylase is a substrate for cAMP-dependent protein kinase." Life Sciences **55**(13): 1045.

http://www.sciencedirect.com/science/article/B6T99-47548Y8-J9/2/dc9a0f329fed636149e1588fa867f2a6

A full-length cDNA clone for rabbit tryptophan hydroxylase (TPH) was modified and subcloned into a bacterial expression vector. Expression of this gene in the protease-deficient strain of bacteria, BL21[DE3], produced TPH immunoreactive protein which exhibited enzyme activity. Treatment of the recombinant enzyme (in bacterial extracts) with the purified catalytic subunit of the cAMP-dependent protein kinase and [y-32P]-ATP resulted in specific phosphorylation of TPH. This expression system provides a means of generating and purifying large amounts of this important enzyme. Moreover, these experiments establish that TPH will serve as an in vitro substrate for cAMP-dependent protein kinase.

Xie, G.-X., T. Meuser, et al. (1999). "Presence of opioid receptor-like (ORL1) receptor mRNA splice variants in peripheral sensory and sympathetic neuronal ganglia." Life Sciences **64**(22): 2029.

http://www.sciencedirect.com/science/article/B6T99-3WWTD97-7/2/acfaf4d19afdbd2d008092822c9c1353

The expression of ORL1 receptor mRNA splice variants is determined in peripheral sensory and sympathetic ganglia and compared to mRNA expression for the three classic opioid receptor subtypes (mu, delta, and kappa) using the method of reverse transcription-polymerase chain reaction. ORL1, mu, delta and kappa receptor subtype mRNAs are present in human dorsal root ganglia (DRG) and trigeminal ganglia and rat DRG. ORL1, mu and delta receptor subtype

mRNAs are present in rat superior cervical ganglia and only ORL1 and delta receptor mRNAs are present in rat lumbar sympathetic ganglia. Both the ORL1 mRNA splice variants are present in sensory and sympathetic ganglia, however, expression of the shorter ORL1 receptor mRNA dominates over expression of the longer splice variant in rat brain and DRG, whereas, expression of the longer splice variant is dominant in sympathetic ganglia.

Xu, Z., B. Dai, et al. (1994). "Gastrin gene expression in human colon cancer cells measured by a simple competitive PCR method." Life Sciences **54**(10): 671.

http://www.sciencedirect.com/science/article/B6T99-474YKY4-1DP/2/2be6dbe850711eed068bb3dbae6a7f12

Gastrin is mitogenic for several colon cancers and is postulated as an autocrine growth factor for colon cancer cells. In the present study we report the development of a simple competitive polymerase chain reaction (PCR) method for measuring relative abundance of gastrin gene expression in colon cancer cells. Primers flanking exons 2 and 3 of the gastrin gene were utilized for co-amplification of cDNA and genomic DNA. The amplification of genomic DNA was distinguished from that of cDNA by the presence of the 130 bp intron sequence which was resolved by electrophoresis on agarose gels. A standard reaction of competitive PCR, using known concentrations of genomic DNA and cDNA, was first established. The steady state levels of gastrin mRNA were next quantitated in three human colon cancer cell lines (HCT-116, Colo-205 and DLD-1) by competitive PCR. Gastrin mRNA levels in these cell lines ranged from ~0.1 to 1.0 fmoles/mg total RNA (~2-25 copies of gastrin mRNA per cell). This low to moderate levels of gastrin were expressed by human colon cancer cell lines which may function as autocrine growth factors for colon cancers.

Yamamoto, J., K. Ihara, et al. (2004). "Characteristic expression of aryl hydrocarbon receptor repressor gene in human tissues: Organ-specific distribution and variable induction patterns in mononuclear cells." Life Sciences **74**(8): 1039.

http://www.sciencedirect.com/science/article/B6T99-4B0T00G-3/2/532bf5586dc34fd737653c127c049dac

To investigate the expression of aryl hydrocarbon receptor repressor (AhRR) and related molecules in various tissues and the effects of aromatic hydrocarbons (AHs) on their expression, we developed a reliable technique of quantification of human AhRR as well as aryl hydrocarbon receptor (AhR), AhR nuclear translocator (ARNT) and cytochrome P450 1A1 (CYP1A1) mRNA by real-time TaqMan PCR method. First, we examined the expression of these genes in human adult or fetal tissues. The levels of AhRR expression were extremely high in testis, very high in lung, ovary, spleen and pancreas from adults, whereas those were low in those from fetuses. On the other hand, CYP1A1 expression was extremely high in lung, and AhR and ARNT were ubiquitously expressed in almost all tissues. Second, we compared the expression levels of these genes in mononuclear cells (MNCs) from various sources. Comparison of the basal expression levels of these genes in MNCs demonstrated that MNCs from umbilical cord blood showed higher AhRR or CYP1A1 expression than those from adults. The induction of AhRR or CYP1A1 expression by 3-methylcholanthrene (3-MC) was observed in MNCs from adults but not from umbilical cord blood. Consequently, there existed characteristic differences in the basal levels of AhRR and CYP1A1 expression in MNCs, as well as in their inducibility by 3-MC among MNCs from various types of human bloods. These results will provide basic information for a possible application of AhRR and CYP1A1 measurements to evaluate AH exposure in vivo.

Ying, S.-Y., S.-Q. Li, et al. (1995). "Expression and localization of inhibin [alpha]-subunit in rat retinal photoreceptor cells." Life Sciences **57**(1): 45.

http://www.sciencedirect.com/science/article/B6T99-3YYTHNJ-BP/2/53a8f632190c92b468921a2ecb1717b1

To determine whether rat retinal photoreceptor cells produce inhibin, a molecule closely related to activin, a multifunctional growth factor in the transforming growth factor [beta] superfamily (TGF[beta]), we have conducted immunohistochemistry using specific antibodies for inhibin which were raised against a synthetic N-terminal fragment of the [alpha]-subunit of inhibin. The mature inhibin molecule was identified at both the inner and outer segments of photoreceptor cells. To determine if messenger RNA for the [alpha]-subunit of inhibin is expressed in the retinal cells, both in situ hybridization with a specific probe and the reverse transcription-polymerase chain reaction (RT-PCR) technique with primers specific for the [alpha]-subunit of inhibin were used. Messenger RNA expression of the [alpha]-subunit of inhibin was detected by RT-PCR and localized in the photoreceptor cells as determined by in situ hybridization. In addition, the identity of the cDNA product of RT-PCR was verified with Southern analysis and DNA sequencing. The localization of mature inhibin protein and its corresponding message to photoreceptor cells suggest that inhibin may have a paracrine function in the retina, perhaps in the photoreceptor cells themselves.

Livestock Production Science (2)

Galov, A., K. Byrne, et al. "Effectiveness of nine polymorphic microsatellite markers in parentage testing in Posavina, Croatian Coldblood and Lipizzaner horse breeds in Croatia." Livestock Production Science **In Press, Corrected Proof** http://www.sciencedirect.com/science/article/B6T9B-4F1GRF8-4/2/ffdc0a02d35a92ad9dc9a3280cb95b82

The global biodiversity crisis extends to autochthonous local breeds of livestock. There is an increasing danger that these rare breeds become extinct and with them their locally adapted gene pool. Modern molecular tools such as parentage testing using microsatellite genotyping are powerful in guiding management and conservation. We tested nine microsatellite markers in three Croatian horse breeds and obtained high exclusion probabilities (EPs) for the most common test scenario 'one parent and offspring known and the other parent tested' (99.9% in Posavina and Croatian Coldblood and 99.3% in Lipizzaner), despite that Lipizzaner has an overall lower genetic variability at microsatellite loci. To become a useful tool in breed management in countries with developing economies, genetic screening systems must be designed to be statistically powerful yet economically viable. Therefore, a suite of six markers that can be run in two multiplex systems and which still gives high exclusion probabilities (99.5% in Posavina and Croatian Coldblood and 98% in Lipizzaner) was chosen.

Kawakita, Y., H. Abe, et al. (2001). "The relation between plasma leptin concentrations and carcass lipid contents in Japanese Black steers." Livestock Production Science **73**(1): 25.

http://www.sciencedirect.com/science/article/B6T9B-44MFPWG-

3/2/52f2e816cde7fdf989cc9534e7d71b6e

We investigated a relation between carcass lipid contents and plasma leptin in 40 Japanese Black steers, which were fed a 12% crude protein diet (CP12) or a 16% crude protein diet (CP16) in the growing phase (from 10 to 18 months of age) and a low starch level diet (LS) or a high starch level diet (HS) in the finishing phase (from 19 to 27 months of age). Plasma leptin concentrations were measured by multi-species leptin radioimmunoassay kit using recombinant bovine leptin as the standard. The CP16 group had greater backfat thickness at slaughter compared with the CP12 group (PPPPr=0.59, P<0.05). However, plasma leptin levels seem to be weak index to predict final adiposity in Japanese Black steers.

Mammalian Biology - Zeitschrift fur Saugetierkunde (1)

Lorenzini, R., R. Fico, et al. "Mitochondrial DNA evidence for a genetic distinction of the native red deer of Mesola, northern Italy, from the Alpine populations and the Sardinian subspecies." Mammalian Biology - Zeitschrift fur Saugetierkunde **In Press, Corrected Proof**
http://www.sciencedirect.com/science/article/B7GX2-4FG898J-4/2/78df556a535b4f9660eab24cad6deaad

Native red deer of Mesola Wood, northern Italy, were compared with the Sardinian subspecies and with some populations from the Italian Alps and Austria using the analysis of restriction fragment length polymorphism (RELP) of mitochondrial (mt) DNA segments. The results highlight the existence of four main genetic lineages, and provide evidence for a structuring of populations according to their geographic occurrence. Two mitochondrial lineages, although highly distantly related, are shared between the populations from the centre-eastern Alps of Italy and Austria, while the other two lineages characterize the Sardinian and Mesola red deer, respectively. The exclusive haplotype found in the Mesola population appears as being an offshot of one of the two main Alpine lineages, suggesting a presumed origin of these deer from a panmictic population which dwelt in mid-southern Europe, prior to the fragmentation of populations caused by human activities and manipulations. Considering their distinctiveness in morphologic and genetic traits, as well as their historical background and biogeographical value, these native deer should be regarded as a national conservation priority. The Sardinian red deer is highly divergent from both Mesola and Alpine populations. However, the controversial question of the phylogeographic origin of this subspecies remains unresolved. The utility of RELP analyses of mtDNA segments as a tool to discriminate among red deer populations as well as to develop effective strategies for their conservation and management.ZusammenfassungMitochondrien-DNA-Daten zur genetischen Unterscheidung der autochthonen Rothirsche von Mesola, Norditalien, von jenen aus alpinen Populationen und von der Unterart auf SardinienAutochthone Rothirsche aus dem Mesola-Waldgebiet in Norditalien wurden mittels Restriktionsfragmenteangenpolymorphismen (RFLPs) von Abschnitten der mitochondrialen (mt)DNA mit Rotwild der sardischen Unterart und Tieren aus alpinen Populationen verglichen. Die Ergebnisse weisen auf die Existenz von vier genetischen Hauptlinien hin und belegen auch eine Strukturierung der Populationen gema[beta] ihrer geographischen Herkunft. Zwei entfernt verwandte mtDNA-Linien wurden in Bestanden aus dem Zentral- und Ostalpenbereich von Italien und Usterreich gefunden. Die beiden anderen Linien waren jewels charakteristisch fur Rotwild auf Sardinien und Rotwild in Mesola. Der exklusive Haplotypes der Mesola-Population scheint ein Abkommling einer der beiden alpinen Hauptlinien zu sein. Dies legt eine Abstammung der Mesola-Rothirsche von einer gro[beta]en, panmiktischen Population nahe, wie sie vor der anthropogen bedingten Fragmentierung und Isolation von Bestanden im Bereich von Mittel- und Sudeuropa existiert haben sollte. Sowohl nach der Eigenstandigkeit in morphologischen und molekularen Merkmalen als auch im Hinblick auf die

Bestandsgeschichte und den biogeographischen Wert sollte der letzten autochthonen Rotwildpopulation in Mesola eine nationale Prioritat im Rahmen von Arterhaltungsprogrammen zugebilligt werden. Das sardinische Rotwild unterschied sich in hohem Ma[beta] von den alpinen Bestanden und der Mesola-Population. Die phylogeographische Herkunft bleibt allerdings unklar. Die Brauchbarkeit von mtDNA-RFLPs zur Abgrenzung von Rothirschbestanden sowie zur Entwicklung von Arterhaltungs- und Management-Konzepten wird diskutiert.

Marine Pollution Bulletin (1)

Schaffelke, B., N. Murphy, et al. (2002). "Using genetic techniques to investigate the sources of the invasive alga Caulerpa taxifolia in three new locations in Australia." Marine Pollution Bulletin **44**(3): 204.

http://www.sciencedirect.com/science/article/B6V6N-455TBHJ-K/2/594c31ff2db871eba8eb3af5940db305

Matrix Biology (15)

Asuncion, L., B. Fogelgren, et al. (2001). "A novel human lysyl oxidase-like gene (LOXL4) on chromosome 10q24 has an altered scavenger receptor cysteine rich domain." Matrix Biology **20**(7): 487.

http://www.sciencedirect.com/science/article/B6VPM-44B213D-9/2/64a14940501849e3d4af5a8c8e8d5050

We have identified a novel 14-exon human lysyl oxidase-like gene, LOXL4, on chromosome 10q24. The cDNA and derived amino acid sequence of LOXL4 demonstrates a conserved C-terminal region including the characteristic copper-binding site, lysyl and tyrosyl residues and a cytokine receptor-like domain. One of the four N-terminal SRCR domains contains a 13 amino acid insertion encoded by a short exon not present within the closely homologous LOXL2 and LOXL3 genes. The 3.5-kb LOXL4 mRNA is present in pancreas and testis and at lower levels in several other tissues. Fibroblasts, smooth muscle and osteosarcoma (HOS) cells express LOXL4. No expression was detected in HCT-116 and DLD-1 colon, MCF-7 breast and DU-145 prostate cancer cell lines.

Azizan, A., J. U. Gaw, et al. (2000). "Chondromodulin I and pleiotrophin gene expression in bovine cartilage and epiphysis." Matrix Biology **19**(6): 521.

http://www.sciencedirect.com/science/article/B6VPM-41JTP8W-8/2/b7c91962c0e234eb8a9de9474362d01e

Pleiotrophin and chondromodulin-I are low molecular weight proteins that are abundant (20 [mu]g/g tissue) in fetal cartilage and difficult to detect in adult cartilage. We characterized their gene and protein expression patterns to gain a better understanding of their roles in the regulation of limb development and growth. In order to compare and contrast the relative amounts of the respective mRNA species within the developing epiphysis, a competitive PCR assay was developed. The results showed that the mRNAs for both proteins were abundant in fetal cartilage and while present in adult cartilage, were at 20-60-fold lower levels. Northern blotting revealed gradients of mRNA for both of these proteins in growth plate cartilage, with the highest levels in the resting zone, and the lowest in the hypertrophic zone. In contrast to pleiotrophin, chondromodulin-1 is down-regulated by retinoic acid with a pattern of expression similar to collagen type II and link protein, and may play a more specific role than pleiotrophin in modulating the chondrocyte phenotype.

Botella, L. M., F. Sanz-Rodriguez, et al. (2004). "Lumican is down-regulated in cells expressing endoglin. Evidence for an inverse correlationship between Endoglin and Lumican expression." Matrix Biology **22**(7): 561.

http://www.sciencedirect.com/science/article/B6VPM-4BSW8KC-5/2/ce9b85ce637808f11e5fc5a99d1ca51a

Endoglin (CD105) is a homodimeric membrane glycoprotein, which acts as a TGF-[beta] coreceptor in the vasculature and plays an important role in cardiovascular development and vascular remodelling. To isolate putative genes regulated by endoglin expression, a PCR-based RNA fingerprinting technique was carried out. Myoblasts stably transfected with endoglin showed a decrease in the expression of lumican both at the RNA and protein levels. Lumican is a proteoglycan of the extracellular matrix, belonging to the SLRP (Small Leucine-Rich Repeat Proteoglycans) family. Lumican down-regulation by endoglin appeared to be controlled, at least in part, at the transcriptional level, as indicated by RT-PCR, and transient transfection experiments using a lumican promoter reporter based vector. This inverse correlation between endoglin and lumican expression was substantiated by immunohistochemical staining of vessels from human tissues. Thus, cells belonging to the high endothelia, such as tonsil, express a large amount of endoglin, and the lumican content of their matrix is considerably reduced. Conversely, in resting endothelia, such as that of large vessels, the expression of endoglin is reduced whereas the amount of lumican is greatly increased. The inverse regulation in the expression of endoglin and lumican was also evident after TGF-[beta] treatments since endoglin was up-regulated, whereas lumican was down-regulated by this cytokine. This report describes for the first time a relationship between endoglin and lumican expression.

Clark, A. G., A. L. Rohrbaugh, et al. (2002). "The effects of ascorbic acid on cartilage metabolism in guinea pig articular cartilage explants." Matrix Biology **21**(2): 175.

http://www.sciencedirect.com/science/article/B6VPM-44PVT88-1/2/1dab29c60aa570ed11d39fc00c27f674

Ascorbic acid has been associated with the slowing of osteoarthritis progression in guinea pig and man. The goal of this study was to evaluate transcriptional and translational regulation of cartilage matrix components by ascorbic acid. Guinea pig articular cartilage explants were grown in the presence of -ascorbic acid (-Asc), -isoascorbic acid (-Asc), sodium -ascorbate (Na -Asc), sodium -isoascorbate (Na -Asc), or ascorbyl-2-phosphate (A2P) to isolate and analyze the acidic and nutrient effects of ascorbic acid. Transcription of type II collagen, prolyl 4-hydroxylase (alpha subunit), and aggrecan increased in response to the antiscorbutic forms of ascorbic acid (-Asc, Na -Asc, and A2P) and was stereospecific to the -forms. Collagen and aggrecan synthesis also

increased in response to the antiscorbutic forms but only in the absence of acidity. All ascorbic acid forms tended to increase oxidative damage over control. This was especially true for the non-nutrient -forms and the high dose -Asc. Finally, we investigated the ability of chondrocytes to express the newly described sodium-dependent vitamin C transporters (SVCTs). We identified transcripts for SVCT2 but not SVCT1 in guinea pig cartilage explants. This represents the first characterization of SVCTs in chondrocytes. This study confirms that ascorbic acid stimulates collagen synthesis and in addition modestly stimulates aggrecan synthesis. These effects are exerted at both transcriptional and post-transcriptional levels. The stereospecificity of these effects is consistent with chondrocyte expression of SVCT2, shown previously to transport -Asc more efficiently than -Asc. Therefore, this transporter may be the primary mechanism by which the -forms of ascorbic acid enter the chondrocyte to control matrix gene activity.

Ilic, M. Z., M. N. Vankemmelbeke, et al. (2000). "Bovine joint capsule and fibroblasts derived from joint capsule express aggrecanase activity." Matrix Biology **19**(3): 257.

http://www.sciencedirect.com/science/article/B6VPM-40WDVPV-8/2/d2f1992f0b009049ad0caaa4773f63fc

Bovine joint capsule was maintained in explant culture in the presence of bovine aggrecan monomer and it was shown that the aggrecan monomer was degraded. Amino-terminal sequence analysis of the resulting aggrecan core protein fragments revealed that the core protein was cleaved at five specific sites attributed to glutamyl endopeptidases referred to as aggrecanase activity. Fibroblast cultures were established from explant cultures of joint capsule and when these cells were exposed to aggrecan, cleavage of the core protein of aggrecan at the aggrecanase sites was observed. Inclusion of either retinoic acid or interleukin-1[alpha] in medium of either joint capsule explant cultures or fibroblast cultures did not increase the rate of cleavage of exogenous aggrecan present in the culture medium. When aggrecan monomer was incubated with conditioned medium from explant cultures of joint capsule maintained in medium, degradation could be detected after 10 min. After a 6-h incubation period the same fragments of aggrecan core protein were observed as those for tissue or cells incubated directly with aggrecan monomer. RT-PCR analysis of mRNA extracted from joint capsule fibroblasts showed that these cells express both aggrecanase-1 and -2 [ADAMTS-2 (Tang) and ADAMTS-5].

Kerkvliet, E. H. M., I. C. Jansen, et al. (2003). "Collagen type I, III and V differently modulate synthesis and activation of matrix metalloproteinases by cultured rabbit periosteal fibroblasts." Matrix Biology **22**(3): 217.

http://www.sciencedirect.com/science/article/B6VPM-48N3JC5-2/2/09b5c676bc243cfa7f1c7add918040b1

In the present study we investigated whether the collagen types I, III and V affect the activity of fibroblasts obtained from rabbit periosteum. The cells were cultured on plates either or not coated with different amounts of collagen type I, III or V and analyzed for their attachment, DNA synthesis and the expression and activity of matrix metalloproteinases (MMPs). Our data show that the three collagen types promoted attachment and spreading of the cells and stimulated DNA synthesis when used in relatively low concentrations. High concentrations of type V--but not of type I or III--proved to inhibit thymidine incorporation. The expression and activity of matrix metalloproteinase 1 (MMP-1; interstitial collagenase) decreased under the influence of relatively low amounts of collagen (1-integrin or echistatin increased the level of MMP-1 but had no effect on MMP-2. All parameters tested were similarly affected by type I and III collagen, whereas the effect of type V was always less. We conclude that the collagen types I, III and V provide different sets of signals for fibroblasts that differently modulate their proliferation and MMP expression.

Konttinen, Y., S. Halinen, et al. (1998). "Matrix metalloproteinase (MMP)-9 type IV collagenase/gelatinase implicated in the pathogenesis of Sjogren's syndrome." Matrix Biology **17**(5): 335.

http://www.sciencedirect.com/science/article/B6VPM-46YXFH8-9/2/20e3abd241f27ab103ecef45e8742c53

Type IV collagenases/gelatinases (matrix metalloproteinases MMP-2 and MMP-9) in labial salivary glands (LSG) and saliva in Sjogren's syndrome (SS) and healthy controls were studied. Zymograms and Western blots disclosed that SS saliva contained 92/82 kD MMP-9/type IV collagenase duplex. Specific activity measurement disclosed 53.1 +/- 9.8 U/mg protein MMP-9 in SS compared to 16.5 +/- 2.6 U/mg in healthy controls (p = 0.01). MMP-2 did not differ between SS and controls. In SS salivary glands, MMP-2 and MMP-9 were also expressed, in addition to stromal fibroblasts and occasional infiltrating neutrophils, respectively, in acinar end piece cells. In addition, an effective proMMP-9 activator, human trypsin-2 (also known as tumor-associated trypsin-2 or TAT-2), was found in acinar end piece cells and in saliva. Interestingly, proteolytically processed MMP-9 was found in saliva (vide supra), and in vivo activated MMP-9 was significantly higher in SS than in controls (p = 0.002). LSGs, particularly in SS, were characterized ultrastructurally by areas containing small cytoplasmic vesicles in the basal parts of the epithelial cells associated with areas of disordered and thickened basal lamina. Based on our results, we conclude here that SS saliva contains increased concentrations of MMP-9, which is of glandular origin in part. Pro MMP-9 is to a large extent proteolytically activated. This is probably mediated by the most potent pro MMP-9 activator found in vivo thus far, namely trypsin-2. Therefore, the MMP-9/trypsin-2 cascade may be resposible for the increased remodelling and/or structural destruction of the basement membrane scaffolding in salivary glands in SS. Due to the role of basal lamina as an important molecular sieve and extracellular matrix-cell signal, these pathological changes may contribute to the pathogenesis of the syndrome.

Lorenzo, P., P. Aman, et al. (1999). "The human CILP gene: exon/intron organization and chromosomal mapping." Matrix Biology **18**(5): 445.

http://www.sciencedirect.com/science/article/B6VPM-3Y3XR41-4/2/29b7d42200c08062426066ea150a7481

The human cDNA for cartilage intermediate layer protein (CILP) codes for a larger precursor protein that consists of CILP and a homologue to porcine Nucleotide pyrophosphohydrolase (NTPPHase) [Lorenzo et al. 1998a. J. Biol. Chem. 273, 23469-23475]. The human gene has now been isolated and characterized. Southern blot analysis indicated a single copy of the CILP gene in the human genome. The gene spans approximately 15.3 kbp of genomic DNA, and is organized in nine exons. The 5' flanking region contains a putative promoter region with a TATA-like box localized from -29 to -23 bp upstream of the transcription start site. Analysis of the putative promoter region revealed potentially cis-regulatory eukaryotic elements such as GATA-1, MyoD, MZF1, and CdxA. The protein coding region begins in exon 2 with the putative signal peptide. CILP is encoded from exon 3 to exon 9. In addition, exon 9 also codes for the entire NTPPHase homologue and contains the 3' untranslated region of the gene. All the introns follow the 'gt-ag' rule, except the last intron, intron 8, that belongs to the minor class of pre-mRNA introns that contain 'at-ac' at their 5' and 3' ends, respectively. The CILP gene was mapped to human chromosome 15q22.

Mates, L., E. Korpos, et al. (2002). "Comparative analysis of the mouse and human genes (Matn2 and

MATN2) for matrilin-2, a filament-forming protein widely distributed in extracellular matrices." Matrix Biology **21**(2): 163.

http://www.sciencedirect.com/science/article/B6VPM-44V2101-1/2/98d11c77df0765fe3767c67d2dd15ab0

We previously identified matrilin-2 (MATN2), the largest member of the novel family of matrilins. These filament-forming adapter proteins expressed in a distinct, but partially overlapping, pattern in all tissues were implicated in the organization of the extracellular matrix. Matrilin-2 functions in a great variety of tissues. Here, we present the genomic organization of the highly conserved mouse and human MATN2 loci, which cover >100 kb and 167.167 kb genomic regions, respectively, and are composed of 19 exons. RT-PCR analysis revealed that alternative transcripts with identical protein coding regions are transcribed from two promoters in both species. The upstream, housekeeping type promoter is functional in all tissues and cell types tested. The activity of the downstream, TATA-like promoter preceded with putative motifs for the homeobox transcription factor PRRX2 is restricted to embryonic fibroblasts and certain cell lines. The oligomerization module is split by an U12-type AT-AC intron found in conserved position in all four matrilin genes. We assigned Matn2 to mouse chromosome 15, linked to Trhr and Sntb1 in a region synthenic to human chromosome 8q22-24.

Mates, L., C. Nicolae, et al. (2004). "Mice lacking the extracellular matrix adaptor protein matrilin-2 develop without obvious abnormalities." Matrix Biology **23**(3): 195.

http://www.sciencedirect.com/science/article/B6VPM-4CPDFTP-5/2/e61391f65a1ed6f4c4943fa2fbebcbb9

Matrilins are putative adaptor proteins of the extracellular matrix (ECM) which can form both collagen-dependent and collagen-independent filamentous networks. While all known matrilins (matrilin-1, -2, -3, and -4) are expressed in cartilage, only matrilin-2 and matrilin-4 are abundant in non-skeletal tissues. To clarify the biological role of matrilin-2, we have developed a matrilin-2-deficient mouse strain. Matrilin-2 null mice show no gross abnormalities during embryonic or adult development, are fertile, and have a normal lifespan. Histological and ultrastructural analyses indicate apparently normal structure of all organs and tissues where matrilin-2 is expressed. Although matrilin-2 co-localizes with matrilin-4 in many tissues, Northern hybridization, semiquantitative RT-PCR, immunohistochemistry and biochemical analysis reveal no significant alteration in the steady-state level of matrilin-4 expression in homozygous mutant mice. Immunostaining of wild-type and mutant skin samples indicate no detectable differences in the expression and deposition of matrilin-2 binding partners including collagen I, laminin-nidogen complexes, fibrillin-2 and fibronectin. In addition, electron microscopy reveals an intact basement membrane at the epidermal-dermal junction and normal organization of the dermal collagen fibrils in mutant skin. These data suggest that either matrilin-2 and matrilin-2-mediated matrix-matrix interactions are dispensable for proper ECM assembly and function, or that they are efficiently compensated by other matrix components including wild-type levels of matrilin-4.

Sztrolovics, R., M. Van Der Rest, et al. (1994). "Identification of type I collagen gene polymorphisms: Tolerance of sequence variation at an [alpha]2(I) Helix Y position." Matrix Biology **14**(1): 9.

http://www.sciencedirect.com/science/article/B6VPM-47T8XVT-3/2/09c76fbc8fd8f57337779f517b6f9721

This study has examined the frequency and distribution of polymorphisms in the type 1 collagen

coding sequences. RNA from a group of human skin fibroblast cell lines, was analyzed by the chemical cleavage mismatch detection method using hydroxylamine, a reagent specific for C base mismatches, and overlapping cDNA probes covering the entire prepro[alpha]1(I) and prepro[alpha]2(I) coding regions. Mismatches were detected at only two nucleotide positions, one in each of the type I collagen sequences, suggesting that polymorphisms are relatively rare within these cDNAs. cDNA sequence analysis demonstrated that the prepro[alpha]1(I) mismatch, detected in only one cell line, was due to a sequence polymorphism involving the wobble position of the codon for arginine residue 59 within the amino-propeptide globular subdomain of the pro[alpha]1(I) chain and not resulting in a change in the polypeptide primary structure. In contrast, the prepro[alpha]2(I) mismatch, detected in 6 of the 16 cell lines, was shown to arise from a sequence polymorphism affecting the identity of Y-position residue 459 of the [alpha]2(I) triple helical domain, resulting in an alanine/proline dimorphism at this position. This study is the first to identify a type I collagen coding sequence polymorphism resulting in an alteration at the level of the amino acid sequence. The data suggest that at least some [alpha]1(I) and [alpha]2(I) helix Y positions may be tolerant of sequence variation, particularly if the replacing amino acid is proline, a residue involved in stabilizing the collagen triple helix.

Sztrolovicz, R., D. L. Rimoin, et al. (1994). "Single-strand conformation polymorphism analysis of human decorin, biglycan and fibromodulin cDNAs." Matrix Biology **14**(4): 307.

http://www.sciencedirect.com/science/article/B6VPM-47GH9YC-5/2/6d785b226dfcb2d2c6a39f04cef75db0

The coding regions of the human decorin, biglycan and fibromodulin cDNAs have been examined utilizing the method of single-strand conformation polymorphism analysis. Analysis of total RNA from a group of eight human skin fibroblast cell lines did not detect any sequence variations in the decorin cDNA. In contrast, the analysis detected three sequence variations in the biglycan cDNA and one in the fibromodulin cDNA from the same group of cell lines. For the biglycan cDNA, one variation involved a position in the 5'-untranslated region, while the other two affected the wobble bases of triplets encoding serine residues 10 and 143 of the mature core protein. For the fibromodulin cDNA, the variation involved the wobble position of the codon for glutamic acid residue 61 of the putative mature core protein. Single-strand conformation polymorphism analysis of these proteoglycan cDNAs was also applied to study patients exhibiting a variety of connective tissue pathologies, including chondrodysplasia punctata, Desbuquois syndrome, Dyggve-Melchior-Clausen syndrome, dyssegmental dysplasia, Ehlers-Danlos syndrome types I and III, Ellis van Creveld syndrome and thanatophoric dysplasia, though no additional sequence variations were detected.

Walker, L. C., M. A. Overstreet, et al. (2005). "Tissue-specific expression and regulation of the alternatively-spliced forms of lysyl hydroxylase 2 (LH2) in human kidney cells and skin fibroblasts." Matrix Biology **23**(8): 515.

http://www.sciencedirect.com/science/article/B6VPM-4F0GR57-1/2/b93bfdf39d962c515c0244713b5e9d57

Lysyl hydroxylases 1, 2, and 3 catalyse the hydroxylation of specific lysines in collagen. A small percentage of these hydroxylysine residues are precursors for the cross-link formation essential for the tensile strength of collagen. Lysyl hydroxylase 2 (LH2) exists as two alternatively-spliced forms; the long transcript (the major ubiquitously-expressed form) includes a 63 bp exon (13A) that is spliced out in the short form (expressed, together with the long form, in human kidney, spleen, liver, and placenta). This study shows that this alternative splicing event can be regulated by both cell density and cycloheximide (CHX). Although only the long form of LH2 is detected in

untreated confluent human skin fibroblasts, after 24 h treatment with CHX the short LH2 transcript is also expressed. In kidney cells, in which both LH2 transcripts are equally expressed, the long LH2 transcript is significantly decreased after 24 h CHX treatment, whereas expression of the short transcript is slightly increased. This suggests that, in kidney cells, the splicing mechanism for the inclusion of exon 13A in LH2 requires a newly-synthesized protein factor that is suppressed by CHX, whereas, in skin fibroblasts in which levels of LH2 (long) are unaffected, CHX appears to suppress a factor that inhibits exclusion of exon 13A, thereby promoting expression of LH2 (short). As these alternate transcripts of LH2 may have specificity for hydroxylation of lysines in either telopeptide or helical collagen domains, their relative expression determines the type of cross-links formed, thereby affecting collagen strength. Therefore, any perturbation of the regulation of LH2 splicing could influence the stability of the extracellular matrix and contribute to specific connective tissue disorders.

Yao, L. Y., C. Moody, et al. (1994). "Identification of the proteoglycan versican in aorta and smooth muscle cells by DNA sequence analysis, in situ hybridization and immunohistochemistry." Matrix Biology **14**(3): 213.

http://www.sciencedirect.com/science/article/B6VPM-47T2M7S-3/2/49c7a02a82d775d3b05fe4fea36e0b23

Versican is a large chondroitin sulfate proteoglycan (CSPG) initially identified in cultured human fibroblasts. Previous studies have shown that there is a versican-like molecule in cultured monkey smooth muscle cells. In this study, we have cloned and sequenced the large CSPG from cultured monkey smooth muscle cells, fetal and juvenile monkey aorta, and human fetal aorta. The cDNA sequence from human fetal aorta is completely homologous to the human fibroblast versican. We obtained 2.5 kb of cDNA sequence from monkey aortic RNA and cultured monkey smooth muscle cell RNA. This sequence covers three distinct domains of versican (hyaluronic acid binding domain, glycosaminoglycan attachment domain and protein binding domain) and demonstrates over 90% homology to the human versican sequence. In situ hybridization histochemistry indicates that the versican RNA transcript is located in the epithelium throughout the tunica media of the aorta. Western blot analysis and immunohistochemistry also confirm the presence of versican in human and monkey aorta.

Yoon, S., H. Kuivaniemi, et al. (2002). "MMP13 promoter polymorphism is associated with atherosclerosis in the abdominal aorta of young black males." Matrix Biology **21**(6): 487.

http://www.sciencedirect.com/science/article/B6VPM-470KM47-4/2/52aa52578ad341bd4c2a9e7b974c6211

Previous studies suggested that remodeling of connective tissue is important in progression of atherosclerosis. We investigated the importance of matrix metalloproteinase 13 (MMP13), in the pathogenesis of atherosclerosis using 995 samples from the Pathobiological Determinants of Atherosclerosis in Youth collection in an association study. We identified two new MMP13 promoter polymorphisms. The genotype for one of the MMP13 polymorphisms was associated with fibrous plaque (P=0.024) in black males. Immunohistochemistry using antibodies for MMP13 showed that MMP13 is expressed in all layers of the aorta. In-vitro transfection experiments with reporter gene constructs and electrophoretic mobility-shift assays showed that the MMP13 polymorphism was a functional variant. MMP13 is therefore, a genetic risk factor for extent of fibrous plaque in the abdominal aorta in young black males. Elucidation of the currently unknown mechanism of the MMP13 polymorphism's action may provide for pharmacological intervention to reduce the severity of atherosclerotic changes in susceptible individuals.

Meat Science (2)

Dooley, J. J., K. E. Paine, et al. (2004). "Detection of meat species using TaqMan real-time PCR assays." Meat Science **68**(3): 431.

http://www.sciencedirect.com/science/article/B6T9G-4CHHR4K-8/2/afd5edff57d3c288e9e57197abb8bad8

Species-specific real-time PCR (TaqMan) assays were developed for detection of beef, pork, lamb, chicken and turkey. Assays were developed around small (amplicons b (cytb) gene. Speciation was achieved using species-specific primers. For detection purposes, two TaqMan probes were developed; the first was specific to the mammalian species (beef, lamb and pork), the second to the poultry species (chicken and turkey). Normal end-point TaqMan PCR conditions were applied; however, PCR was limited to 30 cycles. Applying the assays to DNA extracts from raw meat admixtures, it was possible to detect each species when spiked in any other species at a 0.5% level. The absolute level of detection, for each species, was not determined; however, experimentally determined limits for beef, lamb and turkey were below 0.1%.

Hird, H., R. Goodier, et al. (2003). "Rapid detection of chicken and turkey in heated meat products using the polymerase chain reaction followed by amplicon visualisation with vistra green." Meat Science **65**(3): 1117.

http://www.sciencedirect.com/science/article/B6T9G-4834HXG-2/2/b12ffef2faaafab1082af87bf2899dcb

A rapid and highly specific assay suitable for the routine detection of turkey and chicken in processed meat products has been developed. Based on PCR amplification of species-specific amplicons with rapid visualisation using vistra green, the assay may be completed within 5 h of receipt of sample. DNA was isolated from meat samples by the use of Wizard DNA isolation technology and followed by DNA amplification in the polymerase chain reaction using species specific primers, chicken forward (CF), chicken reverse (CR), turkey forward (TF) and turkey reverse (TR): the production of an amplicon was detected after the end of the PCR in less than 5 min using vistra green and a fluorescence plate reader. The presence of fluorescence denoted the presence of the target species in the sample.

Microbes and Infection (9)

Garin, D., C. Peyrefitte, et al. (2001). "Highly sensitive Taqman(R) PCR detection of Puumala hantavirus." Microbes and Infection **3**(9): 739.

http://www.sciencedirect.com/science/article/B6VPN-43P3WGY-7/2/2b0875a866e3a6f72a37b89217c8527f

An increasing number of clinical cases of Hantavirus infections have been reported from various regions in Asia, Europe and North America. Hantaviruses (family Bunyaviridae, genus Hantavirus) are enveloped and possess a single-stranded trisegmented RNA genome of negative polarity. Rodents or insectivores are natural hosts of hantaviruses and transmit the virus to humans chiefly by aerosolisation. These viruses are the causative agents of haemorrhagic fever with renal and pulmonary syndromes. In the northeast of France, Puumala hantavirus causes, every year, more than 150 mild forms of haemorrhagic fever with a renal syndrome known as nephropathia epidemica. Serological tests may lack sensitivity for diagnosing early stages of infection and virus isolation is limited because it grows poorly in cell culture. Since reverse transcription (RT)-PCR amplification is an efficient method for detecting viral genomes in patient specimens, we developed an assay using a Taqman(R) probe and compared it with the classical RT-PCR amplification. To achieve this goal, a Puumala strain was grown in Vero E6 cells and RNA extracted from the culture supernatant. We found that the semi-nested RT-PCR detected a minimal amount of 300 TCID50 mL-1, while the Taqman(R) PCR allowed detection of less than 10 TCID50 mL-1 and provided a quantitative analysis.

Gomes, J. P., R.-c. Hsia, et al. "Immunoreactivity and differential developmental expression of known and putative Chlamydia trachomatis membrane proteins for biologically variant serovars representing distinct disease groups." Microbes and Infection **In Press, Corrected Proof**
http://www.sciencedirect.com/science/article/B6VPN-4FJNT9Y-3/2/cf6d9e1e4b9fefe6eadb6daef7b81f10

Chlamydia trachomatis is an intracellular bacterium that causes ocular and urogenital diseases worldwide. Membrane proteins have only been partially characterized, and the discovery of a nine-member polymorphic membrane protein gene family has enhanced interest in defining their function. We previously reported two putative insertion sequence-like elements in pmpC for biovariant Ba and one each for G and L2, suggesting horizontal gene transfer. Because of this and the tissue tropism differences for these biovariants, we analyzed by quantitative real-time RT-PCR pmpC expression relative to immunogenic protein genes ompA, groEL and gseA throughout development. Sera from infected adolescents were reacted by immunoblot against recombinant (r)PmpC and rMOMP. ompA and groEL revealed different developmental transcriptome profiles among the biovariants. pmpC expression occurred at 2 h, peaked at 18 for L2 (at 24 for Ba and G), with the highest mRNA levels throughout development for L2. pmpC expression as a function of time paralleled ompA expression with higher mRNA levels compared with groEL later in development. Only sera from D-, E- and G-infected patients reacted to rPmpC; all infected patients reacted to rMOMP. pmpC expression during logarithmic growth suggests a role in membrane building and/or integrity, which is supported by the presence of a signal peptidase and C-terminal phenylalanine in PmpC. Because phylogenetic analyses of pmpC segregate serovars according to tissue tropism, we speculate that biovariant transcriptome differences may contribute to this tropism. The heterogeneous biovariant pmpC expression throughout development and differential PmpC immunoreactivity also suggest a role for pmpC in antigenic variation.

Graefe, S. E. B., T. Jacobs, et al. (2003). "Interleukin-12 but not interleukin-18 is required for immunity to Trypanosoma cruzi in mice." Microbes and Infection **5**(10): 833.

http://www.sciencedirect.com/science/article/B6VPN-491BJJG-2/2/d78218441f2b529df3d08fbb27c99b40

Protective immunity to the parasite Trypanosoma cruzi in mice depends on a pro-inflammatory T

cell response involving the production of interferon-[gamma] (IFN-[gamma]). In conjunction with interleukin-12 (IL-12), IL-18 promotes the synthesis of IFN-[gamma] and a T helper type 1 immune response. We investigated the requirements of IL-12 and IL-18 in murine T. cruzi infection by use of C57BL/6 mice genetically deficient in either cytokine. IL-12p40-/- mice succumbed to infection at doses of 100 parasites, whereas IL-18-/- and wild-type mice resisted infectious doses up to 1000 parasites to the same extent. Levels of parasitemia were comparable between the latter groups, as were tissue parasite burdens according to quantitative real-time PCR. In contrast, IL-12p40-/- mice displayed vastly increased levels of parasites both in blood and in tissue. IFN-[gamma] concentrations in the serum of infected mice and in supernatants of splenocytes stimulated in vitro were decreased in IL-18-/- mice, whereas in IL-12p40-/- mice, IFN-[gamma] was undetectable in the serum and drastically reduced in cell supernatants. Levels of IL-12 production were generally comparable between wild-type and IL-18-/- mice, as were levels of IL-4, IL-2 and nitric oxide. Thus, the requirement for endogenous pro-inflammatory cytokines for a protective murine immune response against T. cruzi is satisfied by the expression of IL-12, while IL-18 is dispensable.

Green, L. C., P. J. Didier, et al. (2004). "Natural and experimental infection of immunocompromised rhesus macaques (Macaca mulatta) with the microsporidian Enterocytozoon bieneusi genotype D." Microbes and Infection **6**(11): 996.

http://www.sciencedirect.com/science/article/B6VPN-4CTJ467-1/2/2d0844cf04fb6a20cb3ebd85d4e0c812

Microsporidia are obligate intracellular parasites that cause opportunistic infections in AIDS and other immunocompromised patients. Eight simian immunodeficiency virus (SIV)-infected rhesus macaque monkeys (Macaca mulatta) were inoculated orally with Enterocytozoon bieneusi spores isolated from intestinal lavage fluid of an AIDS patient (genotype D) to study the natural history of this infection. Four monkeys were already naturally infected with E. bieneusi (also genotype D), and were included to determine if a second inoculum affected the course of illness. Spore shedding was detected in feces of all eight monkeys within the first week of experimental infection. Five monkeys died within 3.5 months of experimental E. bieneusi inoculation. Three of these five monkeys began the study with CD4+CD29+ T cell levels well below 20% of total T lymphocytes. Deaths were due to a variety of AIDS-related manifestations. Microsporidia did not appear to directly contribute to mortality but may have contributed to morbidity. At necropsy, microsporidia were found in bile and tissue sections of the gallbladder but not in the gut, kidneys, or liver. The percent CD4+CD29+ levels of the last three monkeys remained near the level observed at the time of inoculation. These monkeys lived more than 2 years after the end of the study and continued to shed spores. This study corroborates previous reports that E. bieneusi can be reliably transmitted to SIV-infected rhesus monkeys but indicates that the use of SIV-infected monkeys for the study of microsporidiosis is complicated by the confounding effect of other opportunistic or AIDS-related infections.

Hort, G. M., J. Weisenburger, et al. (2003). "Delayed type hypersensitivity-associated disruption of splenic periarteriolar lymphatic sheaths coincides with temporary loss of IFN-[gamma] production and impaired eradication of bacteria in Brucella abortus-infected mice." Microbes and Infection **5**(2): 95.

http://www.sciencedirect.com/science/article/B6VPN-47RB11M-1/2/a6d7f3bd262e202e611386154a1e89da

A major problem of infections with facultative intracellular bacteria is their chronic course. We comprehensively evaluated the host response in murine brucellosis to study mechanisms

contributing to bacterial persistence in the presence of an established immune response. Evidence is presented that the decrease in eradication kinetics, reproducibly occurring 18 d after infection of mice with Brucella abortus S19, is related to a state of downregulation of defense mechanisms. This is not due to a Th1 to Th2 switch or prostaglandin-mediated suppression by macrophages but is most probably caused by a severe disruption of spleen morphology at the height of Brucella-induced delayed type hypersensitivity. This results in a profound depletion of both CD4+ and CD8+ T cells in periarteriolar lymphatic sheaths, a consecutive deleterious shift in the relation of permissive macrophages and protective lymphocytes and an impaired capacity of splenocytes to produce IFN-[gamma] in response to soluble Brucella antigen.

Kawakami, K., Y. Kinjo, et al. (2004). "Interferon-[gamma] production and host protective response against Mycobacterium tuberculosis in mice lacking both IL-12p40 and IL-18." Microbes and Infection **6**(4): 339.

http://www.sciencedirect.com/science/article/B6VPN-4BSWK35-2/2/199bebf1c444a84aa673ae0615d2474d

Interferon (IFN)-[gamma] plays an essential role in host defense against infection with Mycobacterium tuberculosis, and its synthesis is critically regulated by interleukin (IL)-12, IL-18 and the recently identified IL-23. The present study was designed to determine the roles of these cytokines in IFN-[gamma]-mediated host defenses against M. tuberculosis. For this purpose, we compared host protective responses in IL-12p40 and IL-18 double-knockout (DKO) mice (which lacked both IL-12/IL-18 and also IL-23) and IFN-[gamma] gene-disrupted (GKO) mice. DKO mice were more resistant to the infection than GKO mice, as indicated by their extended survival and reduced live colony numbers in spleen, liver and lung. IFN-[gamma] was detected by ELISA in liver and lung homogenates, but not in spleen and serum, and in all organs by RT-PCR in DKO mice at comparable or reduced levels to those in wild-type mice. IFN-[gamma] production was reduced by depletion of CD4+ T cells, but not of natural killer (NK), NKT, [gamma][delta]T and dendritic cells. Neutralization of IFN-[gamma] or TNF-[alpha] by specific monoclonal antibodies (mAbs) significantly shortened the survival time of the infected DKO mice. Furthermore, anti-TNF-[alpha] mAb partially attenuated IFN-[gamma] synthesis in the liver of these mice. Finally, the expression level of inducible nitric oxide synthase (iNOS) mRNA in the spleen, liver and lung was considerable in DKO mice but only marginal or undetected in GKO mice. Our results indicate the presence of IL-12-, IL-18- and IL-23-independent host protective responses against mycobacterial infection mediated by IFN-[gamma], which was secreted from helper T cells.

Lan, R., A. M. Davison, et al. (2003). "AFLP analysis of Salmonella enterica serovar Typhimurium isolates of phage types DT 9 and DT 135: diversity within phage types and its epidemiological significance." Microbes and Infection **5**(10): 841.

http://www.sciencedirect.com/science/article/B6VPN-490R86X-2/2/59272e30454607229f879e4c807612f9

Amplified fragment length polymorphism (AFLP) was applied to 35 and 34 isolates, respectively, of Salmonella enterica serovar Typhimurium phage types DT 9 and DT 135, using eight primer pair combinations. Eight and 17 AFLP types were observed in DT 9 and DT 135, respectively. DT 9 is rare in the UK and common in Australia, but one AFLP form dominated with 28 isolates, comprising 22 of 25 UK isolates, four of five Australian isolates, one Jamaican and one Spanish isolate. Of the others, two UK isolates are closely related to the major form, two from elsewhere are in the major cluster and three isolates from different countries are in a separate cluster. For DT 135, two closely related AFLP types of seven and 11 isolates form the major cluster, which also includes 11 isolates, mostly in single-isolate AFLP types, while five isolates from different

countries form a well-separated minor cluster. For both DTs all isolates are grouped together if only the phage type specific bands identified earlier are used, confirming their value for molecular-based 'phage typing'. Polymorphic markers identified in this study could also be used for subtyping within both phage types. The value of AFLP is in locating DNA fragments useful for typing, but implementation of a replacement typing scheme would probably involve multiplex PCR or microarray technologies.

Lee, B.-J., M. Watanabe, et al. (2003). "Age- and host-dependent control of Borna disease virus spread in the developing brains of gerbils and rats." Microbes and Infection **5**(13): 1195.

http://www.sciencedirect.com/science/article/B6VPN-49JG390-1/2/d643f6f6f5cbb4b42b0bc0a2676f69dd

Borna disease virus (BDV) is a non-cytolytic, neurotropic RNA virus that has a broad host range in warm-blooded animals, probably including humans. Recently, we have demonstrated that the neonatal gerbil is a unique model for analyzing BDV-induced acute neurological disease. In this report, to understand the effects of the brain development of gerbils in BDV-induced neuropathogenesis, as well as to investigate the host-dependent differences in BDV propagation and pathogenesis in the brains, we performed experimental infection of BDV using two different infant rodent models, gerbils and rats. We demonstrated here that most of the gerbils infected with BDV on postnatal days (PD) 14, but not on PD1 and PD7, could survive neurological disorders during the observation period of PD85. Interestingly, the levels of BDV RNA and antigen in surviving PD14 inoculated gerbil brains were extremely low, whereas diseased gerbils and both PD7 and PD14 inoculated rats contained significant amounts of BDV antigen in the central nervous system, suggesting that PD14 gerbils successfully controlled BDV spread in the brain. Furthermore, the viral distribution, as well as the expression levels of cytokine and CD8 mRNAs, in the brains was markedly different between the rodent models and between diseased and non-diseased statuses of the gerbils. These results demonstrated that developmentally regulated and host-specific factors could contribute to the prevention of BDV spread in developing animal brains. Studies using different animal systems would provide novel insights into the mechanisms of host defense responses to neurotropic virus infections.

Wolff, C., T. Kruppa, et al. (2001). "Rapid elimination of GB virus C (hepatitis G virus) in the mosquito Aedes aegypti." Microbes and Infection **3**(9): 683.

http://www.sciencedirect.com/science/article/B6VPN-43P3WGY-1/2/0cc908b05257dd3d0419bdac1d72e2f4

The transmissibility of the GB virus C (hepatitis G virus; HGV), a member of the Flaviviridae, by a typical flavivirus vector was investigated. Female mosquitoes of the species Aedes aegypti were fed with HGV-infected human blood and assayed 1, 24, 48, 72 and 96 h after the blood meal for viral RNA, human glyceraldehyde-3-phosphate dehydrogenase mRNA, human [beta]-actin DNA and A. aegypti actin mRNA by total nucleic acid extraction, reverse transcription and PCR. Viral RNA had already disappeared from nucleic acid extracts 1 h after the blood meal and was not detectable throughout the observation period. Aedes-specific mRNA served as an internal control and was detected in all nucleic acid extracts, whereas human mRNA had disappeared after 24 h, indicating digestion of human cells. From these results we conclude that GB virus C (HGV) cannot replicate in A. aegypti, which is a widespread and competent vector of several other flaviviruses.

Microbial Pathogenesis (2)

Smirnova, N. I., N. B. Cheldyshova, et al. (2004). "Molecular-genetic peculiarities of classical biotype Vibrio cholerae, the etiological agent of the last outbreak Asiatic cholera in Russia." Microbial Pathogenesis **36**(3): 131.

http://www.sciencedirect.com/science/article/B6WN6-4B4HB5D-1/2/beb5fa049ba30acb919949315dfc9bb4

Molecular-genetic properties of classical biotype Vibrio cholerae strains that caused the Asiatic cholera outbreak in 1942 in Russia have been investigated for the first time. Being characterized by high-level production of cholera toxin and toxin-coregulated adhesion pili both of which are the major virulence factors, all the strains studied, in contrast to the typical cholera pathogens, were autographic requiring purine and/or amino acids added to the minimal medium for their growth. Moreover, these strains containing the structural gene hapA, as shown by the polymerase chain reaction, produced no soluble hemagglutinin/protease, which enables the vibrios to get disseminated in the environment. The peculiarities of the natural V. cholerae strains elucidated in the work are likely to be responsible for the unusual infectious and epidemic processes observed during that cholera outbreak.

Weiss, D. J., O. A. Evanson, et al. (2004). "Sequential patterns of gene expression by bovine monocyte-derived macrophages associated with ingestion of mycobacterial organisms." Microbial Pathogenesis **37**(4): 215.

http://www.sciencedirect.com/science/article/B6WN6-4DBSV92-1/2/8a961e1228e023d6ff446280eeb1f501

We investigated mechanisms involved in killing of mycobacterial organisms by comparing the response of bovine monocyte-derived macrophages to ingestion of Mycobacterium avium subsp. paratuberculosis or M. avium subsp. avium organisms. Previous studies have shown that bovine macrophages have the capacity to kill M. avium subsp. avium organisms in vitro but cannot kill M. avium subsp. paratuberculosis organisms. We used bovine cDNA microarray technology to investigate sequential gene expression by bovine monocyte-derived macrophages and function assays to correlate gene expression with biological activity. Results of the gene expression studies indicated substantial differences between macrophages phagocytizing the two organisms. At 2, 6, and 24 h after infection, 12, 53, and 19 genes, respectively, were differentially expressed. Over all time periods, approximately twice as many genes had lower expression in M. avium subsp. paratuberculosis-infected macrophages than had greater expression. Differentially regulated genes of most interest to antimicrobial responses included inflammatory molecules (transforming growth factor-[beta], thrombospondin 1, monocyte chemokine, and cathepsin K), phagosome-lysosome-related genes (H+ ATPases, lysosomal-associated membrane protein 2, vesicle trafficking protein, and solute carrier protein), and apoptosis-related genes (tumor necrosis factor receptor-associated factor 2, and tumor protein p53 binding protein). Function assays indicated that M. avium subsp. avium-infected macrophages had a greater capacity to acidify phagosomes and a greater percentage of apoptotic cells. In conclusion, these results suggest that a complex interaction between macrophages and mycobacterial organisms is involved in determining the fate of the organism. Although multiple genes and metabolic pathways are involved, the capacity of cells to acidify phagosomes and induce apoptosis appears to play a prominent role.

Microbiological Research (1)

El Karkouri, K., F. Martin, et al. (2005). "Diversity of ectomycorrhizal fungi naturally established on containerised Pinus seedlings in nursery conditions." Microbiological Research **160**(1): 47.

http://www.sciencedirect.com/science/article/B7GJ8-4DTKYNR-4/2/834062b757f2da0e896cf2020c1961fb

SummaryThe study examined the diversity of ectomycorrhizal fungi, naturally established on roots of containerised Pinus seedlings in a nursery, using PCR-RFLP and sequencing of the nuclear ribosomal internal transcribed spacer. Seventy-two samples, including ectomycorrhizae and fruit bodies, were examined. Molecular typing assigned the fungal symbionts to four ectomycorrhizal Boletales: Rhizopogon rubescens, Suillus bovinus, S. variegatus, and R. luteolus. R. rubescens was abundant (37.5%), while Suillus and R. luteolus species were moderately established (25-26%) and rare (2.8%), respectively. In addition, Rhizopogon species colonised P. nigra ssp. salzmannii seedlings, whereas Suillus species were identified on Pinus nigra ssp. nigra seedlings. The diversity and the ability of these naturally established symbionts under artificial nursery conditions were discussed. The molecular survey investigated here should contribute to successful monitoring of mycorrhizal application under both nursery and plantation conditions.

Microbiology (22)

Ahren, D., M. Tholander, et al. (2005). "Comparison of gene expression in trap cells and vegetative hyphae of the nematophagous fungus Monacrosporium haptotylum." Microbiology **151**(3): 789-803.

http://mic.sgmjournals.org/cgi/content/abstract/151/3/789

Nematode-trapping fungi enter the parasitic stage by developing specific morphological structures called traps. The global patterns of gene expression in traps and mycelium of the fungus Monacrosporium haptotylum were compared. The trap of this fungus is a unicellular spherical structure called the knob, which develops on the apex of a hyphal branch. RNA was isolated from knobs and mycelium and hybridized to a cDNA array containing probes of 2822 EST clones of M. haptotylum. Despite the fact that the knobs and mycelium were grown in the same medium, there were substantial differences in the patterns of genes expressed in the two cell types. In total, 23{middle dot}3 % (657 of 2822) of the putative genes were differentially expressed in knobs versus mycelium. Several of these genes displayed sequence similarities to genes known to be involved in regulating morphogenesis and cell polarity in fungi. Among them were several putative homologues for small GTPases, such as rho1, rac1 and ras1, and a rho GDP dissociation inhibitor (rdi1). Several homologues to genes involved in stress response, protein synthesis and protein degradation, transcription, and carbon metabolism were also differentially expressed. In the last category, a glycogen phosphorylase (gph1) gene homologue, one of the most

upregulated genes in the knobs as compared to mycelium, was characterized. A number of the genes that were differentially expressed in trap cells are also known to be regulated during the development of infection structures in plant-pathogenic fungi. Among them, a gas1 (mas3) gene homologue (designated gks1), which is specifically expressed in appressoria of the rice blast fungus, was characterized.

Alice, A. F., G. Perez-Martinez, et al. (2003). "Phosphoenolpyruvate phosphotransferase system and N-acetylglucosamine metabolism in Bacillus sphaericus." Microbiology **149**(7): 1687-1698.

http://mic.sgmjournals.org/cgi/content/abstract/149/7/1687

Bacillus sphaericus, a bacterium of biotechnological interest due to its ability to produce mosquitocidal toxins, is unable to use sugars as carbon source. However, ptsHI genes encoding HPr and EI proteins belonging to a PTS were cloned, sequenced and characterized. Both HPr and EI proteins were fully functional for phosphoenolpyruvate-dependent transphosphorylation in complementation assays using extracts from Staphylococcus aureus mutants for one of these proteins. HPr(His6) was purified from wild-type and a Ser46/Gln mutant of B. sphaericus, and used for in vitro phosphorylation experiments using extracts from either B. sphaericus or Bacillus subtilis as kinase source. The results showed that both phosphorylated forms, P-Ser46-HPr and P-His15-HPr, could be obtained. The findings also proved indirectly the existence of an HPr kinase activity in B. sphaericus. The genetic structure of these ptsHI genes has some unusual features, as they are co-transcribed with genes encoding metabolic enzymes related to N-acetylglucosamine (GlcNAc) catabolism (nagA, nagB and an undetermined orf2). In fact, this bacterium was able to utilize this amino sugar as carbon and energy source, but a ptsH null mutant had lost this characteristic. Investigation of GlcNAc uptake and streptozotocin inhibition in both a wild-type and a ptsH null mutant strain led to the proposal that GlcNAc is transported and phosphorylated by an EIINag element of the PTS, as yet uncharacterized. In addition, GlcNAc-6-phosphate deacetylase and GlcN-6-phosphate deaminase activities were determined; both were induced in the presence of GlcNAc. These results, together with the authors' recent findings of the presence of a phosphofructokinase activity, are strongly indicative of a glycolytic pathway in B. sphaericus. They also open new possibilities for genetic improvements in industrial applications.

Anlezark, G. M., T. Vaughan, et al. (2002). "Bacillus amyloliquefaciens orthologue of Bacillus subtilis ywrO encodes a nitroreductase enzyme which activates the prodrug CB 1954." Microbiology **148**(1): 297-306.

http://mic.sgmjournals.org/cgi/content/abstract/148/1/297

A nitroreductase with distinct properties that can activate the prodrug 5-aziridinyl-2,4-dinitrobenzamide (CB 1954) was isolated from Bacillus amyloliquefaciens. The encoding gene was identified as a homologue of the ywrO of Bacillus subtilis, and was obtained as a PCR product by reverse genetics, cloned and the entire nucleotide sequence determined. The gene was found to reside between homologues of the B. subtilis alsD and yswB genes; however, the ywrO and yswB genes of B. amyloliquefaciens were not separated by a fourth gene, ywsA. The B. amyloliquefaciens ywrO gene was overexpressed, the recombinant protein purified and its properties were compared with those of two CB 1954-activating enzymes, Escherichia coli B nitroreductase (NTR) and Walker DT-diaphorase (DTD). In common with these enzymes menadione was an electron acceptor (Km 3 {micro}M) and activity with this substrate was inhibited by the presence of dicoumarol (Ki 1{middle dot}0 {micro}M). In contrast, YwrO showed a marked preference for NADPH as a cofactor (Km 40 {micro}M) and therefore could not be classified as a DTD (EC 1.6.99.2). The flavin FMN was an acceptor with high affinity. B.

amyloliquefaciens YwrO was shown to be a flavoprotein with a monomeric molecular mass of 21{middle dot}5 kDa by calculation and SDS-PAGE. The cytotoxic 4-hydroxylamine derivative was the single CB 1954 reduction product, but B. amyloliquefaciens YwrO was inactive with the bischloroethyl analogue of CB 1954, SN 23862. In both of these properties B. amyloliquefaciens YwrO more closely resembles DTD than NTR. Its Km for CB 1954 was lower than that of NTR (617 {micro}M compared to 862 {micro}M). Enhanced in vitro cytotoxicity of CB 1954 was demonstrated on incubation of V79 cells with prodrug, NADPH and B. amyloliquefaciens YwrO. The work has led to the identification of a previously unknown nitroreductase, B. amyloliquefaciens YwrO, with distinct properties which will aid the rational selection of appropriate genes for applications in directed enzyme prodrug therapy (DEPT).

Bardarov, S., S. Bardarov, Jr., et al. (2002). "Specialized transduction: an efficient method for generating marked and unmarked targeted gene disruptions in Mycobacterium tuberculosis, M. bovis BCG and M. smegmatis." Microbiology **148**(10): 3007-3017.

http://mic.sgmjournals.org/cgi/content/abstract/148/10/3007

The authors have developed a simple and highly efficient system for generating allelic exchanges in both fast- and slow-growing mycobacteria. In this procedure a gene of interest, disrupted by a selectable marker, is cloned into a conditionally replicating (temperature-sensitive) shuttle phasmid to generate a specialized transducing mycobacteriophage. The temperature-sensitive mutations in the mycobacteriophage genome permit replication at the permissive temperature of 30 {degrees}C but prevent replication at the non-permissive temperature of 37 {degrees}C. Transduction at a non-permissive temperature results in highly efficient delivery of the recombination substrate to virtually all cells in the recipient population. The deletion mutations in the targeted genes are marked with antibiotic-resistance genes that are flanked by {gamma}{delta}-res (resolvase recognition target) sites. The transductants which have undergone a homologous recombination event can be conveniently selected on antibiotic-containing media. To demonstrate the utility of this genetic system seven different targeted gene disruptions were generated in three substrains of Mycobacterium bovis BCG, three strains of Mycobacterium tuberculosis, and Mycobacterium smegmatis. Mutants in the lysA, nadBC, panC, panCD, leuCD, Rv3291c and Rv0867c genes or operons were isolated as antibiotic-resistant (and in some cases auxotrophic) transductants. Using a plasmid encoding the {gamma}{delta}-resolvase (tnpR), the resistance genes could be removed, generating unmarked deletion mutations. It is concluded from the high frequency of allelic exchange events observed in this study that specialized transduction is a very efficient technique for genetic manipulation of mycobacteria and is a method of choice for constructing isogenic strains of M. tuberculosis, BCG or M. smegmatis which differ by defined mutations.

Becker, S., P. K. Hayes, et al. (2005). "Different gvpC length variants are transcribed within single filaments of the cyanobacterium Planktothrix rubescens." Microbiology **151**(1): 59-67.

http://mic.sgmjournals.org/cgi/content/abstract/151/1/59

Transcripts of the gas vesicle genes gvpA and gvpC were detected in single filaments of the cyanobacterium Planktothrix rubescens using reverse transcription and quantitative real-time PCR. Primers were designed to amplify short sequences within gvpA and three length variants of gvpC. With genomic template DNA, and using Sybr Green to monitor product accumulation, similar amplification efficiencies were observed for each of these genes. The relative copy numbers of gvpC length variants in genomic DNA from five Planktothrix gas vesicle genotypes determined by real-time PCR were similar to those indicated by sequencing the gas vesicle gene clusters. The precipitation of gvp cDNA reverse-transcribed from cellular RNA from single

filaments was required before amplification of the gene fragments; without this step it was not possible to detect the accumulation of the expected amplicons by dissociation analysis. Precipitation was also necessary to ensure the generation of product curves that allowed linear regression in an early stage of PCR, a prerequisite for the quantification of low-input cDNA amounts without the need for standard curves. This report shows that different gvpC length variants are transcribed within single Planktothrix filaments, both from laboratory cultures and from natural samples taken from Lake Zurich. This has implications for the efficiency of buoyancy provision by the possible production of gas vesicles of different strengths within individual cyanobacterial filaments. The hypothesis that post-transcriptional regulation may influence the type of protein (GvpC) present in gas vesicles is presented.

Creuzburg, K., B. Kohler, et al. (2005). "Genetic structure and chromosomal integration site of the cryptic prophage CP-1639 encoding Shiga toxin 1." Microbiology **151**(3): 941-950.

http://mic.sgmjournals.org/cgi/content/abstract/151/3/941

The sequence of 50 625 bp of chromosomal DNA derived from Shiga-toxin (Stx)-producing Escherichia coli (STEC) O111: H- strain 1639/77 was determined. This DNA fragment contains the cryptic Stx1-encoding prophage CP-1639 and its flanking chromosomal regions. The genome of CP-1639 basically resembles that of lambdoid phages in structure, but contains three IS629 elements, one of which disrupts the gene of a tail fibre component. The prophage genome lacks parts of the recombination region including integrase and excisionase genes. Moreover, a capsid protein gene is absent. CP-1639 is closely associated with an integrase gene of an ancient integrative element. This element consists of three ORFs of unknown origin and a truncated integrase gene homologous to intA of CP4-57. By PCR analysis and sequencing, it was shown that this integrative element is present in a number of non-O157 STEC serotypes and in non-STEC strains, where it is located at the 3'-end of the chromosomal ssrA gene. Whereas in most E. coli O111: H- strains, prophages are inserted in this site, E. coli O26 strains contain the integrative element not connected to a prophage. In E. coli O103 strains, the genetic structure of this region is variable. Comparison of DNA sequences of this particular site in E. coli O157: H7 strain EDL933, E. coli O111: H- strain 1639/77 and E. coli K-12 strain MG1655 showed that the ssrA gene is associated in all cases with the presence of foreign DNA. The results of this study have shown that the cryptic prophage CP-1639 is associated with an integrative element at a particular site in the E. coli chromosome that possesses high genetic variability.

Fernandez, M., Y. Cuadrado, et al. (2002). "Characterization of the hom-thrC-thrB cluster in aminoethoxyvinylglycine-producing Streptomyces sp. NRRL 5331." Microbiology **148**(5): 1413-1420.

http://mic.sgmjournals.org/cgi/content/abstract/148/5/1413

Three genes from the aminoethoxyvinylglycine (AVG)-producing Streptomyces sp. NRRL 5331 involved in threonine biosynthesis, hom, thrB and thrC, encoding homoserine dehydrogenase (HDH), homoserine kinase (HK) and threonine synthase (TS), respectively, have been cloned and sequenced. The hom and thrC genes appear to be organized in a bicistronic operon as deduced by disruption experiments. The thrB gene, however, is transcribed as a monocistronic transcript. The encoded proteins are quite similar to the HDH, HK and TS proteins from other bacterial species. The overall organization of these three genes, in the order hom-thrC-thrB, differs from that in other bacteria and is similar to that reported in the Streptomyces coelicolor genome sequence. This is the first time in which the gene cluster for the three last steps of threonine biosynthesis has been characterized from a streptomycete. Disruption of thrC indicated that threonine is not a direct precursor for AVG biosynthesis in Streptomyces sp. NRRL 5331 and

suggested that the branching point of the aspartic acid-derived biosynthetic route of this metabolite should lie earlier on the threonine biosynthetic route.

Gomez-Gil, B., S. Soto-Rodriguez, et al. (2004). "Molecular identification of Vibrio harveyi-related isolates associated with diseased aquatic organisms." Microbiology **150**(6): 1769-1777.

http://mic.sgmjournals.org/cgi/content/abstract/150/6/1769

Fifty strains belonging to Vibrio harveyi, Vibrio campbellii, and the recently described Vibrio rotiferianus, were analysed using phenotypic and genomic techniques with the aim of analysing the usefulness of the different techniques for the identification of V. harveyi-related species. The species V. harveyi and V. campbellii were phenotypically indistinguishable by more than 100 phenotypic features. Thirty-nine experimental strains were phenotypically identified as V. harveyi, but FAFLP, REP-PCR, IGS-PCR and DNA-DNA hybridization proved that they in fact belong to the species V. campbellii. Similar groupings were found among all fingerprinting methodologies (except IGS-PCR). Thirty-two experimental strains clustered with the V. campbellii type and one reference strain; seven strains clustered with the V. harveyi type and three reference strains; and the type and four reference strains of V. rotiferianus grouped together. The correlations between DNA-DNA hybridization and the genomic fingerprinting by FAFLP and (GTG)5-PCR were found to be above 0{middle dot}68 and statistically significant, suggesting the value of the latter techniques for the reliable identification of V. harveyi-related species. The results presented indicate that strains phenotypically identified as V. harveyi are in fact V. campbellii; these findings position V. campbellii as an important species involved in diseases of reared aquatic organisms.

Hardy, K. J., D. W. Ussery, et al. (2004). "Distribution and characterization of staphylococcal interspersed repeat units (SIRUs) and potential use for strain differentiation." Microbiology **150**(12): 4045-4052.

http://mic.sgmjournals.org/cgi/content/abstract/150/12/4045

Variable-number tandem repeats (VNTRs) have been shown to be a powerful tool in the determination of evolutionary relationships and population genetics of bacteria. The sequencing of a number of Staphylococcus aureus genomes has allowed the identification of novel VNTR sequences in S. aureus, which are similar to those used in the study of the evolution of Mycobacterium tuberculosis clades. Seven VNTRs, termed staphylococcal interspersed repeat units (SIRUs), distributed around the genome are described, occurring in both unique and multiple sites, and varying in length from 48 to 159 bp. Variations in copy numbers were observed in all loci, within both the sequenced genomes and the UK epidemic methicillin-resistant S. aureus (EMRSA) isolates. Clonally related UK EMRSA isolates were clustered using SIRUs, which provided a greater degree of discrimination than multi-locus sequence typing, indicating that VNTRs may be a more appropriate evolutionary marker for studying transmission events and the geographical spread of S. aureus clades.

Hobb, R. I., H.-J. Tseng, et al. (2002). "Molecular analysis of a haemagglutinin of Haemophilus paragallinarum." Microbiology **148**(7): 2171-2179.

http://mic.sgmjournals.org/cgi/content/abstract/148/7/2171

The gene encoding a haemagglutinin of H. paragallinarum, hagA, has been identified and the full-

length nucleotide sequence determined. A [~]39 kDa protein, recognized by an anti-haemagglutinin monoclonal antibody, mAb4D, was purified from H. paragallinarum strain 0083 and the N-terminal sequence obtained. The full-length nucleotide sequence was obtained by inverse PCR and the deduced amino acid sequence of the protein encoded was shown to be similar to other outer-membrane proteins of closely related organisms in the HAP group (Haemophilus, Actinobacillus, Pasteurella), especially the P5 protein of Haemophilus influenzae. The hagA gene was cloned into a His-tag expression vector and overexpressed in Escherichia coli strain M15(pREP4). The identity of the purified recombinant protein as a H. paragallinarum haemagglutinin was confirmed by haemagglutination of chicken red blood cells and reactivity, in a Western blot, with the monoclonal antibody specific for the serovar A haemagglutinin.

Ichinose, K., M. Ozawa, et al. (2003). "Cloning, sequencing and heterologous expression of the medermycin biosynthetic gene cluster of Streptomyces sp. AM-7161: towards comparative analysis of the benzoisochromanequinone gene clusters." Microbiology **149**(7): 1633-1645.

http://mic.sgmjournals.org/cgi/content/abstract/149/7/1633

Medermycin is a Streptomyces aromatic C-glycoside antibiotic classified in the benzoisochromanequinones (BIQs), which presents several interesting biosynthetic problems concerning polyketide synthase (PKS), post-PKS tailoring and deoxysugar pathways. The biosynthetic gene cluster for medermycin (the med cluster) was cloned from Streptomyces sp. AM-7161. Completeness of the clone was proved by the heterologous expression of a cosmid carrying the entire med cluster in Streptomyces coelicolor CH999 to produce medermycin. The DNA sequence of the cosmid (36 202 bp) revealed 34 complete ORFs, with an incomplete ORF at either end. Functional assignment of the deduced products was made for PKS and biosynthetically related enzymes, tailoring steps including strereochemical control, oxidation, angolosamine pathway, C-glycosylation, and regulation. The med cluster was estimated to be about 30 kb long, covering 29 ORFs. An unusual characteristic of the cluster is the disconnected organization of the minimal PKS genes: med-ORF23 encoding the acyl carrier protein is 20 kb apart from med-ORF1 and med-ORF2 for the two ketosynthase components. Secondly, the six genes (med-ORF14, 15, 16, 17, 18 and 20) for the biosynthesis of the deoxysugar, angolosamine, are all contiguous. Finally, the finding of a glycosyltransferase gene, med-ORF8, suggests a possible involvement of conventional C-glycosylation in medermycin biosynthesis. Comparison among the three complete BIQ gene clusters - med and those for actinorhodin (act) and granaticin (gra) - revealed some common genes whose deduced functions are unavailable from database searches (the unknowns'). An example is med-ORF5, a homologue of actVI-ORF3 and gra-ORF18, which was highlighted by a recent proteomic analysis of S. coelicolor A3(2).

Manos, J. and R. Belas (2004). "Transcription of Proteus mirabilis flaAB." Microbiology **150**(9): 2857-2863.

http://mic.sgmjournals.org/cgi/content/abstract/150/9/2857

Proteus mirabilis, a Gram-negative urinary tract pathogen, has two highly homologous, tandemly arranged flagellin-encoding genes, flaA and flaB. flaA is transcribed from a{sigma} 28 promoter, while flaB is a silent allele. Previous studies have demonstrated the presence of a family of hybrid flagellin genes, referred to as flaAB. These genes are composed of the 5' end of flaA and the 3' end of flaB, and are produced through excision of the intervening DNA between the two genes. Although the existence of flaAB DNA has been documented, it was not known if transcription of flaAB occurs in wild-type P. mirabilis. In this study, proof of flaAB transcription was obtained from a combination of RNA dot-blots and RT-PCR assays using specific primers and probes for flaAB and flaA. The RNA data were further supported by the demonstration of phenotypic switching of

the locus using a FlaAB-detector strain. The results show that flaAB mRNA is transcribed and is 1/64 as abundant as flaA in the population of wild-type cells, suggesting that flaAB constitutes 1{middle dot}0-1{middle dot}5 % of the total flagellin message. Nucleotide sequence analysis of flaAB products produced by RT-PCR from the wild-type confirms previous reports of a variable fusion site between flaA and flaB resulting in a hybrid flagellin transcript. These data support the hypothesis that the production of FlaAB is integral to the physiology of P. mirabilis.

Marceau, M., F. Sebbane, et al. (2004). "The pmrF polymyxin-resistance operon of Yersinia pseudotuberculosis is upregulated by the PhoP-PhoQ two-component system but not by PmrA-PmrB, and is not required for virulence." Microbiology **150**(12): 3947-3957.

http://mic.sgmjournals.org/cgi/content/abstract/150/12/3947

The Yersinia pseudotuberculosis chromosome contains a seven-gene polycistronic unit (the pmrF operon) whose products share extensive homologies with their pmrF counterparts in Salmonella enterica serovar Typhimurium (S. typhimurium), another Gram-negative bacterial enteropathogen. This gene cluster is essential for addition of 4-aminoarabinose to the lipid moiety of LPS, as demonstrated by MALDI-TOF mass spectrometry of lipid A from both wild-type and pmrF-mutated strains. As in S. typhimurium, 4-aminoarabinose substitution of lipid A contributes to in vitro resistance of Y. pseudotuberculosis to the antimicrobial peptide polymyxin B. Whereas pmrF expression in S. typhimurium is mediated by both the PhoP-PhoQ and PmrA-PmrB two-component regulatory systems, it appears to be PmrA-PmrB-independent in Y. pseudotuberculosis, with the response regulator PhoP interacting directly with the pmrF operon promoter region. This result reveals that the ubiquitous PmrA-PmrB regulatory system controls different regulons in distinct bacterial species. In addition, pmrF inactivation in Y. pseudotuberculosis has no effect on bacterial virulence in the mouse, again in contrast to the situation in S. typhimurium. The marked differences in pmrF operon regulation in these two phylogenetically close bacterial species may be related to their dissimilar lifestyles.

Monod, M., B. Lechenne, et al. (2005). "Aminopeptidases and dipeptidyl-peptidases secreted by the dermatophyte Trichophyton rubrum." Microbiology **151**(1): 145-155.

http://mic.sgmjournals.org/cgi/content/abstract/151/1/145

The nature of secreted aminopeptidases in Trichophyton rubrum was investigated by using a reverse genetic approach. T. rubrum genomic and cDNA libraries were screened with Aspergillus spp. and Saccharomyces cerevisiae aminopeptidase genes as the probes. Two leucine aminopeptidases, ruLap1 and ruLap2, and two dipeptidyl-peptidases, ruDppIV and ruDppV, were characterized and compared to orthologues secreted by Aspergillus fumigatus using a recombinant protein from Pichia pastoris. RuLap1 is a 33 kDa nonglycosylated protein, while ruLap2 is a 58-65 kDa glycoprotein. The hydrolytic activity of ruLap1, ruLap2 and A. fumigatus orthologues showed various preferences for different aminoacyl-7-amido-4-methylcoumarin substrates, and various sensitivities to inhibitors and cations. ruDppIV and ruDppV showed similar activities to A. fumigatus orthologues. In addition to endopeptidases, the four aminopeptidases ruLap1, ruLap2, ruDppIV and ruDppV were produced by T. rubrum in a medium containing keratin as the sole nitrogen source. Synergism between endo- and exopeptidases is likely to be essential for dermatophyte virulence, since these fungi grow only in keratinized tissues.

Puskarova, A., P. Ferianc, et al. (2002). "Regulation of yodA encoding a novel cadmium-induced protein in Escherichia coli." Microbiology **148**(12): 3801-3811.

http://mic.sgmjournals.org/cgi/content/abstract/148/12/3801

Bacterial accommodation to moderate concentrations of cadmium is accompanied by transient activation of general stress proteins as well as a sustained induction of other proteins of hitherto unknown functions. One of the latter proteins was previously identified as the product of the Escherichia coli yodA ORF. The yodA ORF encodes 216 aa residues (the YodA protein) and the increased synthesis of YodA during cadmium stress was found probably to be a result of transcriptional activation from one single promoter upstream of the structural yodA gene. Analysis of a transcriptional gene fusion, PyodA-lacZ, demonstrated that basal expression of yodA is low during exponential growth and expression is increased greater than 50-fold by addition of cadmium to growing cells. However, challenging cells with additional metals such as zinc, copper, cobalt and nickel did not increase the level of yodA expression. In addition, hydrogen peroxide also increased yodA expression whereas the superoxide-generating agent paraquat failed to do so. Surprisingly, cadmium-induced transcription of yodA is dependent on soxS and fur, but independent of oxyR. Moreover, a double relA spoT mutation abolished induction of yodA during cadmium exposure but ppGpp is not sufficient to induce yodA since expression of the gene is not elevated during stationary phase. After 45 min of cadmium exposure the YodA protein was primarily detected in the cytoplasmic fraction but was later (150 min) found in both the cytoplasmic and periplasmic compartments.

Rakotoarivonina, H., G. Jubelin, et al. (2002). "Adhesion to cellulose of the Gram-positive bacterium Ruminococcus albus involves type IV pili." Microbiology **148**(6): 1871-1880.

http://mic.sgmjournals.org/cgi/content/abstract/148/6/1871

This study was aimed at characterizing a cell-surface 25 kDa glycoprotein (GP25) that was previously shown to be underproduced by a spontaneous adhesion-defective mutant D5 of Ruminococcus albus 20. An antiserum against wild-type strain 20 was adsorbed with the mutant D5 to enrich it in antibodies specific' to adhesion structures of R. albus 20. The resulting antiserum, called anti-Adh serum, blocked adhesion of R. albus 20 and reacted mainly with GP25 in bacterial and extracellular protein fractions of R. albus 20. The N-terminal sequence of purified GP25 was identical to that of CbpC, a 21 kDa cellulose-binding protein (CBP) of R. albus 8. The nucleotide sequence of the gp25 gene was determined by PCR and genomic walking procedures. The gp25 gene encoded a protein of 165 aa with a calculated molecular mass of 16940 Da that showed 72{middle dot}9% identity with CbpC and presented homologies with type IV pilins of Gram-negative pathogenic bacteria. Negative-staining electron microscopy revealed fine and flexible pili surrounding R. albus 20 cells while mutant cells were not piliated. In addition, immunoelectron microscopy showed that the anti-Adh serum probing mainly GP25, completely decorated the pili surrounding R. albus 20, thereby showing that GP25 was a major pilus subunit. This study shows for the first time the presence of pili at the surface of R. albus and identifies GP25 as their major protein subunit. Though GP25 was not identified as a CBP, isolated pili were shown to bind cellulose. In conclusion, these pili, which belong to the family of type IV pili, mediate adhesion of R. albus 20 to cellulose.

Terry, T. D., Y. M. Zalucki, et al. (2003). "Genetic analysis of a plasmid encoding haemocin production in Haemophilus paragallinarum." Microbiology **149**(11): 3177-3184.

http://mic.sgmjournals.org/cgi/content/abstract/149/11/3177

The full sequence of plasmid p250, isolated from Haemophilus paragallinarum strain HP250, has been obtained. The plasmid contains seven ORFs: a putative integrase, a putative replication protein (repB) and five ORFs similar to those from the haemocin (bacteriocin) hmcDCBAI operon from Haemophilus influenzae. Of 19 other non-plasmid-containing H. paragallinarum strains screened (11 serovar reference strains and 8 field isolates), 17 strains produced haemocin and were resistant to killing by strain HP250. These strains, unlike strain HP250, have a chromosomally encoded haemocin operon. A number of other members of the family Pasteurellaceae were tested for haemocin sensitivity. Pasteurella avium, Pasteurella volantium and Pasteurella species A, all non-pathogenic bacteria found in the respiratory tract of chickens suffering from respiratory diseases, were sensitive to H. paragallinarum haemocin. However, amongst the pathogenic Pasteurellaceae, 50 % of P. multocida isolates and all five isolates of Pasteurella haemolytica tested were sensitive to the haemocin. Given the prevalence of haemocin production in H. paragallinarum strains, it may play a role in aiding colonization by inhibiting other Gram-negative bacteria that are associated with the respiratory tract in chickens. The origin of replication from plasmid p250 has been used to generate an Escherichia coli-H. paragallinarum shuttle vector which may be useful in genetically manipulating H. paragallinarum.

Xu, J., G. Luo, et al. (2002). "Multiple origins of hybrid strains of Cryptococcus neoformans with serotype AD." Microbiology **148**(1): 203-212.

http://mic.sgmjournals.org/cgi/content/abstract/148/1/203

Cryptococcus neoformans is a major pathogen of humans throughout the world. Using commercial mAbs to capsular epitopes, strains of C. neoformans manifest five distinct serotypes - A, B, C, D and AD. Previous studies demonstrated significant divergence among serotypes A, B, C and D, which are thought to be haploid. In this study the origins and evolution of strains of serotype AD were investigated. A portion (537 bp) of the laccase gene was cloned and sequenced from 14 strains of serotype AD. Each strain contained two different alleles and sequences for both alleles were obtained. These sequences were compared to those from serotypes A, B, C and D. This analysis indicated that each of the 14 serotype AD strains contained two phylogenetically distinct haplotypes: one haplotype was highly similar to the serotype A group and the other to the serotype D group. To explain the origins of these serotype AD strains, genealogical analysis is consistent with at least three recent and independent hybridization events. The results demonstrate that the evolution of C. neoformans is continuing and dynamic.

Xu, J. and T. G. Mitchell (2003). "Comparative gene genealogical analyses of strains of serotype AD identify recombination in populations of serotypes A and D in the human pathogenic yeast Cryptococcus neoformans." Microbiology **149**(8): 2147-2154.

http://mic.sgmjournals.org/cgi/content/abstract/149/8/2147

Cryptococcus neoformans is a major pathogen of humans throughout the world. Using commercial monoclonal antibodies to capsular epitopes, strains of C. neoformans manifest five serotypes: A, B, C, D and AD. Previous studies demonstrated significant divergence among serotypes A, B, C and D, which are typically haploid. In contrast, most strains of serotype AD are diploid or aneuploid and result from recent hybridization between strains of serotypes A and D. Whether serotypes A, B, C and D represent strictly asexual lineages is not known. Using comparative genealogical analyses of two genes, the authors investigated whether recombination occurred among strains within serotypes A and D. For each of 14 serotype AD strains, a portion (642 bp) of the orotidine monophosphate pyrophosphorylase (URA5) gene was cloned and sequenced. Each of these 14 strains contained two different alleles and sequences for both

alleles were obtained. The URA5 gene genealogy was compared to that derived from the laccase (LAC) gene, which was reported recently for the same 14 strains. For both genes, each of the 14 serotype AD strains contained two phylogenetically distinct alleles: one allele was highly similar to those from serotype A strains and the other to alleles from serotype D strains. However, within both the serotype A allelic group and the serotype D allelic group, there was significant incongruence between genealogies derived from URA5 and LAC. The results suggest recombination in natural populations of both serotypes A and D.

Yebra, M. J. and G. Perez-Martinez (2002). "Cross-talk between the L-sorbose and D-sorbitol (D-glucitol) metabolic pathways in Lactobacillus casei." Microbiology **148**(8): 2351-2359.

http://mic.sgmjournals.org/cgi/content/abstract/148/8/2351

A gene encoding sorbitol-6-phosphate dehydrogenase (SorF) belonging to the sorbose operon (sorFABCDG) has been characterized in Lactobacillus casei. Inactivation of this gene revealed the presence of another sorbitol-6-phosphate dehydrogenase that was induced by D-sorbitol (D-glucitol). The gene encoding this activity (gutF) has also been isolated, sequenced and disrupted. The sorbitol-6-phosphate dehydrogenase genes (sorF, gutF) were required for growth on L-sorbose and D-sorbitol, respectively. Biochemical and transcriptional analyses of the wild-type and mutant strains demonstrated that L-sorbose and D-sorbitol induced sorF and the gene encoding the sorbose operon activator (sorR), while the expression of gutF was only activated by D-sorbitol. Furthermore, these studies indirectly suggested that a common metabolite of the L-sorbose and D-sorbitol metabolic pathways (probably D-sorbitol 6-phosphate) would act as the effector of SorR. The same effector would also be the inducer of gutF, although the two pathways seem to be subject to distinct regulatory mechanisms.

Zhou, X., S. J. Bent, et al. (2004). "Characterization of vaginal microbial communities in adult healthy women using cultivation-independent methods." Microbiology **150**(8): 2565-2573.

http://mic.sgmjournals.org/cgi/content/abstract/150/8/2565

The normal microbial flora of the vagina plays an important role in preventing genital and urinary tract infections in women. Thus an accurate understanding of the composition and ecology of the ecosystem is important to understanding the aetiology of these diseases. Common wisdom is that lactobacilli dominate the normal vaginal microflora of post-pubertal women. However, this conclusion is based on methods that require cultivation of microbial populations; an approach that is known to yield a biased and incomplete assessment of microbial community structure. In this study cultivation-independent methods were used to analyse samples collected from the mid-vagina of five normal healthy Caucasian women between the ages of 28 and 44. Total microbial community DNA was isolated following resuspension of microbial cells from vaginal swabs. To identify the constituent numerically dominant populations in each community 16S rRNA gene libraries were prepared following PCR amplification using the 8f and 926r primers. From each library, the DNA sequences of approximately 200 16S rRNA clones were determined and subjected to phylogenetic analyses. The diversity and kinds of organisms that comprise the vaginal microbial community varied among women. Species of Lactobacillus appeared to dominate the communities in four of the five women. However, the community of one woman was dominated by Atopobium sp., whereas a second woman had appreciable numbers of Megasphaera sp., Atopobium sp. and Leptotrichia sp., none of which have previously been shown to be common members of the vaginal ecosystem. Of the women whose communities were dominated by lactobacilli, there were two distinct clusters, each of which consisted of a single species. One class consisted of two women with genetically divergent clones that were related to Lactobacillus crispatus, whereas the second group of two women had clones of

Lactobacillus iners that were highly related to a single phylotype. These surprising results suggest that culture-independent methods can provide new insights into the diversity of bacterial species found in the human vagina, and this information could prove to be pivotal in understanding risk factors for various infectious diseases.

Zhu, P., M. J. Klutch, et al. (2002). "Genetic diversity of three lgt loci for biosynthesis of lipooligosaccharide (LOS) in Neisseria species." Microbiology **148**(6): 1833-1844.

http://mic.sgmjournals.org/cgi/content/abstract/148/6/1833

Lipooligosaccharide (LOS) is a major virulence factor of the pathogenic Neisseria. Nine lgt genes at three chromosomal loci (lgt-1, 2, 3) encoding the glycosyltransferases responsible for the biosynthesis of LOS oligosaccharide chains were examined in 26 Neisseria meningitidis, 51 Neisseria gonorrhoeae and 18 commensal Neisseria strains. DNA hybridization, PCR and nucleotide sequence data were compared to previously reported lgt genes. Analysis of the genetic organization of the lgt loci revealed that in N. meningitidis, the lgt-1 and lgt-3 loci were hypervariable genomic regions, whereas the lgt-2 locus was conserved. In N. gonorrhoeae, no variability in the composition or organization of the three lgt loci was observed. lgt genes were detected only in some commensal Neisseria species. The genetic organization of the lgt-1 locus was classified into eight types and the lgt-3 locus was classified into four types. Two types of arrangement at lgt-1 (II and IV) and one type of arrangement at lgt-3 (IV) were novel genetic organizations reported in this study. Based on the three lgt loci, 10 LOS genotypes of N. meningitidis were distinguished. Phylogenetic analysis revealed a gene cluster, lgtH, which separated from the homologous genes lgtB and lgtE. The lgtH and lgtE genes were mutually exclusive and were located at the same position in lgt-1. The data demonstrated that pathogenic and commensal Neisseria share a common lgt gene pool and horizontal gene transfer appears to contribute to the genetic diversity of the lgt loci in Neisseria.

Microchemical Journal (1)

Geoun, M. B., K. S. Choi, et al. (2002). "Characterization of single-stranded DNA separation by capillary gel electrophoresis." Microchemical Journal **72**(3): 305.

http://www.sciencedirect.com/science/article/B6W6H-46SXWG2-5/2/bfdf97bf07cb7d1bf2400f5d68b2e8f7

We have investigated the effect of polymer gel reconditioning, the shape of the capillary, the applied electric field, and the capillary length for single-stranded DNA. The polyethylene oxide gel had deformed under the high electric field causing the degradation of the separation power. By the reintroduction of the fresh polyethylene oxide gel for the next run, one-base resolution was recovered. It turned out that the tip of the capillary at the injection side needed to be clean and symmetric for much improved resolution. Changing DNA motion by the pulsed electric field resulted in the separation of DNA far more than 500 bases.

Mol. Biol. Cell (10)

Askjaer, P., V. Galy, et al. (2002). "Ran GTPase Cycle and Importins alpha and beta Are Essential for Spindle Formation and Nuclear Envelope Assembly in Living Caenorhabditis elegans Embryos." Mol. Biol. Cell **13**(12): 4355-4370.

http://www.molbiolcell.org/cgi/content/abstract/13/12/4355

The small GTPase Ran has been found to play pivotal roles in several aspects of cell function. We have investigated the role of the Ran GTPase cycle in spindle formation and nuclear envelope assembly in dividing Caenorhabditis elegans embryos in real time. We found that Ran and its cofactors RanBP2, RanGAP, and RCC1 are all essential for reformation of the nuclear envelope after cell division. Reducing the expression of any of these components of the Ran GTPase cycle by RNAi leads to strong extranuclear clustering of integral nuclear envelope proteins and nucleoporins. Ran, RanBP2, and RanGAP are also required for building a mitotic spindle, whereas astral microtubules are normal in the absence of these proteins. RCC1(RNAi) embryos have similar abnormalities in the initial phase of spindle formation but eventually recover to form a bipolar spindle. Irregular chromatin structures and chromatin bridges due to spindle failure were frequently observed in embryos where the Ran cycle was perturbed. In addition, connection between the centrosomes and the male pronucleus, and thus centrosome positioning, depends upon the Ran cycle components. Finally, we have demonstrated that both IMA-2 and IMB-1, the homologues of vertebrate importin [alpha] and [beta], are essential for both spindle assembly and nuclear formation in early embryos.

Chou, W.-C., V. Prokova, et al. (2003). "Mechanism of a Transcriptional Cross Talk between Transforming Growth Factor-beta -regulated Smad3 and Smad4 Proteins and Orphan Nuclear Receptor Hepatocyte Nuclear Factor-4." Mol. Biol. Cell **14**(3): 1279-1294.

http://www.molbiolcell.org/cgi/content/abstract/14/3/1279

We have shown previously that the transforming growth factor-[beta] (TGF[beta])-regulated Sma-Mad (Smad) protein 3 and Smad4 proteins transactivate the apolipoprotein C-III promoter in hepatic cells via a hormone response element that binds the nuclear receptor hepatocyte nuclear factor 4 (HNF-4). In the present study, we show that Smad3 and Smad4 but not Smad2 physically interact with HNF-4 via their Mad homology 1 domains both in vitro and in vivo. The synergistic transactivation of target promoters by Smads and HNF-4 was shown to depend on the specific promoter context and did not require an intact [beta]-hairpin/DNA binding domain of the Smads. Using glutathione S-transferase interaction assays, we established that two regions of HNF-4, the N-terminal activation function 1 (AF-1) domain (aa 1-24) and the C-terminal F domain (aa 388-455) can mediate physical Smad3/HNF-4 interactions in vitro. In vivo, Smad3 and Smad4 proteins enhanced the transactivation function of various GAL4-HNF-4 fusion proteins via the AF-1 and the adjacent DNA binding domain, whereas a single tyrosine to alanine substitution in AF-1 abolished coactivation by Smads. The findings suggest that the transcriptional cross talk between the TGF[beta]-regulated Smads and HNF-4 is mediated by specific functional domains in the two types of transcription factors. Furthermore, the specificity of this interaction for certain target promoters may play an important role in various hepatocyte functions, which are regulated by TGF[beta] and the Smads.

Eisenkolb, M., C. Zenzmaier, et al. (2002). "A Specific Structural Requirement for Ergosterol in Long-

chain Fatty Acid Synthesis Mutants Important for Maintaining Raft Domains in Yeast." Mol. Biol. Cell **13**(12): 4414-4428.

http://www.molbiolcell.org/cgi/content/abstract/13/12/4414

Fungal sphingolipids contain ceramide with a very-long-chain fatty acid (C26). To investigate the physiological significance of the C26-substitution on this lipid, we performed a screen for mutants that are synthetically lethal with ELO3. Elo3p is a component of the ER-associated fatty acid elongase and is required for the final elongation cycle to produce C26 from C22/C24 fatty acids. elo3[Delta] mutant cells thus contain C22/C24- instead of the natural C26-substituted ceramide. We now report that under these conditions, an otherwise nonessential, but also fungal-specific, structural modification of the major sterol of yeast, ergosterol, becomes essential, because mutations in ELO3 are synthetically lethal with mutations in ERG6. Erg6p catalyzes the methylation of carbon atom 24 in the aliphatic side chain of sterol. The lethality of an elo3[Delta] erg6[Delta] double mutant is rescued by supplementation with ergosterol but not with cholesterol, indicating a vital structural requirement for the ergosterol-specific methyl group. To characterize this structural requirement in more detail, we generated a strain that is temperature sensitive for the function of Erg6p in an elo3[Delta] mutant background. Examination of raft association of the GPI-anchored Gas1p and plasma membrane ATPase, Pma1p, in the conditional elo3[Delta] erg6ts double mutant, revealed a specific defect of the mutant to maintain raft association of preexisting Pma1p. Interestingly, in an elo3[Delta] mutant at 37{degrees}C, newly synthesized Pma1p failed to enter raft domains early in the biosynthetic pathway, and upon arrival at the plasma membrane was rerouted to the vacuole for degradation. These observations indicate that the C26 fatty acid substitution on lipids is important for establishing raft association of Pma1p and stabilizing the protein at the cell surface. Analysis of raft lipids in the conditional mutant strain revealed a selective enrichment of ergosterol in detergent-resistant membrane domains, indicating that specific structural determinants on both sterols and sphingolipids are required for their association into raft domains.

Lee, K. M., I. Miklos, et al. (2005). "Impairment of the TFIIH-associated CDK-activating Kinase Selectively Affects Cell Cycle-regulated Gene Expression in Fission Yeast." Mol. Biol. Cell: E04-11-0982.

http://www.molbiolcell.org/cgi/content/abstract/E04-11-0982v1

Monitoring Editor: Tim StearnsThe fission yeast Mcs6-Mcs2-Pmh1 complex, homologous to metazoan Cdk7-cyclin H-Mat1, has dual functions in cell division and transcription: as a partially redundant CDK-activating kinase (CAK) that phosphorylates the major cell-cycle CDK, Cdc2, on Thr-167; and as the RNA polymerase (Pol) II carboxyl-terminal domain (CTD) kinase associated with transcription factor (TF) IIH. We analyzed conditional mutants of mcs6 and pmh1, which activate Cdc2 normally but cannot complete cell division at restrictive temperature and arrest with decreased CTD phosphorylation. Transcriptional profiling by microarray hybridization revealed only modest effects on global gene expression: a one-third reduction in a severe mcs6 mutant after prolonged incubation at 36{degrees}C. In contrast, a small subset of transcripts ([~]5%) decreased by >2-fold after Mcs6-complex function was compromised. The signature of repressed genes overlapped significantly with those of cell-separation mutants sep10 and sep15. Sep10, a component of the Pol II Mediator complex, becomes essential in mcs6 or pmh1 mutant backgrounds. Moreover, transcripts dependent on the forkhead transcription factor Sep1, which are expressed coordinately during mitosis, were repressed in Mcs6-complex mutants, and Mcs6 also interacts genetically with Sep1. Thus the Mcs6 complex, a direct activator of Cdc2, also influences the cell-cycle transcriptional program, possibly through its TFIIH-associated kinase function.

Park, E. K., N. Warner, et al. (2004). "Ectopic EphA4 Receptor Induces Posterior Protrusions via FGF Signaling in Xenopus Embryos." Mol. Biol. Cell **15**(4): 1647-1655.

http://www.molbiolcell.org/cgi/content/abstract/15/4/1647

The Eph family of receptor tyrosine kinases regulates numerous biological processes. To examine the biochemical and developmental contributions of specific structural motifs within Eph receptors, wild-type or mutant forms of the EphA4 receptor were ectopically expressed in developing Xenopus embryos. Wild-type EphA4 and a mutant lacking both the SAM domain and PDZ binding motif were constitutively tyrosine phosphorylated in vivo and catalytically active in vitro. EphA4 induced loss of cell adhesion, ventro-lateral protrusions, and severely expanded posterior structures in Xenopus embryos. Moreover, mutation of a conserved SAM domain tyrosine to phenylalanine (Y928F) enhanced the ability of EphA4 to induce these phenotypes, suggesting that the SAM domain may negatively regulate some aspects of EphA4 activity in Xenopus. Analysis of double mutants revealed that the Y928F EphA4 phenotypes were dependent on kinase activity; juxtamembrane sites of tyrosine phosphorylation and SH2 domain-binding were required for cell dissociation, but not for posterior protrusions. The induction of protrusions and expansion of posterior structures is similar to phenotypic effects observed in Xenopus embryos expressing activated FGFR1. Furthermore, the budding ectopic protrusions induced by EphA4 express FGF-8, FGFR1, and FGFR4a. In addition, antisense morpholino oligonucleotide-mediated loss of FGF-8 expression in vivo substantially reduced the phenotypic effects in EphA4Y928F expressing embryos, suggesting a connection between Eph and FGF signaling.

Rudge, S. A., D. M. Anderson, et al. (2004). "Vacuole Size Control: Regulation of PtdIns(3,5)P2 Levels by the Vacuole-associated Vac14-Fig4 Complex, a PtdIns(3,5)P2-specific Phosphatase." Mol. Biol. Cell **15**(1): 24-36.

http://www.molbiolcell.org/cgi/content/abstract/15/1/24

In the budding yeast Saccharomyces cerevisiae, phosphatidylinositol 3,5-bisphosphate (PtdIns(3,5)P2) is synthesized by a single phosphatidylinositol 3-phosphate 5-kinase, Fab1. Cells deficient in PtdIns(3,5)P2 synthesis exhibit a grossly enlarged vacuole morphology, whereas increased levels of PtdIns(3,5)P2 provokes the formation of multiple small vacuoles, suggesting a specific role for PtdIns(3,5)P2 in vacuole size control. Genetic studies have indicated that Fab1 kinase is positively regulated by Vac7 and Vac14; deletion of either gene results in ablation of PtdIns(3,5)P2 synthesis and the formation of a grossly enlarged vacuole. More recently, a suppressor of vac7{Delta} mutants was identified and shown to encode a putative phosphoinositide phosphatase, Fig4. We demonstrate that Fig4 is a magnesium-activated PtdIns(3,5)P2-selective phosphoinositide phosphatase in vitro. Analysis of a Fig4-GFP fusion protein revealed that the Fig4 phosphatase is localized to the limiting membrane of the vacuole. Surprisingly, in the absence of Vac14, Fig4-GFP no longer localizes to the vacuole. However, Fig4-GFP remains localized to the grossly enlarged vacuoles of vac7 deletion mutants. Consistent with these observations, we found that Fig4 physically associates with Vac14 in a common membrane-associated complex. Our studies indicate that Vac14 both positively regulates Fab1 kinase activity and directs the localization/activation of the Fig4 PtdIns(3,5)P2 phosphatase.

Sciorra, V. A., A. Audhya, et al. (2005). "Synthetic Genetic Array Analysis of the PtdIns 4-kinase Pik1p Identifies Components in a Golgi-specific Ypt31/rab-GTPase Signaling Pathway." Mol. Biol. Cell **16**(2): 776-793.

http://www.molbiolcell.org/cgi/content/abstract/16/2/776

Phosphorylated derivatives of phosphatidylinositol are essential regulators of both endocytic and exocytic trafficking in eukaryotic cells. In Saccharomyces cerevisiae, the phosphatidylinositol 4-kinase, Pik1p generates a distinct pool of PtdIns(4)P that is required for normal Golgi structure and secretory function. Here, we utilize a synthetic genetic array analysis of a conditional pik1 mutant to identify candidate components of the Pik1p/PtdIns(4)P signaling pathway at the Golgi. Our data suggest a mechanistic involvement for Pik1p with a specific subset of Golgi-associated proteins, including the Ypt31p rab-GTPase and the TRAPPII protein complex, to regulate protein trafficking through the secretory pathway. We further demonstrate that TRAPPII specifically functions in a Ypt31p-dependent pathway and identify Gyp2p as the first biologically relevant GTPase activating protein for Ypt31p. We propose that multiple stage-specific signals, which may include Pik1p/PtdIns(4)P, TRAPPII and Gyp2p, impinge upon Ypt31 signaling to regulate Golgi secretory function.

Stang, E., F. D. Blystad, et al. (2004). "Cbl-dependent Ubiquitination Is Required for Progression of EGF Receptors into Clathrin-coated Pits." Mol. Biol. Cell **15**(8): 3591-3604.

http://www.molbiolcell.org/cgi/content/abstract/15/8/3591

Ligand binding causes the EGF receptor (EGFR) to become ubiquitinated by Cbl upon association with the adaptor protein Grb2. We have investigated the role of ubiquitin and Grb2 in ligand-induced endocytosis of the EGFR. Incubation of cells with EGF on ice caused translocation of Grb2 and Cbl from the cytosol to the rim of coated pits. Grb2 with point mutations in both SH3 domains inhibited recruitment of the EGFR to clathrin-coated pits, in a Ras-independent manner. On overexpression of the Cbl-binding protein Sprouty, ubiquitination of the EGFR was inhibited, the EGFR was recruited only to the rim of coated pits, and endocytosis of the EGFR was inhibited. Conjugation-defective ubiquitin similarly inhibited recruitment of EGF-EGFR to clathrin-coated pits. Even though this does not prove that cargo must be ubiquitinated, this indicates the importance of interaction of ubiquitinated protein(s) with proteins harboring ubiquitin-interacting domains. We propose that Grb2 mediates transient anchoring of the EGFR to an Eps15-containing molecular complex at the rim of coated pits and that Cbl-induced ubiquitination of the EGFR allows relocation of EGFR from the rim to the center of clathrin-coated pits.

Tatzer, V., G. Zellnig, et al. (2002). "Lipid-dependent Subcellular Relocalization of the Acyl Chain Desaturase in Yeast." Mol. Biol. Cell **13**(12): 4429-4442.

http://www.molbiolcell.org/cgi/content/abstract/13/12/4429

The degree of acyl chain desaturation of membrane lipids is a critical determinant of membrane fluidity. Temperature-sensitive mutants of the single essential acyl chain desaturase, Ole1p, of yeast have previously been isolated in screens for mitochondrial inheritance mutants (Stewart, L.C., and Yaffe, M.P. (1991). J. Cell Biol. 115, 1249-1257). We now report that the mutant desaturase relocalizes from its uniform ER distribution to a more punctuate localization at the cell periphery upon inactivation of the enzyme. This relocalization takes place within minutes at nonpermissive conditions, a time scale at which mitochondrial morphology and inheritance is not yet affected. Relocalization of the desaturase is fully reversible and does not affect the steady state localization of other ER resident proteins or the kinetic and fidelity of the secretory pathway, indicating a high degree of selectivity for the desaturase. Relocalization of the desaturase is energy independent but is lipid dependent because it is rescued by supplementation with

unsaturated fatty acids. Relocalization of the desaturase is also observed in cells treated with inhibitors of the enzyme, indicating that it is independent of temperature-induced alterations of the enzyme. In the absence of desaturase function, lipid synthesis continues, resulting in the generation of lipids with saturated acyl chains. A model is discussed in which the accumulation of saturated lipids in a microdomain around the desaturase could induce the observed segregation and relocalization of the enzyme.

Vedrenne, C., C. Giroud, et al. (2002). "Two Related Subpellicular Cytoskeleton-associated Proteins in Trypanosoma brucei Stabilize Microtubules." Mol. Biol. Cell **13**(3): 1058-1070.

http://www.molbiolcell.org/cgi/content/abstract/13/3/1058

The subpellicular microtubules of the trypanosome cytoskeleton are cross-linked to each other and the plasma membrane, creating a cage-like structure. We have isolated, from Trypanosoma brucei, two related low-molecular-weight cytoskeleton-associated proteins (15- and 17-kDa), called CAP15 and CAP17, which are differentially expressed during the life cycle. Immunolabeling shows a corset-like colocalization of both CAPs and tubulin. Western blot and electron microscope analyses show CAP15 and CAP17 labeling on detergent-extracted cytoskeletons. However, the localization of both proteins is restricted to the anterior, microtubule minus, and less dynamic half of the corset. CAP15 and CAP17 share properties of microtubule-associated proteins when expressed in heterologous cells (Chinese hamster ovary and HeLa), colocalization with their microtubules, induction of microtubule bundle formation, cold resistance, and insensitivity to nocodazole. When overexpressed in T. brucei, both CAP15 and CAP17 cover the whole subpellicular corset and induce morphological disorders, cell cycle-based abnormalities, and subsequent asymmetric cytokinesis.

Molecular Phylogenetics and Evolution (49)

Arnedo, M. A., J. Coddington, et al. (2004). "From a comb to a tree: phylogenetic relationships of the comb-footed spiders (Araneae, Theridiidae) inferred from nuclear and mitochondrial genes." Molecular Phylogenetics and Evolution **31**(1): 225.

http://www.sciencedirect.com/science/article/B6WNH-49CN2XB-6/2/0d2acf3be8260aefca161ee5393996b9

The family Theridiidae is one of the most diverse assemblages of spiders, from both a morphological and ecological point of view. The family includes some of the very few cases of sociality reported in spiders, in addition to bizarre foraging behaviors such as kleptoparasitism and araneophagy, and highly diverse web architecture. Theridiids are one of the seven largest families in the Araneae, with about 2200 species described. However, this species diversity is currently grouped in half the number of genera described for other spider families of similar species richness. Recent cladistic analyses of morphological data have provided an undeniable advance in identifying the closest relatives of the theridiids as well as establishing the family's monophyly. Nevertheless, the comb-footed spiders remain an assemblage of poorly defined genera, among which hypothesized relationships have yet to be examined thoroughly. Providing a robust cladistic structure for the Theridiidae is an essential step towards the clarification of the taxonomy of the group and the interpretation of the evolution of the diverse traits found in the

family. Here we present results of a molecular phylogenetic analysis of a broad taxonomic sample of the family (40 taxa in 33 of the 79 currently recognized genera) and representatives of nine additional araneoid families, using approximately 2.5 kb corresponding to fragments of three nuclear genes (Histone 3, 18SrDNA, and 28SrDNA) and two mitochondrial genes (16SrDNA and CoI). Several methods for incorporating indel information into the phylogenetic analysis are explored, and partition support for the different clades and sensitivity of the results to different assumptions of the analysis are examined as well. Our results marginally support theridiid monophyly, although the phylogenetic structure of the outgroup is unstable and largely contradicts current phylogenetic hypotheses based on morphological data. Several groups of theridiids receive strong support in most of the analyses: latrodectines, argyrodines, hadrotarsines, a revised version of spintharines and two clades including all theridiids without trace of a colulus and those without colular setae. However, the interrelationships of these clades are sensitive to data perturbations and changes in the analysis assumptions.

Barker, F. K. (2004). "Monophyly and relationships of wrens (Aves: Troglodytidae): a congruence analysis of heterogeneous mitochondrial and nuclear DNA sequence data." Molecular Phylogenetics and Evolution **31**(2): 486.

http://www.sciencedirect.com/science/article/B6WNH-49NVGDS-7/2/be744d24eafd71e40b03b7e0806e4e3a

The wrens (Aves: Troglodytidae) are a group of primarily New World insectivorous birds, the monophyly of which has long been recognized, but whose intergeneric relationships are essentially unknown. In order to test the monophyly of the group, and to attempt to resolve relationships among genera within it, sequences from the mitochondrial cytochrome b gene and the fourth intron of the nuclear [beta]-fibrinogen gene were obtained from nearly all genera of wrens, from their relatives as suggested by traditional taxonomy and DNA-DNA hybridization analyses, and from additional passerines. Maximum likelihood analysis of the two data sets yielded maximal congruence between independently derived estimates of relationship, outperforming a variety of weighted parsimony methods. Hierarchical likelihood ratio tests indicated that the two gene regions differed significantly in every estimated parameter of sequence evolution, and combined analysis of the two data sets was accomplished using a heterogeneous-model Bayesian approach. Independent and simultaneous analyses of both data sets supported monophyly of the wrens (excluding one recently added member, the monotypic genus Donacobius) and a sister-group relationship between wrens and the gnatcatchers (Polioptila). Additionally, strong support was found for paraphyly of the genus Thryothorus, and for a sister-group relationship between the genera Cistothorus and Troglodytes. Analyses of these data failed to resolve basal relationships within wrens, possibly due to ambiguity in rooting with a distant, species-poor outgroup. Analysis of the combined data for wrens alone yielded results which were largely congruent with relationships inferred using the complete data set, with the benefit of stronger support for relationships within the group. However, alternative rootings of this ingroup tree were weakly supported by nucleotide substitution data. Insertion-deletion events suggest that the genus Salpinctes may be sister to all other wrens.

Bickham, J. W., J. C. Patton, et al. (2004). "Molecular phylogenetics, karyotypic diversity, and partition of the genus Myotis (Chiroptera: Vespertilionidae)." Molecular Phylogenetics and Evolution **33**(2): 333.

http://www.sciencedirect.com/science/article/B6WNH-4D2WMC3-4/2/68117290843c09cfbb228e5e217634af

Nucleotide sequences of the mitochondrial cytochrome b gene are reported from bats of the

genus Myotis including species of the endemic southern African subgenus Cistugo, Myotis (Cistugo) sebrai and Myotis (Cistugo) lesueuri. We also examined Myotis annectans from Southeast Asia, and Myotis macropus from Australia. The two species of Cistugo and Myotis annectans represent the only species of Myotis to differ in chromosome number from the common 2n = 44 found in >40 species. Our results show that the two species of Cistugo are more divergent from the other species of Myotis than several other well-recognized genera and we recommend elevating Cistugo to full generic rank. Myotis annectans groups well within Myotis, clustering with other Southeast Asian and Japanese species, and thus represents the only species of Myotis known to have diverged from the common 2n = 44 karyotype. Myotis macropus clusters within a clade that includes Southeast Asian species.

Burger, J., W. Rosendahl, et al. (2004). "Molecular phylogeny of the extinct cave lion Panthera leo spelaea." Molecular Phylogenetics and Evolution **30**(3): 841.

http://www.sciencedirect.com/science/article/B6WNH-49S7XSK-2/2/282c38530dc3d082751bc69ab73bac42

To reconstruct the phylogenetic position of the extinct cave lion (Panthera leo spelaea), we sequenced 1 kb of the mitochondrial cytochrome b gene from two Pleistocene cave lion DNA samples (47 and 32 ky B.P.). Phylogenetic analysis shows that the ancient sequences form a clade that is most closely related to the extant lions from Africa and Asia; at the same time, cave lions appear to be highly distinct from their living relatives. Our data show that these cave lion sequences represent lineages that were isolated from lions in Africa and Asia since their dispersal over Europe about 600 ky B.P., as they are not found among our sample of extant populations. The cave lion lineages presented here went extinct without mitochondrial descendants on other continents. The high sequence divergence in the cytochrome b gene between cave and modern lions is notable.

Calcagnotto, D., S. A. Schaefer, et al. "Relationships among characiform fishes inferred from analysis of nuclear and mitochondrial gene sequences." Molecular Phylogenetics and Evolution **In Press, Corrected Proof** http://www.sciencedirect.com/science/article/B6WNH-4FDJRVG-2/2/c9c2b46cb8831c6e1aa1da4a1fdd2e49

Suprafamilial relationships among characiform fishes and implications for the taxonomy and biogeographic history of the Characiformes were investigated by parsimony analysis of four nuclear and two mitochondrial genes across 124 ingroup and 11 outgroup taxa. Simultaneous analysis of 3660 aligned base pairs from the mitochondrial 16S and cytochrome b genes and the nuclear recombination activating gene (RAG2), seven in absentia (sia), forkhead (fkh), and [alpha]-tropomyosin (trop) gene loci confirmed the non-monophyly of the African and Neotropical assemblages and corroborated many suprafamilial groups proposed previously on the basis of morphological features. The African distichodontids plus citharinids were strongly supported as a monophyletic Citharinoidei that is the sistergroup to all other characiforms, which form a monophyletic Characoidei composed of two large clades. The first represents an assemblage of both African and Neotropical taxa, wherein a monophyletic African Alestidae is sister to a smaller clade comprised of the Neotropical families Ctenolucidae, Lebiasinidae, and the African Hepsetidae, with that assemblage sister to a strictly Neotropical clade comprised of the Crenuchidae and Erythrinidae. The second clade within the Characoidei is strictly Neotropical and includes all other Characiformes grouped into two well supported major clades. The first, corresponding to a traditional definition of the Characidae, is congruent with some groupings previously supported by morphological evidence. The second clade comprises a monophyletic Anostomoidea that is sister to a clade formed by the families Hemiodontidae, Parodontidae, and Serrasalmidae, with that assemblage, in turn, the sistergroup of the Cynodontidae.

Serrasalmidae, traditionally regarded as a subfamily of Characidae, was recovered as the sistergroup of (Anostomoidea (Parodontidae + Hemiodontidae)) and the family Cynodontidae was recovered with strong support as the sistergroup to this assemblage. Our results reveal three instances of trans-continental sistergroup relationships and, in light of the fossil evidence, suggest that marine dispersal cannot be ruled out a priori and that a simple model of vicariance does not readily explain the biogeographic history of the characiform fishes.

Carson, R. J. and G. S. Spicer (2003). "A phylogenetic analysis of the emberizid sparrows based on three mitochondrial genes." Molecular Phylogenetics and Evolution **29**(1): 43.

http://www.sciencedirect.com/science/article/B6WNH-48KFF3P-2/2/47bc8c274a60106c3dba8f8504a28f5f

Previous molecular phylogenetic studies have examined the taxonomic relationships among a number of typical emberizid sparrow genera. To help clarify these relationships, we sequenced a 1673 base pair fragment for the complete sequence of three mitochondrial genes: adenosine triphosphatase (Atp8 and Atp6) and cytochrome oxidase subunit III (COIII) for 38 sparrow species, along with Passerina amoena (Cardinalidae) and Piranga ludoviciana (Thraupidae) which were selected as the outgroups. Our analysis confirms the monophyly of traditional genera such as Junco, Melospiza, and Zonotrichia. Although Calcarius and Plectrophenax are often thought to be putative emberizids, all our analyses placed these genera basal to all other sparrows examined. As observed with Calcarius, Spizella did not form a monophyletic group, with S. arborea being the sister-taxon to Passerella iliaca. Our analyses also suggest that Aimophila ruficeps is probably more closely related to the "brown towhees" (Pipilo aberti, P. crissalis, and P. fuscus) than its putative congeners. The genus Ammodramus was also not monophyletic, since it appears that Passerculus sandwichensis is more closely related to A. henslowii and A. leconteii then either one is related to its congener A. savannarum. Finally, our analyses exhibited other unsuspected associations, such as the sister-taxon relationships between Amphispiza bilineata and the Chondestes grammacus/Calamospiza melanocorys clade, and Amphispiza belli and Pooecetes gramineus.

Cheviron, Z. A., S. J. Hackett, et al. "Complex evolutionary history of a Neotropical lowland forest bird (Lepidothrix coronata) and its implications for historical hypotheses of the origin of Neotropical avian diversity." Molecular Phylogenetics and Evolution **In Press, Corrected Proof**
http://www.sciencedirect.com/science/article/B6WNH-4FMBK98-4/2/dd9ecca5ecd445b7ad6894c87e554024

Here we apply a combination of phylogeographic and historical demographic analyses to the study of mtDNA sequence variation within the Blue-crowned Manakin (Lepidothrix coronata), a widespread Neotropical bird. A high degree of phylogeographic structure allowed us to demonstrate that several vicariant events, including Andean uplift, the formation of riverine barriers, and climatically induced vegetational shifts, as well as a non-vicariant process, range expansion, have all acted, at varying spatial and temporal scales, to influence genetic structure within L. coronata, suggesting that current historical hypotheses of the origin of Neotropical avian diversity that focus on single vicariant mechanisms may be overly simplistic. Our data also support an origin (>2 mybp) that is substantially older than the late Pleistocene for the genetic structure within this species and indicate that phylogeographic patterns within the species are not concordant with plumage-based subspecific taxonomy. These data add to a growing body of evidence suggesting that the origin of several Neotropical avian species may have occurred in the mid-Pliocene, thus, geological arguments surrounding putative Pleistocene vicariant events, while interesting in their own right, may have little relevance to Neotropical avian diversification at the species level.

Cortes-Ortiz, L., E. Bermingham, et al. (2003). "Molecular systematics and biogeography of the Neotropical monkey genus, Alouatta." Molecular Phylogenetics and Evolution **26**(1): 64.

http://www.sciencedirect.com/science/article/B6WNH-4783K6K-1/2/047833090815bf05218ec4905e7105c8

Despres, L., L. Gielly, et al. (2003). "Using AFLP to resolve phylogenetic relationships in a morphologically diversified plant species complex when nuclear and chloroplast sequences fail to reveal variability." Molecular Phylogenetics and Evolution **27**(2): 185.

http://www.sciencedirect.com/science/article/B6WNH-47TNSB8-2/2/67e6e755288629e3efff2e3d9290e75a

Inferring phylogenetic relationships among closely related plant species is often difficult due to the lack of molecular markers exhibiting enough nucleotide variability at this taxonomic level. Moreover, gene tree does not necessary represent the true species tree because of random sorting of polymorphic alleles in different lineages. A solution to these problems is to use many amplified fragment length polymorphisms (AFLP) distributed throughout the whole genome, to infer cladistic and phenetic among-species relationships. Phylogenetic relationships among interfertile species of Trollius L. (Ranunculaceae) were investigated using nuclear DNA (ITS1 + 5.8S rRNA + ITS2) and chloroplast DNA (trnL intron and trnL-trnF intergene spacer) sequences, and AFLP markers. ITS sequences were not informative at the intrageneric level, but confirmed the sister relationship between Trollius and Adonis genera, and provided new information on the phylogenetic relationships among five Ranunculaceae genera. Chloroplast DNA was more informative among Trollius species, but not consistent with the sections previously described. AFLP proved to be a powerful tool to resolve the complex genetic relationships between the morphological entities constituting the genus Trollius. Although as much as 76.1% of the total AFLP variability was found within a priori defined morphological groups, the remaining 23.9% variability differentiating groups was sufficient to generate congruent and robust cladistic and phenetic trees. Several morphological traits, independent from those used to define groups, were mapped onto the molecular phylogeny, and their evolution discussed in relation to the absence/presence of pollinator-seed parasite Chiastocheta flies.

Dick, M. H., A. Herrera-Cubilla, et al. (2003). "Molecular phylogeny and phylogeography of free-living Bryozoa (Cupuladriidae) from both sides of the Isthmus of Panama." Molecular Phylogenetics and Evolution **27**(3): 355.

http://www.sciencedirect.com/science/article/B6WNH-4840SR4-8/2/d39c006a1bb2682e98390aaa90427967

Genetic data were used to identify Recent species of free-living bryozoans (Cupuladriidae) from both sides of the Isthmus of Panama, and to examine their phylogenetic relationships, species richness, and population structures. An approximately 480 bp fragment of the 16S mitochondrial rRNA gene was sequenced from 182 individuals from Panama, the Gulf of Mexico, and El Salvador. Ten haplotype groups (Cupuladria 4, 5, and 6; Discoporella 1, 2, 3A, 3B, 3C, 7, and 8) were identified. Genetic distances between haplotype groups (3.2-26.5%; K2P + [Gamma]) were 1-2 orders of magnitude greater than within groups (0.1-1.4%). Seven of the haplotype groups represent morphologically distinct species; Discoporellas 3A-C appear to be cryptic species. Phylogenetic analyses identified two pairs of transisthmian sister clades. An average divergence

rate derived from other taxa suggests that Cupuladrias 4 and 5 diverged [ap]7 Ma, a Discoporella 7 clade diverged from a 3A-C clade [ap]11 Ma, and the 3A-C clade radiated [ap]6-4 Ma; these events all predated final closure of the isthmus? 3 Ma. The Caribbean side of the isthmus, with 5 species, is only marginally richer in cupuladriids than the Pacific side, with 4, but has greater phylogenetic depth. The Caribbean retains lineages stemming from a New World Miocene radiation that are not represented in the eastern Pacific; extant eastern Pacific cupuladriids share most recent common ancestry with only two of the Caribbean lineages. Species in the eastern Pacific tend to show shallow population structures, with high levels of gene flow between geographically separate populations, whereas Caribbean species tend to show deeper populations structures, with indications of restricted gene flow between Bocas del Toro/Gulf of Mosquitos and Costa Arriba/San Blas. The population structures derive from Pleistocene histories and may be of limited value in interpreting the macroevolutionary pattern, as our results provide no evidence of speciation on either side of the isthmus following closure in the late Pliocene.

Eberhard, J. R. and E. Bermingham "Phylogeny and comparative biogeography of Pionopsitta parrots and Pteroglossus toucans." Molecular Phylogenetics and Evolution **In Press, Corrected Proof** http://www.sciencedirect.com/science/article/B6WNH-4FR3NDB-1/2/29c3a5ca15b05b74361027e4b751f9cf

Studies of Neotropical birds, and their distributions and areas of endemism, in particular, have been central in the formulation of hypotheses proposed to explain the high species diversity in the Neotropics. We used mtDNA sequence data (ATPase 6 and 8, COI, and cyt b) to reconstruct the species-level phylogenies for two genera, Pionopsitta (Aves: Psittacidae) and Pteroglossus (Aves: Ramphastidae), compare our results with previous morphology-based phylogenetic analyses, and estimate the absolute timing of lineage and biogeographic divergences. Both the Pionopsitta and Pteroglossus phylogenies support a hypothesis of area relationships in which a divergence of the Serra do Mar (Atlantic Forest, Brazil) region of endemism is followed by the divergence of cis- and trans-Andean regions, then a split between the upper and lower Amazon basin, next the divergence of the Guyana area, and finally diversification of taxa in the upper Amazon basin's areas of endemism. Phylogenies of both genera support a hypothesis of area relationships that is similar to that proposed by Prum [XIX International Ornithological Congress (1988), 2562] for high-vagility species, but while they agree on the relative timing of area divergence (vicariance) events, they yield different absolute time estimates for those divergences when the typical avian mtDNA clock calibration is used. Taken at face value, the time estimates indicate that both genera began to diversify before the start of the Pleistocene, and that climatic and habitat shifts alone do not account for the diversification of these taxa.

Garza, J. C. and D. S. Woodruff (1992). "A phylogenetic study of the gibbons (Hylobates) using DNA obtained noninvasively from hair." Molecular Phylogenetics and Evolution **1**(3): 202.

http://www.sciencedirect.com/science/article/B6WNH-4DXK9Y9-4/2/8c14e9413b8620da324e88fa78947692

Variation in a 252-nucleotide segment of the cytochrome b gene from 26 gibbons is described. DNA was extracted from hair, amplified, and directly sequenced. These sequences represent seven of the nine nominal species and three of the four hylobatid subgenera. Variation was observed at 55 sites, 42 of which are phylogenetically informative. Levels of transitional and transversional divergence between the taxa are similar to those reported for homologous mtDNA sequences in other mammals. Parsimony, maximum likelihood, and bootstrap analyses (1) support some traditional phylogenetic hypotheses (monophyly of the concolor gibbons), (2) suggest previously unrecognized affinities between the lar species group and Hylobates klossi and between H. lar and H. agijis unko, and (3) show that this segment does not contain

information sufficient for completely resolving gibbon relationships at the subgeneric level. The study demonstrates the great potential of noninvasive DNA sampling for phylogenetic analyses of mammals.

Gentile, A., M. S. Rossi, et al. (2005). "Origin, divergence, and phylogeny of epichloe endophytes of native Argentine grasses." Molecular Phylogenetics and Evolution **35**(1): 196.

http://www.sciencedirect.com/science/article/B6WNH-4FFH20D-2/2/f8d7492acf2a57c846109cc68cb80970

The epichloe endophytes are systemic, constitutive, and often vertically transmitted fungal symbionts of grass species in subfamily Pooideae. Prior studies indicate that several asexual epichloe endophytes (Neotyphodium species) have evolved directly from sexual (Epichloe) species, whereas others evolved by hybridization between two or more endophyte species. In this paper, we investigate the phylogenies of 27 Neotyphodium spp. isolates from 10 native grass species (in 4 tribes) in 22 populations throughout Argentina. Relationships among these fungi and a worldwide collection of epichloe endophytes were estimated by phylogenetic analysis of sequences from variable portions (mainly introns) of genes for [beta]-tubulin (tub2) and translation elongation factor 1-[alpha] (tef1). Most of the Argentine endophyte isolates were interspecific hybrids of Epichloe festucae and E. typhina. Only one isolate was a hybrid of a different ancestry, and three isolates were apparently non-hybrid endophytes. These results indicate that interspecific hybridization, which promotes genetic variation, was common during the evolution of the endophytes of Argentine grasses.

Gomez-Zurita, J. (2004). "Molecular systematics and time-scale for the evolution of Timarcha, a leaf-beetle genus with a disjunct Holarctic distribution." Molecular Phylogenetics and Evolution **32**(2): 647.

http://www.sciencedirect.com/science/article/B6WNH-4C2NKD5-1/2/91844db8219e55d1830a8d405d8525e0

In recent years we have investigated the evolution of the Holarctic leaf-beetle genus Timarcha using molecular approaches, but to date several important questions remained unanswered, including its systematic arrangement in a temporal context, or the phylogenetic placement of the Nearctic taxa. Here I present a reanalysis of available genetic data together with newly generated data for key taxa (markers 16S rDNA, CO2, ITS-2, and 18S rDNA), including the Nearctic species (subgenus Americanotimarcha), using direct optimization-based phylogenetic reconstructions. Lineage ages are estimated using maximum likelihood branch-length estimates and the molecular clock calibration derived from several presumed vicariance events in the Mediterranean. Phylogenetic analyses and 18S rDNA divergences suggest the ancient divergence of the Nearctic and Palaearctic lineages, related to the North Atlantic opening in the middle Eocene. The diversification of the Palaearctic Timarcha seems closely related to the geological evolution of the Mediterranean area during the Tertiary, with Pleistocenic climate changes affecting species ranges and lineage extinction, but not resulting in extensive speciation.

Hay, J. M., C. H. Daugherty, et al. (2003). "Low genetic divergence obscures phylogeny among populations of Sphenodon, remnant of an ancient reptile lineage." Molecular Phylogenetics and Evolution **29**(1): 1.

http://www.sciencedirect.com/science/article/B6WNH-48Y069B-1/2/2e37be624691a1f854385553d1a4aa1d

Tuatara (two species of Sphenodon) are the last representatives of a branch of an ancient reptilian lineage, Sphenodontia, that have been isolated on the New Zealand landmass for 82 million years. We present analyses of geographic variation in allozymes, mitochondrial DNA, nuclear DNA sequences, and one-way albumin immunological comparisons. These all confirm a surprisingly low level of genetic diversity within Sphenodon for such an ancient lineage. We hypothesise a recent extended population bottleneck, probably during the Pliocene/Pleistocene glaciation cycles, to explain the current paucity of variation. All data sets reveal clear genetic differentiation between the northern populations and those in Cook Strait, but offer conflicting views of the history and taxonomic relationships of the Cook Strait population on North Brother Island, currently recognised as Sphenodon guntheri. Allozymes show this population to be the most divergent of all tuatara populations, but preliminary mitochondrial DNA data indicate few differences between S. guntheri and Cook Strait Sphenodon punctatus. Interpretation of the trees is confounded by the lack of a suitable outgroup. As in other cases of conflicting nuclear and mitochondrial data sets, the different data sets likely reveal different aspects of the animals' evolutionary history, and introgression is not uncommon between species pairs.

Hyvonen, J., S. Koskinen, et al. (2004). "Phylogeny of the Polytrichales (Bryophyta) based on simultaneous analysis of molecular and morphological data." Molecular Phylogenetics and Evolution **31**(3): 915.

http://www.sciencedirect.com/science/article/B6WNH-4BBMT6T-1/2/bc37a00303f555e9b77f997d9cc9bc11

Phylogenetic analyses of Polytrichales were conducted using morphology and sequence data from the chloroplast genes rbcL and rps4 plus the trnL-F gene region, part of the mitochondrial nad5 and the nuclear-encoded 18S rDNA. Our analyses included 46 species representing all genera of Polytrichales. Phylogenetic trees were constructed with simultaneous parsimony analyses of all sequences plus morphology and separate combinations of sequence data only. Results lend support for recognition of Polytrichales as a monophyletic entity. Oedipodium griffithianum appears as a sister taxon to Polytrichales or as a sister taxon of all mosses excluding Sphagnales and Andreaeles. Within Polytrichales, Alophosia and Atrichopsis, species without the adaxial lamellae (in Atrichopsis present but poorly developed on male gametophyte) otherwise typical of the group are sister to the remaining species followed by a clade including Bartramiopsis and Lyellia, species with adaxial lamellae covering only the central portion of the leaves. Six taxa with an exclusively Southern Hemisphere distribution form a grade between the basal lineages and a clade including genera that are mostly confined to the Northern Hemisphere.

Joseph, L., T. Wilke, et al. (2004). "Towards a phylogenetic framework for the evolution of shakes, rattles, and rolls in Myiarchus tyrant-flycatchers (Aves: Passeriformes: Tyrannidae)." Molecular Phylogenetics and Evolution **31**(1): 139.

http://www.sciencedirect.com/science/article/B6WNH-49CN2XB-5/2/49a692ddb4c88198c0a1971faf9dd4aa

A phylogeny of 19 of the 22 currently recognized species of Myiarchus tyrant-flycatchers is presented. It is based on 842 bp of mitochondrial DNA (mtDNA) sequences from the ATPase subunit 8 and ATPase subunit 6 genes. Except for the morphologically distinct M. semirufus,

mtDNAs of the remaining 18 species fall into either of two clades. One comprises predominantly Caribbean and Central and North American taxa (Clade I), and the other is of predominantly South American taxa (Clade II). The phylogeny is only very broadly concordant with some vocal characters and also with the limited morphological diversity for which the group is well known. Paraphyly in several species (M. swainsoni, M. tuberculifer, M. ferox, M. phaeocephalus, M. sagrae, M. stolidus) suggests that morphological evolution, albeit resulting in limited morphological diversity, has been more rapid than that of mtDNA, or that current taxonomy is faulty, or both. A South American origin for Myiarchus is likely. Dispersal and vicariance both appear to have been involved in generating the present-day distribution of some species. Relatively recent dispersal events out of South America are inferred to have brought species of Clades I and II into broad sympatry. Jamaica has been colonized independently at least twice by members of Clades I and II. The phylogeny brings a historical perspective that in turn suggests that ecological study of closely related species from within each major clade where they are sympatric will be especially rewarding.

Kizirian, D., A. Trager, et al. (2004). "Evolution of Galapagos Island Lava Lizards (Iguania: Tropiduridae: Microlophus)." Molecular Phylogenetics and Evolution **32**(3): 761.

http://www.sciencedirect.com/science/article/B6WNH-4CHRY76-1/2/6e102df9ebc94bffc074ddbf3d459d03

Nucleotide sequences of mitochondrial genes (ND1, ND2, COI, and tRNAs) were determined for 38 samples representing 15 taxa of tropidurid lizards from the Galapagos Islands and mainland South America. Phylogenetically informative characters (759 of 1956) were analyzed under Bayesian, maximum likelihood, and parsimony frameworks. This study supports the hypothesis that tropidurid lizards dispersed to the Galapagos on at least two separate occasions. One dispersal event involved an eastern Galapagos clade (Microlophus habelii and M. bivittatus, on Marchena and San Cristobal islands, respectively) the sister taxon of which is M. occipitalis from coastal Ecuador and Peru; the closest mainland relative of the western Galapagos clade was not unambiguously identified. The wide-ranging M. albemarlensis is revealed to be a complex of weakly divergent lineages that is paraphyletic with respect to the insular species M. duncanensis, M. grayii, and M. pacificus.

Lavoue, S. and J. P. Sullivan (2004). "Simultaneous analysis of five molecular markers provides a well-supported phylogenetic hypothesis for the living bony-tongue fishes (Osteoglossomorpha: Teleostei)." Molecular Phylogenetics and Evolution **33**(1): 171.

http://www.sciencedirect.com/science/article/B6WNH-4CPVM0R-1/2/ceac61e394db4e7fae7f4c459ec66f4a

Fishes of the Superorder Osteoglossomorpha (the "bonytongues") constitute a morphologically heterogeneous group of basal teleosts, including highly derived subgroups such as African electric fishes, the African butterfly fish, and Old World knifefishes. Lack of consensus among hypotheses of osteoglossomorph relationships advanced during the past 30 years may be due in part to the difficulty of identifying shared derived characters among the morphologically differentiated extant families of this group. In this study, we present a novel phylogenetic hypothesis for this group, based on the analysis of more than 4000 characters from five molecular markers (the mitochondrial cytochrome b, 12S and 16S rRNA genes, and the nuclear genes RAG2 and MLL). Our taxonomic sampling includes one representative of each extant non-mormyrid osteoglossomorph genus, one representative for the monophyletic family Mormyridae, and four outgroup taxa within the basal Teleostei. Maximum parsimony analysis of combined and equally weighted characters from the five molecular markers and Bayesian analysis provide a

single, well-supported, hypothesis of osteoglossomorph interrelationships and show the group to be monophyletic. The tree topology is the following: (Hiodon alosoides, (Pantodon buchholzi, (((Osteoglossum bicirrhosum, Scleropages sp.), (Arapaima gigas, Heterotis niloticus)), ((Gymnarchus niloticus, Ivindomyrus opdenboschi), ((Notopterus notopterus, Chitala ornata), (Xenomystus nigri, Papyrocranus afer)))))). We compare our results with previously published phylogenetic hypotheses based on morpho-anatomical data. Additionally, we explore the consequences of the long terminal branch length for the taxon Pantodon buchholzi in our phylogenetic reconstruction and we use the obtained phylogenetic tree to reconstruct the evolutionary history of electroreception in the Notopteroidei.

Liu, H. and A. T. Beckenbach (1992). "Evolution of the mitochondrial cytochrome oxidase II gene among 10 orders of insects." Molecular Phylogenetics and Evolution **1**(1): 41.

http://www.sciencedirect.com/science/article/B6WNH-4DYVPKS-6/2/d24d2bdf5b50478cb857053f28947e5d

We examine the complete nucleotide sequences of the mitochondrial cytochrome oxidase II gene of 13 species of insects, representing 10 orders. The genes range from 673 to 690 by in length, encoding 226 to 229 amino acids. Several insertion or deletion events, each involving one or two codons, can be observed. The 3' end of the gene is extremely variable in both length and sequence, making alignment of the ends unreliable. Using the first 639 nucleotide positions, for which unambiguous alignments could be obtained, we examine the neighbor-joining trees based on nucleotide divergences and based on conserved subsets of that data, including transversion and amino acid and second codon position divergences. Each of these subsets produces different trees, none of which can be easily reconciled with trees constructed using morphology and the fossil record. Bootstrap analysis using second codon positions strongly supports affinities between the order Blatteria (cockroaches) and the order Isoptera (termites) and between a wasp and the published honeybee sequence (Order Hymenoptera). The divergence of insect orders is very ancient and may have occurred too rapidly for easy resolution using mitochondrial protein sequences. Unambiguous resolution of insect orders will probably require analysis of many additional taxa, using the COII gene and other conserved sequences.

Luo, J., D. Yang, et al. (2004). "Molecular phylogeny and biogeography of Oriental voles: genus Eothenomys (Muridae, Mammalia)." Molecular Phylogenetics and Evolution **33**(2): 349.

http://www.sciencedirect.com/science/article/B6WNH-4CYWM12-4/2/3f623876e95346a6ddf5416c49aede69

Oriental voles of the genus Eothenomys are predominantly distributed along the Southeastern shoulder of the Qinghai-Tibetan Plateau. Based on phylogenetic analyses of the mitochondrial cytochrome b gene (1143 bp) obtained from 23 specimens (eight species) of Oriental voles collected from this area, together with nucleotide sequences from six specimens (two species) of Japanese red-backed voles (Eothenomys andersoni and Eothenomys smithii) and five species of the closely related genus Clethrionomys, we revised the systematic status of Eothenomys. We also tested if vicariance could explain the observed high species diversity in this area by correlating estimated divergence times to species distribution patterns and corresponding paleo-geographic events. Our results suggest that: (1) the eight species of Oriental voles form a monophyletic group with two distinct clades, and that these two clades should be considered as valid subgenera--Eothenomys and Anteliomys; (2) Eothenomys eleusis and Eothenomys miletus are not independent species; (3) Japanese red-backed voles are more closely related to the genus Clethrionomys than to continental Asian Eothenomys taxa; and (4) the genus Clethrionomys, as presently defined, is paraphyletic. In addition, the process of speciation of

Oriental voles appears to be related to the Trans-Himalayan formation via three recent uplift events of the Qinghai-Tibetan Plateau within the last 3.6 million years, as well as to the effects of the mid-Quaternary ice age.

Mansion, G. and L. Struwe (2004). "Generic delimitation and phylogenetic relationships within the subtribe Chironiinae (Chironieae: Gentianaceae), with special reference to Centaurium: evidence from nrDNA and cpDNA sequences." Molecular Phylogenetics and Evolution **32**(3): 951.

http://www.sciencedirect.com/science/article/B6WNH-4CJ46KB-2/2/923b3a969f96b65bf0dced47cc7d4e7e

To better understand the evolutionary history of the genus Centaurium and its relationship to other genera of the subtribe Chironiinae (Gentianaceae: Chironieae), molecular analyses were performed using 80 nuclear ribosomal ITS and 76 chloroplast trnLF (both the trnL UAA intron and the trnL-F spacer) sequences. In addition, morphological, palynological, and phytochemical characters were included to a combined data matrix to detect possible non-molecular synapomorphies. Phylogenetic reconstructions support the monophyly of the Chironiinae and an age estimate of ca. 22 million years for the subtribe. Conversely, both molecular data sets reveal a polyphyletic Centaurium, with four well-supported main clades hereafter treated as separate genera. The primarily Mediterranean Centaurium s.s. is closely related to southern African endemics Chironia and Orphium, and to the Chilean species Centaurium cachanlahuen. The resurrected Mexican and Central American genus Gyrandra is closely related to Sabatia (from eastern North America). Lastly, the monospecific genus Exaculum (Mediterranean) forms a monophyletic group together with the two new genera: Schenkia (Mediterranean and Australian species) and Zeltnera (all other indigenous American centauries). Several biogeographical patterns can be inferred for this group, supporting a Mediterranean origin followed by dispersals to (1) North America, Central America, and South America, (2) southern Africa (including the Cape region), and (3) Australia and Pacific Islands.

Markos, S. and B. G. Baldwin (2002). "Structure, molecular evolution, and phylogenetic utility of the 5' region of the external transcribed spacer of 18S-26S rDNA in Lessingia (Compositae, Astereae)." Molecular Phylogenetics and Evolution **23**(2): 214.

http://www.sciencedirect.com/science/article/B6WNH-4627PGJ-B/2/8c6d9b365e492fbdf04bc620aededc37

Marks, B. D., S. J. Hackett, et al. (2002). "Historical relationships among Neotropical lowland forest areas of endemism as determined by mitochondrial DNA sequence variation within the Wedge-billed Woodcreeper (Aves: Dendrocolaptidae: Glyphorynchus spirurus)." Molecular Phylogenetics and Evolution **24**(1): 153.

http://www.sciencedirect.com/science/article/B6WNH-4698TKT-G/2/0fb9bf50aed09e6c4f9518016b4fb7e0

Maxmen, A. B., B. F. King, et al. (2003). "Evolutionary relationships within the protostome phylum Sipuncula: a molecular analysis of ribosomal genes and histone H3 sequence data." Molecular Phylogenetics and Evolution **27**(3): 489.

http://www.sciencedirect.com/science/article/B6WNH-4894317-1/2/14a8677a2ae2e3967cd007c7f17b3092

The phylogenetic relationships of the members of the phylum Sipuncula are investigated by means of DNA sequence data from three nuclear markers, two ribosomal genes (18S rRNA and the D3 expansion fragment of 28S rRNA), and one protein-coding gene, histone H3. Phylogenetic analysis via direct optimization of DNA sequence data using parsimony as optimality criterion is executed for 12 combinations of parameter sets accounting for different indel costs and transversion/transition cost ratios in a sensitivity analysis framework. Alternative outgroup analyses are also performed to test whether they affected rooting of the sipunculan topology. Nodal support is measured by parsimony jackknifing and Bremer support values. Results from the different partitions are highly congruent, and the combined analysis for the parameter set that minimizes overall incongruence supports monophyly of Sipuncula, but nonmonophyly of several higher taxa recognized for the phylum. Mostly responsible for this is the split of the family Sipunculidae in three main lineages, with the genus Sipunculus being the sister group to the remaining sipunculans, the genus Phascolopsis nesting within the Golfingiiformes, and the genus Siphonosoma being associated to the Phascolosomatidea. Other interesting results are the position of Phascolion within Golfingiidae and the position of Antillesoma within Aspidosiphonidae. These results are not affected by the loci selected or by the outgroup chosen. The position of Apionsoma is discussed, although more data would be needed to better ascertain its phylogenetic affinities. Monophyly of the genera with multiple representatives (Themiste, Aspidosiphon, and Phascolosoma) is well supported, but not the monophyly of the genera Nephasoma or Golfingia. Interesting phylogeographic questions arise from analysis of multiple representatives of a few species.

Morris, D. C., M. P. Schwarz, et al. (2002). "Phylogenetics of Australian Acacia thrips: the evolution of behaviour and ecology." Molecular Phylogenetics and Evolution **25**(2): 278.

http://www.sciencedirect.com/science/article/B6WNH-470M3Y9-1/2/79ecefc87b39a47604f03bfa1025d1fb

The species of thrips found on Acacia constitute a major component of the Australian thrips fauna, with at least 235 species in more than 30 genera, many of these being in the process of description as new. These thrips exhibit social behaviours, ranging from solitary and colonial species to a variety of more complex social organisations. Furthermore, the domiciliary habits of these species include domicile construction, gall induction, and opportunistic use of abandoned galls and domiciles. This suite of thrips also includes a variety of inquiline and kleptoparasitic taxa. To understand how these various traits have evolved and interact in this diverse group, we have reconstructed a phylogeny for 42 species of thrips associated with Acacia around Australia. We obtained DNA sequence data from two nuclear genes (Elongation Factor-1[alpha] and wingless) and one mitochondrial gene (cytochrome oxidase I) and analysed these using maximum parsimony and maximum likelihood methods. A phylogeny resulting from such analysis allows inference of evolutionary transitions in domiciliary habits, social organisations, and parasitic behaviours. Gall induction and parasitic behaviour are postulated to each have a single origin, with no losses of either trait. Once parasitism evolved a remarkable radiation followed that allowed exploitation of very diverse hosts. Our data do not allow hypotheses of single versus multiple origins of domicile building to be resolved while opportunistic gall use appears to have arisen several times.

Morrison, C. L., R. Rios, et al. (2004). "Phylogenetic evidence for an ancient rapid radiation of Caribbean sponge-dwelling snapping shrimps (Synalpheus)." Molecular Phylogenetics and Evolution **30**(3): 563.

http://www.sciencedirect.com/science/article/B6WNH-49CN2XB-2/2/d5feac373acf096efc1b2459d5287e21

A common challenge in reconstructing phylogenies involves a high frequency of short internal branches, which makes basal relationships difficult to resolve. Often it is not clear whether this pattern results from insufficient or inappropriate data, versus from a rapid evolutionary radiation. The snapping shrimp genus Synalpheus, which contains in excess of 100 species and is a prominent component of coral-reef faunas worldwide, provides an example. Its taxonomy has long been problematic due to the subtlety of diagnostic characters and apparently widespread variability within species. Here we use partial mt COI and 16S rRNA sequences and morphological characters to reconstruct relationships among 31 species in the morphologically well-defined gambarelloides species group, a putative clade of obligate sponge associates that is mostly endemic to the Caribbean and contains the only known eusocial marine animals. Analysis of the combined data produced a single tree with good support for many terminal clades and for relationships with outgroups, but poor support for branches near the base of the gambarelloides group. Most basal branches are extremely short and terminal branches are long, suggesting a relatively ancient, but rapid radiation of the gambarelloides group. This hypothesis is supported by significant departure from a null model of temporally random cladogenesis. Calibration of divergence times among gambarelloides-group species using data from three geminate pairs of Synalpheus species separated by the isthmus of Panama suggests a major radiation between ~5 and 7 Mya, a few My before final closure of the Panamanian seaway during a period of spreading carbonate environments in the Caribbean; a second, smaller radiation occurred ~4 Mya. This molecular evidence for a rapid radiation among Caribbean marine organisms in the late Miocene/early Pliocene is strikingly similar to patterns documented from fossil data for several other Caribbean reef-associated invertebrate taxa. The similar patterns and timing of cladogenesis evidenced by molecular and fossil data for different Caribbean and East Pacific taxa suggests that the radiation involved a wide range of organisms, and strengthens the case that poor basal resolution in the gambarelloides group of Synalpheus reflects a real evolutionary phenomenon. The rapid radiation also helps explain the historical difficulty of diagnosing species in Synalpheus.

Moulton, J. K. and B. M. Wiegmann (2004). "Evolution and phylogenetic utility of CAD (rudimentary) among Mesozoic-aged Eremoneuran Diptera (Insecta)." Molecular Phylogenetics and Evolution **31**(1): 363.

http://www.sciencedirect.com/science/article/B6WNH-49M0ND6-3/2/08e25741cdd948f7c3fc576d336dd2c7

We sequenced nearly the entire carbomoylphosphate synthase (CPS) domain of CAD, or rudimentary, (ca. 4 kb) from 29 species of flies representing all major clades within Eremoneura, or higher flies, and several orthorrhaphous brachyceran outgroups. We compared these sequences with orthologs from Anopheles gambiae and Drosophila melanogaster to assess structure, compositional bias, and phylogenetic utility. CAD is large (6.6+ kb), complex (comprised of three major and myriad minor functional domains) and relatively free of introns, extreme nucleotide bias (except third codon positions), and large hypervariable regions. The CPS domain possesses moderate levels of nonsynonymous divergence among taxa of intermediate evolutionary age and conveys considerable phylogenetic signal. Phylogenetic analysis of CPS sequences under varying methods and assumptions resulted in well-resolved, strongly supported trees concordant with many traditional ideas about higher dipteran phylogeny and with prior inferences from 28S rDNA. The most robustly supported major eremoneuran clades were Cyclorrhapha, Platypezoidea, Eumuscomorpha, Empidoidea, Atelestidae, Empidoidea exclusive of Atelestidae, Hybotidae s.l., Microphoridae + Dolichopodidae, and Empididae s. str. Because CAD is ubiquitous, apparently single copy (at least within holometabolous insects), readily obtained from several insect orders using primers described herein, and exhibits considerable

phylogenetic utility, it should have wide applicability in insect molecular systematics.

Nichols, S. A. (2005). "An evaluation of support for order-level monophyly and interrelationships within the class Demospongiae using partial data from the large subunit rDNA and cytochrome oxidase subunit I." Molecular Phylogenetics and Evolution **34**(1): 81.

http://www.sciencedirect.com/science/article/B6WNH-4DTKC5P-4/2/312b682cd042c5f188b175463f2f0487

Large subunit ribosomal DNA (LSU rDNA) sequence data from 120 taxa and cytochrome oxidase subunit 1(COI) sequence data from 27 taxa are analyzed separately and together to estimate the internal phylogeny of the class Demospongiae and to evaluate how consistent these data are with pre-existing hypotheses of relationship concerning order-level monophyly and relationships. The monophyly of Porifera is only slightly inconsistent with LSU data, which do not support the monophyly of the class Demospongiae regardless of the inclusion or exclusion of Homoscleromopha (this result is likely due to the placement of a single hexactinellid taxon within the Demospongiae), however, no LSU support is found for the monophyly of Silicea (Demospongiae + Hexactinellida) unless homoscleromorphs are excluded. Neither the subclasses Ceractinomorpha and Tetractinomorpha, nor the orders Halichondrida, Hadromerida, and Haplosclerida are supported as monophyletic under any data partition. The haplosclerid suborders Haplosclerina and Petrosina are supported as monophyletic to the exclusion of the suborder Spongillina, and the orders Dictyoceratida, Verongida, Poecilosclerida, Astrophorida, Spirophorida, Homosclerophorida, and Agelasida are largely reconstructed as monophyletic, with the exception of few anomalously placed taxa. Few inter-order relationships are strongly supported by any data partition, but there is moderate support for a verongid + chondrosid clade and a tetractinellid + halichondrid clade. Furthermore, LSU data strongly support the existence of two novel clades that do not correspond to the existing classification and that show no morphological uniformity. Finally, every data partition supports the monophyly of a clade that includes the order Agelasida, some members of the genus Axinella, and two taxa tentatively identified as belonging to the orders Hadromerida and Halichondrida.

Oh, S.-H. and D. Potter (2003). "Phylogenetic utility of the second intron of LEAFY in Neillia and Stephanandra (Rosaceae) and implications for the origin of Stephanandra." Molecular Phylogenetics and Evolution **29**(2): 203.

http://www.sciencedirect.com/science/article/B6WNH-48GF21T-K/2/a44881d415a6bb4987ee8aa137a63be8

A homeotic gene, LEAFY, has been suggested to be a single-copy gene in diploid angiosperms. Nucleotide sequences of the second intron of this gene, along with those of several regions of the chloroplast genome (trnL-trnF, trnD-trnY-trnE-trnT, and matK-trnK) and nuclear ribosomal ITS, were obtained from the species of Neillia and Stephanandra to examine the phylogenetic utility of the intron and to elucidate the phylogenetic relationships among species of the two genera. PCR amplification of the second intron of LEAFY using universal degenerate primers produced PCR products in sufficient quantity for successful direct sequencing. The length of the intron ranged from 591 to 622 base pairs (bp) in Neillia and Stephanandra, except in N. thibetica (ca. 1370 bp), and sequence analysis of this region from multiple accessions revealed low levels of infraspecific variation. Comparison of the LEAFY data with ITS and cpDNA data demonstrated that the LEAFY intron was the most variable and useful for phylogenetic analysis at the species level, providing many more phylogenetically informative characters per 100 bp (7.4) than either ITS (3.2) or cpDNA (0.7). Phylogenetic analyses of LEAFY data using both maximum parsimony and likelihood methods generated well supported and highly resolved gene trees with few

homoplasies (CI=0.97). Stephanandra is monophyletic and is nested within Neillia in both LEAFY and cpDNA trees, while the relationship is poorly resolved by ITS data. LEAFY and cpDNA data, however, strongly conflicted with each other with respect to the position of Stephanandra: LEAFY trees placed Stephanandra as sister to the ((N. affinis, N. gracilis), N. thyrsiflora) clade whereas cpDNA data suggested Stephanandra is sister to N. uekii. Both gene trees, however, are nearly identical to each other when Stephanandra is excluded. A hybrid origin of Stephanandra is suggested as a plausible hypothesis to explain the incongruence between LEAFY and cpDNA data sets, though gene duplication/loss and lineage sorting events cannot be ruled out as possibilities.

Perdices, A., E. Bermingham, et al. (2002). "Evolutionary history of the genus Rhamdia (Teleostei: Pimelodidae) in Central America." Molecular Phylogenetics and Evolution **25**(1): 172.

http://www.sciencedirect.com/science/article/B6WNH-46RD1H5-1/2/4bf7fbe07bc70e7c2c0724472c26ff56

We constructed phylogenetic hypotheses for Mesoamerican Rhamdia, the only genus of primary freshwater fish represented by sympatric species across Central America. Phylogenetic relationships were inferred from analysis of 1990 base pairs (bp) of mitochondrial DNA (mtDNA), represented by the complete nucleotide sequences of the cytochrome b (cyt b) and the ATP synthase 8 and 6 (ATPase 8/6) genes. We sequenced 120 individuals from 53 drainages to provide a comprehensive geographic picture of Central American Rhamdia systematics and phylogeography. Phylogeographic analysis distinguished multiple Rhamdia mtDNA lineages, and the geographic congruence across evolutionarily independent Rhamdia clades indicated that vicariance has played a strong role in the Mesoamerican diversification of this genus. Phylogenetic analyses of species-level relationships provide strong support for the monophyly of a trans-Andean clade of three evolutionarily equivalent Rhamdia taxa: R. guatemalensis, R. laticauda, and R. cinerascens. Application of fish-based mitochondrial DNA clocks ticking at 1.3-1.5% sequence divergence per million years (Ma), suggests that the split between cis- and trans-Andean Rhamdia extends back about 8 Ma, and the three distinct trans-Andean Rhamdia clades split about 6 Ma ago. Thus the mtDNA divergence observed between cis- and trans-Andean Rhamdia species is too low to support an ancient colonization of Central America in the Late Cretaceous or Paleocene as had been hypothesized in one colonization model for Mesoamerican fishes. Rather the mtDNA data indicate that Rhamdia most likely colonized Central America in the late Miocene or Pliocene, promoting a strong role for the Isthmus of Panama in the Mesoamerican expansion of this genus. Basal polytomies suggest that both the R. laticauda and R. guatemalensis clades spread rapidly across the Central American landscape, but differences in the average mtDNA genetic distances among clades comprising the two species, indicate that the R. laticauda spread and diversified across Mesoamerica about 1 million years before R. guatemalensis.

Pinou, T., S. Vicario, et al. (2004). "Relict snakes of North America and their relationships within Caenophidia, using likelihood-based Bayesian methods on mitochondrial sequences." Molecular Phylogenetics and Evolution **32**(2): 563.

http://www.sciencedirect.com/science/article/B6WNH-4C5HRR5-1/2/3e347039028d538cfe2b3153047698e0

This paper focuses on the phylogenetic relationships of eight North American caenophidian snake species (Carphophis amoena, Contia tenuis, Diadophis punctatus, Farancia abacura, Farancia erytrogramma, Heterodon nasicus, Heterodon platyrhinos, and Heterodon simus) whose phylogenetic relationships remain controversial. Past studies have referred to these "relict" North

American snakes either as colubrid, or as Neotropical dipsadids and/or xenodontids. Based on mitochondrial DNA ribosomal gene sequences and a likelihood-based Bayesian analysis, our study suggests that these North American snakes are not monophyletic and are nested within a group (Dipsadoidea) that contains the Dipsadidae, Xenodontidae, and Natricidae. In addition, we use the relationships proposed here to highlight putative examples of parallel evolution of hemipenial morphology among snake clades.

Plocik, A., J. Layden, et al. (2004). "Comparative analysis of NBS domain sequences of NBS-LRR disease resistance genes from sunflower, lettuce, and chicory." Molecular Phylogenetics and Evolution **31**(1): 153.

http://www.sciencedirect.com/science/article/B6WNH-49M0ND6-1/2/87fb955d1c9b8609a6d0f53dcc32b97a

Plant resistance to many types of pathogens and pests can be achieved by the presence of disease resistance (R) genes. The nucleotide binding site-leucine rich repeat (NBS-LRR) class of R-genes is the most commonly isolated class of R-genes and makes up a super-family, which is often arranged in the genome as large multi-gene clusters. The NBS domain of these genes can be targeted by polymerase chain reaction (PCR) amplification using degenerate primers. Previous studies have used PCR derived NBS sequences to investigate both ancient R-gene evolution and recent evolution within specific plant families. However, comparative studies with the Asteraceae family have largely been ignored. In this study, we address recent evolution of NBS sequences within the Asteraceae and extend the comparison to the Arabidopsis thaliana genome. Using multiple sets of primers, NBS fragments were amplified from genomic DNA of three species from the family Asteraceae: Helianthus annuus (sunflower), Lactuca sativa (lettuce), and Cichorium intybus (chicory). Analysis suggests that Asteraceae species share distinct families of R-genes, composed of genes related to both coiled-coil (CC) and toll-interleukin-receptor homology (TIR) domain containing NBS-LRR R-genes. Between the most closely related species, (lettuce and chicory) a striking similarity of CC subfamily composition was identified, while sunflower showed less similarity in structure. These sequences were also compared to the A. thaliana genome. Asteraceae NBS gene subfamilies appear to be distinct from Arabidopsis gene clades. These data suggest that NBS families in the Asteraceae family are ancient, but also that gene duplication and gene loss events occur and change the composition of these gene subfamilies over time.

Quandt, D., S. Huttunen, et al. (2004). "Molecular phylogenetics of the Meteoriaceae s. str.: focusing on the genera Meteorium and Papillaria." Molecular Phylogenetics and Evolution **32**(2): 435.

http://www.sciencedirect.com/science/article/B6WNH-4CJ46KB-1/2/c3fb43da1314a188e6d1e66fcdabafb2

In order to delimit and understand the evolution of the Meteoriaceae, we provide phylogenetic analyses using the internal transcribed spacer 2 (ITS2) of nuclear ribosomal DNA in combination with two plastid markers, trnL-F and psbT-H. In contrast to the widely used trnL-F region, the psbT-H gene cluster, coding for proteins of photosystem II, has been rarely used to address systematic questions among the different land plant lineages. To overcome the problem of potential ambiguous alignments of non-coding DNA regions, the data were independently analyzed using direct optimization. The comparison and evaluation of the obtained results showed that the inferred cladograms based on the different phylogenetic approaches are very similar, with only minor differences. In combination with morphological characters, generic relationships as well as taxonomic and nomenclatural problems, especially regarding the key genera Meteorium and Papillaria are discussed in detail. New insights into generic relationships

of the Meteoriaceae are provided, such as the exclusion of the monospecific southern South American genera Ancistrodes and Cryphaeophilum, which are subsequently transferred to the Hookeriaceae and Cryphaeaceae, respectively. Phylogenetic reconstructions using maximum likelihood as well as parsimony approaches reveal that at the familial level the Meteoriaceae s. l. are polyphyletic, if the formerly recognized "Trachypodaceae" are considered as a separate family. Based on our results we favor the synonym of the Trachypodaceae with the Meteoriaceae.

Quenouille, B., E. Bermingham, et al. (2004). "Molecular systematics of the damselfishes (Teleostei: Pomacentridae): Bayesian phylogenetic analyses of mitochondrial and nuclear DNA sequences." Molecular Phylogenetics and Evolution **31**(1): 66.

http://www.sciencedirect.com/science/article/B6WNH-49M0ND6-2/2/16716c181922f3479428cedc5bde9ece

Damselfishes in the family Pomacentridae represent one of the few families of reef fishes found on coral reefs irrespective of location. At a local scale, damselfishes are often the most abundant coral reef fish, and their study has provided much of our current understanding of the ecology of tropical reef animals. The study of phylogenetic relationships among the Pomacentridae has lagged ecological investigation of the group, thus limiting historical perspective on the remarkable species richness of the family. In this study, we used 1989 bp of DNA sequence representing three mitochondrial genes and 1500 bp of the single copy nuclear RAG1 region to infer hypotheses of relationship for the group. Our analysis includes 103 Pomacentridae species in 18 genera, and three of the four named subfamilies: Amphriprioninae, Chrominae, and Pomacentrinae. The Bayesian method of phylogenetic reconstruction was applied to the data, because even with a large number of sequences it is an efficient means of analysis that provides intuitive measures of support for tree topologies and for the parameters of the nucleotide substitution model. Four Pomacentridae clades were identified with high statistical support whether the data were analyzed from a mtDNA, RAG1 or combined perspective, and in all analyses the current subfamilial classification of the Pomacentridae was rejected. At the genus level, Amphiprion, Chromis, and Chrysiptera were also rejected as natural groups. Abudefduf, Amblyglyphidodon, Dascyllus, Neoglyphidodon, Neopomacentrus, and Pomacentrus were each strongly supported as monophyletic genera but the support for monophyly is nonetheless compromised by sample size, except in the case of Dascyllus and Abudefduf for which we have sampled almost all of the described species.

Rees, D. J., M. Dioli, et al. (2003). "Molecules and morphology: evidence for cryptic hybridization in African Hyalomma (Acari: Ixodidae)." Molecular Phylogenetics and Evolution **27**(1): 131.

http://www.sciencedirect.com/science/article/B6WNH-47HK3HT-4/2/7a02a57a6345395797585b5574c2bea8

The role of natural hybridization and introgression as part of the evolutionary process is of increasing interest to zoologists, particularly as more examples of gene exchange among species are identified. We present mitochondrial and nuclear sequence data for Hyalomma dromedarii, Hyalomma truncatum, and Hyalomma marginatum rufipes (Acari: Ixodidae) collected from one-humped camels in Ethiopia. These species are well differentiated morphologically and genetically; sequence data from the mitochondrial DNA (mtDNA) cytochrome oxidase I gene indicates 10-14% divergence between the species. However, incongruence between morphology and the mtDNA phylogeny was observed, with multiple individuals of H. dromedarii and H. truncatum present on the same mtDNA lineage as H. marginatum rufipes. Thus, individuals with morphology of H. dromedarii and H. truncatum are indistinguishable from H. marginatum rufipes on the basis of mtDNA. Multiple copies of ITS-2 were subsequently cloned and sequenced for a

subset of individuals from the mtDNA phylogeny, representing both 'normal' and 'putative hybrid' individuals. Very low sequence divergence (0.3%) was observed within 'normal' individuals of both H. dromedarii and H. truncatum relative to the 'putative hybrid' individuals (6 and 2.7%, respectively). The pattern of intra-individual variation in ITS-2 within 'putative hybrid' individuals, particularly in H. dromedarii, strongly suggests that gene flow has occurred among these Hyalomma species, but no indication of this is given by the morphology of the individuals.

Saux, C., B. L. Fisher, et al. (2004). "Dracula ant phylogeny as inferred by nuclear 28S rDNA sequences and implications for ant systematics (Hymenoptera: Formicidae: Amblyoponinae)." Molecular Phylogenetics and Evolution **33**(2): 457.

http://www.sciencedirect.com/science/article/B6WNH-4D0Y3T4-2/2/9cff2b5e674430ff63f21a597f23128f

Ants are one of the most ecologically and numerically dominant families of organisms in almost every terrestrial habitat throughout the world, though they include only about 1% of all described insect species. The development of eusociality is thought to have been a driving force in the striking diversification and dominance of this group, yet we know little about the evolution of the major lineages of ants and have been unable to clearly determine their primitive characteristics. Ants within the subfamily Amblyoponinae are specialized arthropod predators, possess many anatomically and behaviorally primitive characters and have been proposed as a possible basal lineage within the ants. We investigate the phylogenetic relationships among the members of the subfamily, using nuclear 28S rDNA sequence data. Outgroups for the analysis include members of the poneromorph and leptanillomorph (Apomyrma, Leptanilla) ant subfamilies, as well as three wasp families. Parsimony, maximum likelihood, and Bayesian analyses provide strong support for the monophyly of a clade containing the two genera Apomyrma + Mystrium (100% bpp; 97% ML bs; and 97% MP bs), and moderate support for the monophyly of the Amblyoponinae as long as Apomyrma (Apomyrminae) is included (87% bpp; 57% ML bs; and 76% MP bs). Analyses did not recover evidence of monophyly of the Amblyopone genus, while the monophyly of the other genera in the subfamily is supported. Based on these results we provide a morphological diagnosis of the Amblyoponinae that includes Apomyrma. Among the outgroup taxa, Typhlomyrmex grouped consistently with Ectatomma, supporting the recent placement of Typhlomyrmex in the Ectatomminae. The results of this present study place the included ant subfamilies into roughly two clades with the basal placement of Leptanilla unclear. One clade contains all the Amblyoponinae (including Apomyrma), Ponerinae, and Proceratiinae (Poneroid clade). The other clade contains members from subfamilies Cerapachyinae, Dolichoderinae, Ectatomminae, Formicinae, Myrmeciinae, and Myrmicinae (Formicoid clade).

Siddall, M. E., S. L. Perkins, et al. (2004). "Leech mycetome endosymbionts are a new lineage of alphaproteobacteria related to the Rhizobiaceae." Molecular Phylogenetics and Evolution **30**(1): 178.

http://www.sciencedirect.com/science/article/B6WNH-48WPS6T-1/2/3a576ccfa3363585d3fe4cee4414fec7

Mycetomal organs attached to the esophagus of hematophagous leeches which are known to harbor endosymbiotic bacteria were removed from three species in the leech family Glossiphoniidae. Anatomical observations indicated that placobdellid mycetomes are paired and caecate, inserting into the esophagus posterior to the proboscis. Light and electron microscopy demonstrated that there is a single layer of mycetome epithelial cells harboring Gram-negative rods and that these epithelial cells are ultrastructurally distinct from neighboring esophageal epithelial cells. Fluorescent in situ hybridization with eubacterial and alphaproteobacterial probes

localized the bacteria solely to the mycetomes both in adult and in unfed juvenile leeches whereas a gammaproteobacterial probe did not yield a bound fluorescencent signal. DNA was isolated from these tissues and subjected to PCR amplification using bacteria-specific primers for 16S and 23S rDNA. Results from sequencing the amplification products and phylogenetic analysis with other Alphaproteobacteria revealed that the bacteria resident in these organs comprise a new genus of Alphaproteobacteria, Reichenowia n. gen., closely related to the nitrogen-fixing, nodule-forming Rhizobiaceae. The three bacterial strains, though different from each other were each other's closest relatives, suggesting a history of close coevolution with their leech hosts.

Steiper, M. E. and M. Ruvolo (2003). "New World monkey phylogeny based on X-linked G6PD DNA sequences." Molecular Phylogenetics and Evolution **27**(1): 121.

http://www.sciencedirect.com/science/article/B6WNH-47HK3HT-5/2/7c38c4ea76ffaf0797640536304f7b10

The Platyrrhini, or New World monkeys, are an infraorder of Primates comprised of 16 genera. Molecular phylogenetic analyses have consistently sorted these genera into three groups: the Pitheciidae (e.g., saki and titi monkeys), Atelidae (e.g., spider and howler monkeys), and Cebidae (e.g., night monkeys, squirrel monkeys, and tamarins). No consensus has emerged on the relationships among the three groups or within the Cebidae. Here, ~0.8 kb of newly generated intronic DNA sequence data from the X-linked glucose-6-phosphate dehydrogenase (G6PD) locus have been collected from nine New World monkey taxa to examine these relationships. These data are added to 1.3 kb of previously generated G6PD intronic DNA sequence data [Mol. Phylogenet. Evol. 11 (1999) 459]. Using distance and parsimony-based techniques, G6PD sequences provide support for an initial bifurcation between the Pitheciidae and the remaining platyrrhines, linking Atelidae and Cebidae as sister taxa. Bayesian methods provided a conflicting phylogeny with Atelidae as outgroup. Within the Cebidae, a sister relation between Aotus and the Cebus/Saimiri clade is favored by parsimony analysis, but not by other analyses. Potential reasons for the difficulty in resolving family level New World monkey phylogenetics are discussed.

Steppan, S. J., B. L. Storz, et al. (2004). "Nuclear DNA phylogeny of the squirrels (Mammalia: Rodentia) and the evolution of arboreality from c-myc and RAG1." Molecular Phylogenetics and Evolution **30**(3): 703.

http://www.sciencedirect.com/science/article/B6WNH-4967F2T-2/2/48f4439f6e122d16606e39f6dfd9ed0f

Although the family Sciuridae is large and well known, phylogenetic analyses are scarce. We report on a comprehensive molecular phylogeny for the family. Two nuclear genes (c-myc and RAG1) comprising approximately 4500 bp of data (most in exons) are applied for the first time to rodent phylogenetics. Parsimony, likelihood, and Bayesian analyses of the separate gene regions and combined data reveal five major lineages and refute the conventional elevation of the flying squirrels (Pteromyinae) to subfamily status. Instead, flying squirrels are derived from one of the tree squirrel lineages. C-myc indels corroborate the sequence-based topologies. The common ancestor of extant squirrels appears to have been arboreal, confirming the fossil evidence. The results also reveal an unexpected clade of mostly terrestrial squirrels with African and Holarctic centers of diversity. We present a revised classification of squirrels. Our results demonstrate the phylogenetic utility of relatively slowly evolving nuclear exonic data even for relatively recent clades.

Stone, K. D. and J. A. Cook (2002). "Molecular evolution of Holarctic martens (genus Martes, Mammalia: Carnivora: Mustelidae)." Molecular Phylogenetics and Evolution **24**(2): 169.

http://www.sciencedirect.com/science/article/B6WNH-46CS03K-1/2/b8ff70778e19d9cba7229387ab76c489

Thacker, C. E. (2003). "Molecular phylogeny of the gobioid fishes (Teleostei: Perciformes: Gobioidei)." Molecular Phylogenetics and Evolution **26**(3): 354.

http://www.sciencedirect.com/science/article/B6WNH-47S6M2C-2/2/e7535d0f07bc07da50e3c06f69281281

The phylogeny of groups within Gobioidei is examined with molecular sequence data. Gobioidei is a speciose, morphologically diverse group of teleost fishes, most of which are small, benthic, and marine. Efforts to hypothesize relationships among the gobioid groups have been hampered by the prevalence of reductive evolution among goby species; such reduction can make identification of informative morphological characters particularly difficult. Gobies have been variously grouped into two to nine families, several with included subfamilies, but most existing taxonomies are not phylogenetic and few cladistic hypotheses of relationships among goby groups have been advanced. In this study, representatives of eight of the nine gobioid familes (Eleotridae, Odontobutidae, Xenisthmidae, Gobiidae, Kraemeriidae, Schindleriidae, Microdesmidae, and Ptereleotridae), selected to sample broadly from the range of goby diversity, were examined. Complete sequence from the mitochondrial ND1, ND2, and COI genes (3573 bp) was used in a cladistic parsimony analysis to hypothesize relationships among the gobioid groups. A single most parsimonious topology was obtained, with decay indices indicating strong support for most nodes. Major phylogenetic conclusions include that Xenisthmidae is part of Eleotridae, and Eleotridae is paraphyletic with respect to a clade composed of Gobiidae, Microdesmidae, Ptereleotridae, Kraemeriidae, and Schindleriidae. Within this five-family clade, two clades are recovered. One includes Gobionellinae, which is paraphyletic with respect to Kraemeriidae, Sicydiinae, Oxudercinae, and Amblyopinae. The other contains Gobiinae, also paraphyletic, and including Microdesmidae, Ptereleotridae, and Schindleriidae. Previous morphological evidence for goby groupings is discussed; the phylogenetic hypothesis indicates that the morphological reduction observed in many goby species has been derived several times independently.

Van Borm, S., T. Wenseleers, et al. (2003). "Cloning and sequencing of wsp encoding gene fragments reveals a diversity of co-infecting Wolbachia strains in Acromyrmex leafcutter ants." Molecular Phylogenetics and Evolution **26**(1): 102.

http://www.sciencedirect.com/science/article/B6WNH-475B9D7-3/2/0bbab6a302941e820c97890b65266546

Wall, D. P. (2002). "Use of the nuclear gene glyceraldehyde 3-phosphate dehydrogenase for phylogeny reconstruction of recently diverged lineages in Mitthyridium (Musci: Calymperaceae)." Molecular Phylogenetics and Evolution **25**(1): 10.

http://www.sciencedirect.com/science/article/B6WNH-46WNYGM-

3/2/daef3b413ab5e1d0d2843c69bb6296dd

A portion of the nuclear gene glyceraldehyde 3-phosphate dehydrogenase (gpd) was sequenced in 26 representatives of the paleotropical moss, Mitthyridium, and a group of 20 outgroup taxa to assess its utility for phylogenetic reconstruction compared with the better understood chloroplast markers, rps4 and trnL. Primers based on plant and fungal sequences were designed to amplify gpd in plants universally with the exclusion of fungal contaminants. The piece amplified spanned 4 introns and 3 of 9 exons, based on comparisons with complete sequence from Arabidopsis. Size variation in gpd ranged from 891 to 1007 bp, in part attributable to 6 indels of variable length found within the introns. Intron 6 contributed most of the length variation and contained a variable purine-repeat motif of possible use as a microsatellite. Phylogenetic analyses of the full gpd amplicon yielded well-resolved trees that were in nearly full accord with the trees derived from the cpDNA partitions for analyses of both the ingroup and ingroup + outgroup taxon sets. Pairwise nucleotide substitution rates of gpd were as much as 2.2 times higher than those in rps4 and 2.8 times higher than in trnL. Excision of the introns left suitable numbers of parsimony informative characters and demonstrated that the full gpd amplicon could be compartmentalized to provide resolution for both shallow and deep phylogenetic branches. Exons of gpd were found to behave in a clock-like fashion for the 26 ingroup taxa and select outgroups. In general, gpd was found to hold great promise not only for improving resolution of chloroplast-derived phylogenies, but also for phylogenetic reconstruction of recent, diversifying lineages.

Weksler, M. (2003). "Phylogeny of Neotropical oryzomyine rodents (Muridae: Sigmodontinae) based on the nuclear IRBP exon." Molecular Phylogenetics and Evolution **29**(2): 331.

http://www.sciencedirect.com/science/article/B6WNH-48KFF3P-5/2/d09fea423931fb9beff14d8a7536865f

Sigmodontine rodents are the most diverse family-level mammalian clade in the Neotropical region, with about 70 genera and 320 recognized species. Partial sequences (1266 bp) from the first exon of the nuclear gene encoding the Interphotoreceptor Retinoid Binding Protein (IRBP) were used to infer the phylogenetic relationships among 44 species representing all 16 currently recognized genera of the largest sigmodontine tribe, the Oryzomyini. Monophyly of the tribe was assessed relative to 15 non-oryzomyine sigmodontine taxa representing all major sigmodontine lineages. Twelve taxa from seven muroid subfamilies were used as outgroups. The resulting matrix included 71 taxa and 386 parsimony-informative characters. Phylogenetic analysis of this matrix resulted in 16 equally parsimonious cladograms, which contained the following well-supported groups: (i) a monophyletic Oryzomyini, (ii) a clade containing all oryzomyines except Scolomys and Zygodontomys, (iii) a clade containing Oecomys, Handleyomys, and several species of forest-dwelling Oryzomys, and (iv) a clade containing the remaining oryzomyine taxa. The last clade is composed of two large subclades, each with lower nodal support, containing the following taxa: (i) Microryzomys, Oligoryzomys, Neacomys, and Oryzomys balneator; (ii) Holochilus, Lundomys, Pseudoryzomys, Nectomys, Amphinectomys, Sigmodontomys, and several species of open-vegetation or semiaquatic Oryzomys. Regarding relationships among non-oryzomyine taxa, sigmodontines, neotomines, and tylomyines do not form a monophyletic group; a clade containing Rheomys and Sigmodon is basal relative to all other sigmodontines; and the remaining sigmodontines are grouped in three clades: the first containing Thomasomyini, Akodontini, and Reithrodon; the second containing Abrothrichini, and Phyllotini, plus Wiedomys, Juliomys, Irenomys, and Delomys; and the third containing the oryzomyines. No conflict is observed between IRBP results and previous robust hypotheses from mitochondrial data, while a single case of incongruence is present between the IRBP topology and robust hypothesis from morphological studies.

Wesson, D. M., C. H. Porter, et al. (1992). "Sequence and secondary structure comparisons of ITS rDNA in mosquitoes (Diptera: Culicidae)." Molecular Phylogenetics and Evolution **1**(4): 253.

http://www.sciencedirect.com/science/article/B6WNH-4DXK9Y9-B/2/aa000f97baf606884d8eecb21770d436

Sequences of the internal transcribed spacers (ITS1 and ITS2) of the mosquito Aedes aegypti, and the ITS2 of six related species, A. simpsoni, A. albopictus, A. vexans, A. triseriatus, Haemagogus mesodentatus, and Psorophora ferox are reported. Intraspecific variation in A. aegypti ITS1 is 1.07% among four clones from three individuals, and in the ITS2 is 1.17% among 15 clones from four individuals. In A. simpsoni, intraspecific ITS2 variation is 0.46% among 10 clones from a single individual. Alignment of the ITS2 sequence of the seven species reveals several homologous domains. Secondary structure predictions for the ITS2 region indicate that these domains base pair to form a core region central to several stem features. The sequence outside the ITS2 homologous domains tends to be GC-rich and characteristically slippage generated; these areas preserve or add to the stem length of the predicted secondary structures. These ITS2 intraspacer variable regions resemble previously described expansion segments of the 28S gene region. Evolutionary analysis of the ITS2 of these species, using both sequence and secondary structure information, leads to the prediction of divergence in the mosquito tribe Aedini that is not clearly reflected in current taxonomic designations.

Williams, S. T., D. G. Reid, et al. (2003). "A molecular phylogeny of the Littorininae (Gastropoda: Littorinidae): unequal evolutionary rates, morphological parallelism, and biogeography of the Southern Ocean." Molecular Phylogenetics and Evolution **28**(1): 60.

http://www.sciencedirect.com/science/article/B6WNH-48FK4VN-1/2/b9140e4a7cf75e4f5c9a8d95913e23c6

A molecular phylogeny is presented for the subfamily Littorininae (including representatives of all subgeneric taxa and all members of a group of southern-temperate species formerly classified as 'Nodilittorina'), based on sequence data from two nuclear (18S rRNA, 28S rRNA) and two mitochondrial (12S rRNA, CO1) genes. The phylogeny shows considerable disagreement with earlier hypotheses derived from morphological data. In particular, 'Nodilittorina' is polyphyletic and is here divided into four genera (Echinolittorina, Austrolittorina, Afrolittorina new genus, and the monotypic Nodilittorina s.s.). The phylogenetic relationships of 'Littorina' striata have been controversial and it is here transferred to the genus Tectarius, a surprising relationship for which there is little morphological support. The relationships of the enigmatic Mainwaringia remain poorly resolved, but it is not a basal member of the subfamily. The two living species of Mainwaringia are remarkable for a greatly elevated rate of evolution in all four genes examined; it is suggested that this may be connected with their protandrous hermaphroditism, which is unique in the family. The molecular phylogeny provides a new framework for the adaptive radiation of the Littorininae, showing more frequent shifts between habitats and climatic regimes than previously suspected, and striking parallelism of morphological characters. The fossil record of littorinids is poor, but ages of clades are estimated using a calibration based on a Lower Eocene age of the genus Littoraria. Using these estimates, the antitropical distribution of Littorina and Afrolittorina is an ancient pattern of possibly Cretaceous age. The five members of Austrolittorina show a Gondwanan distribution in Australia, New Zealand, and South America. Based on the morphological uniformity within this clade, relatively recent (Plio-Pleistocene) trans-Pacific dispersal events seemed a likely explanation, as proposed for numerous other congeneric marine taxa. However, molecular estimation of ages of divergence suggest an initial vicariance between Australian and South American lineages at 40-73 Ma, contemporary with the later stages of fragmentation of the Gondwanan supercontinent, followed by more recent (but still mid-Cenozoic) dispersal events across the Tasman Sea and the Pacific Ocean. Afrolittorina is another Cretaceous clade, now restricted to southern Africa and southern Australia, but divergence

between these lineages (29-55 Ma) post-dates Gondwanan fragmentation. Within both Austrolittorina and Afrolittorina all sister-species divergences are estimated to fall in the range 10-47 Ma, so that there is no evidence for speciation events in the Plio-Pleistocene.

Winkworth, R. C. and M. J. Donoghue (2004). "Viburnum phylogeny: evidence from the duplicated nuclear gene GBSSI." Molecular Phylogenetics and Evolution **33**(1): 109.

http://www.sciencedirect.com/science/article/B6WNH-4CT5YFS-4/2/87b46543eff7302df012b11c010371d0

DNA sequencing studies of the granule-bound starch synthase gene (GBSSI) indicate the presence of two loci in Viburnum. Gene trees from separate and combined phylogenetic analyses of the GBSSI paralogues are generally congruent with each other and with trees from previous analyses, especially those of Donoghue et al. [Syst. Bot. 29 (2004) 188] based on nuclear ribosomal ITS and chloroplast trnK intron DNA sequences. Specifically, our GBSSI trees confirm (i) the monophyly of some and non-monophyly of other traditionally recognized taxonomic sections, (ii) the presence of three major supra-sectional lineages within Viburnum, and (iii) the resolution of many species relationships within the section-level clades. Analyses of GBSSI also provide greater resolution of relationships within the largest supra-sectional lineage. Relationships at the base of the Viburnum phylogeny remain uncertain; in particular, the position of the root, relationships among the supra-sectional clades, and the exact placement of several smaller groups (e.g., Viburnum clemensiae, Viburnum urceolatum, and section Pseudotinus). In two lineages each GBSSI paralogue is represented by two distinct sequences. The presence of additional copies appears to be correlated with polyploidy in these clades. Placement of the homoeologues in our gene trees suggests the possibility of a hybrid origin for these polyploids.

Yang, Y., Y.-p. Zhang, et al. (2004). "Phylogenetic relationships of Drosophila melanogaster species group deduced from spacer regions of histone gene H2A-H2B." Molecular Phylogenetics and Evolution **30**(2): 336.

http://www.sciencedirect.com/science/article/B6WNH-49H1KWC-1/2/deae7d1c950b45c0a903f2f56914abad

Nucleotide sequences of the spacer region of the histone gene H2A-H2B from 36 species of Drosophila melanogaster species group were determined. The phylogenetic trees were reconstructed with maximum parsimony, maximum likelihood, and Bayesian methods by using Drosophila pseudoobscura as the out group. Our results show that the melanogaster species group clustered in three main lineages: (1) montium subgroup; (2) ananassae subgroup; and (3) the seven oriental subgroups, among which the montium subgroup diverged first. In the third main lineage, suzukii and takahashii subgroups formed a clade, while eugracilis, melanogaster, elegans, ficusphila, and rhopaloa subgroups formed another clade. The bootstrap values at subgroup levels are high. The phylogenetic relationships of these species subgroups derived from our data are very different from those based on some other DNA data and morphology data.

Mycologia (21)

Alves, A., A. Correia, et al. (2004). "Botryosphaeria corticola, sp. nov. on Quercus species, with notes and description of Botryosphaeria stevensii and its anamorph, Diplodia mutila." Mycologia **96**(3): 598-613.

http://www.mycologia.org/cgi/content/abstract/96/3/598

Botr yosphaeria stevensii frequently has been associated with dieback and canker diseases of oak, mainly in the western Mediterranean area but more rarely in other regions. The species concept of B. stevensii has been unclear, and it is possible that some collections were identified incorrectly. A collection of fungal strains isolated from diseased oak trees and initially identified as B. stevensii was characterized on the basis of morphology and ITS nucleotide sequences. Morphology was compared with the type specimens of Physalospora mutila (= B. stevensii) and its anamorph, Diplodia mutila. It was concluded that the isolates from oak differed from B. stevensii in having larger ascospores and conidia as well as different spore shapes and represented an as yet undescribed species, which is described here as B. corticola. Moreover, ITS sequence data separated B. corticola from all other known species of Botryosphaeria. Amended descriptions of B. stevensii and its anamorph are provided to differentiate B. stevensii from B. corticola and to clarify some of the earlier taxonomic uncertainties.

Binder, M. and A. Bresinsky (2002). "Derivation of a polymorphic lineage of Gasteromycetes from boletoid ancestors." Mycologia **94**(1): 85-98.

http://www.mycologia.org/cgi/content/abstract/94/1/85

The phylogeny of selected gasteromycetes and hymenomycetes was inferred from partial nuclear large subunit rDNA (nuc-lsu, 28S) sequences, delimited by primers LR0R and LR5. Taxon sampling with emphasis on relationships within the Boletales further included some gasteroid groups, which obviously have evolved convergent fruiting body morphology, and therefore remained controversial in taxonomy. This study confirms the close relationship of Geastrales, Gauteriales and Phallales and the presumable derivation of Nidulariales and Tulostomatales within the euagarics clade, as widely accepted. In addition, four Hymenogaster species investigated were found to be in the euagarics clade and a relationship to the Cortinariaceae was indicated. The gasteroid fungus Zelleromyces stephensii is an example for maintaining morphological linkage by a lactiferous hyphal system to the genus Lactarius in the Russulales, and this relationship was affirmed in the sequence analysis. Several previously suggested relationships of gasteromycetes and Boletales were reproducible by analyzing nuc-lsu sequences. As a new result, Astraeus hygrometricus, the barometer earth star, is an additional representative of the Boletales. Together with Boletinellus, Phlebopus, Pisolithus, Calostoma, Gyroporus, Scleroderma, and Veligaster, Astraeus forms an unusual group comprising pileate-stipitate hymenomycetes and polymorphic gasteromycetes. This group is a major lineage within the Boletales and we propose the new suborder Sclerodermatineae, including the six families Boletinellaceae fam. nov. (Boletinellus and Phlebopus), Gyroporaceae (Singer) fam. nov. (Gyroporus), Pisolithaceae (Pisolithus), Astraeaceae (Astraeus), Calostomataceae (Calostoma), and the typus subordinis Sclerodermataceae (Scleroderma and Veligaster). Morphological and ecological characters, and pigment synthesis support the delimitation of the Sclerodermatineae, and indicate the radiation of different lineages in the Boletales originating from fungi with primitive tubular hymenophores. We regard such boletes with gyroid-boletinoid hymenophores, like Boletinellus, Gyrodon, Gyroporus, Paragyrodon and Phlebopus as key taxa in the evolution of Paxillineae, Sclerodermatineae and Boletineae.

Brayford, D., B. M. Honda, et al. (2004). "Neonectria and Cylindrocarpon: the Nectria mammoidea group and species lacking microconidia." Mycologia **96**(3): 572-597.

http://www.mycologia.org/cgi/content/abstract/96/3/572

Neonectria (Hypocreales: Nectriaceae) species having Cylindrocarpon anamorphs that lack microconidia and chlamydospores include: Neo. discophora var. discophora, Neo. discophora var. rubi, stat nov. et comb. nov., Neo. lucida, comb. nov., Neo. viridispora, sp. nov. and Neo. westlandica, comb. nov. Perithecia of these species are red and perithecial anatomyis of the N. mammoidea type, with a palisade of hypha-like cells in the outer perithecial wall. These species occur on recently dead or dying trees. Perithecia of Neo. betulae, sp. nov and Neo. dumontii, sp. nov. are anatomically and biologically similar to those of Neo. discophora. The only known culture of Neo. betulae remained sterile, while Neo. dumontii has not been cultured; their anamorphs are presumed to be Cylindrocarpon. Analyses of mit ssu rDNA sequences indicate that Neonectria/Cylindrocarpon is monophyletic. Within the genus, species having N. mammoidea type perithecia are paraphyletic. Most species cluster with Neo. discophora, but Neo. westlandica and Neo. trachosa are basal to a clade that includes species that do not have a N. mammoidea-type perithecium. Nectria fuckeliana clusters independently of Neonectria and Nectria. Although reported to have a Cylindrocarpon anamorph, fresh ascospore isolates of N. fuckeliana did not produce Cylindrocarpon macroconidia but produced acremonium- or verticillium-like anamorphs. A key to nectriaceous species of Neonectria that have Cylindrocarpon anamorphs that lack microconidia and chlamydospores and/or that have a N. mammoidea type perithecial wall anatomy is presented. New combinations are proposed for other species formerly included in Nectria that have non-microconidial Cylindrocarpon anamorphs: Neonectria cinnamomea, Neo. jungneri, Neo. platycephala, Neo. phaeodisca and Neo. verrucospora.

Castlebury, L. A., A. Y. Rossman, et al. (2002). "A preliminary overview of the Diaporthales based on large subunit nuclear ribosomal DNA sequences." Mycologia **94**(6): 1017-1031.

http://www.mycologia.org/cgi/content/abstract/94/6/1017

The ascomycete order Diaporthales includes a number of plant pathogenic fungi such as Cryphonectria parasitica, the chestnut blight fungus, as well as many asexually reproducing fungi without known sexual states. Relationships among genera in the Diaporthales were evaluated as a basis for the recognition of families and to provide a taxonomic framework for the asexually reproducing diaporthalean fungi. Phylogenetic relationships were determined based on analyses of large subunit (LSU) nuclear ribosomal DNA (nrDNA) sequences. Within the Diaporthales 82 sequences representing 69 taxa were analyzed. Results suggest the presence of at least six major lineages within the Diaporthales recognized as the Gnomoniaceae sensu stricto, Melanconidaceae sensu stricto, Schizoparme complex including the anamorph genera Coniella and Pilidiella, Cryphonectria-Endothia complex, Valsaceae sensu stricto, and Diaporthaceae sensu stricto. In addition, six teleomorphic and anamorphic taxa fell within the Diaporthales but were not allied with any of the six lineages.

Ceresini, P. C., H. D. Shew, et al. (2002). "Genetic diversity of Rhizoctonia solani AG-3 from potato and tobacco in North Carolina." Mycologia **94**(3): 437-449.

http://www.mycologia.org/cgi/content/abstract/94/3/437

Anastomosis group 3 (AG-3) of Rhizoctonia solani (teleomorph = Thanatephorus cucumeris) is frequently associated with diseases of potato (AG-3 PT) and tobacco (AG-3 TB). Although isolates of R. solani AG-3 from these two Solanaceous hosts are somatically related based on anastomosis reaction and taxonomically related based on fatty acid, isozyme and DNA characters, considerable differences are evident in their biology, ecology, and epidemiology.

However, genetic diversity among field populations of R. solani AG-3 PT and TB has not been documented. In this study, the genetic diversity of field populations of R. solani AG-3 PT and AG-3 TB in North Carolina was examined using somatic compatibility and amplified fragment length polymorphism (AFLP) criteria. A sample of 32 isolates from potato and 36 isolates from tobacco were paired in all possible combinations on PDA plus activated charcoal and examined for their resulting somatic interactions. Twenty-eight and eight distinct somatic compatibility groups (SCG) were identified in the AG-3 PT and AG-3 TB samples, respectively. AFLP analyses indicated that each of the 32 AG-3 PT isolates had a distinct AFLP phenotype, whereas 28 AFLP phenotypes were found among the 36 isolates of AG-3 TB. None of the AG-3 PT isolates were somatically compatible or shared a common AFLP phenotype with any AG-3 TB isolate. Clones (i.e., cases where two or more isolates were somatically compatible and shared the same AFLP phenotype) were identified only in the AG-3 TB population. Four clones from tobacco represented 22% of the total population. All eight SCG from tobacco were associated with more than one AFLP phenotype. Compatible somatic interactions between AG-3 PT isolates occurred only between certain isolates from the same field (two isolates in each of four different fields), and when this occurred AFLP phenotypes were similar but not identical.

Ceresini, P. C., H. D. Shew, et al. (2002). "Genetic structure of populations of Rhizoctonia solani AG-3 on potato in eastern North Carolina." Mycologia **94**(3): 450-460.

http://www.mycologia.org/cgi/content/abstract/94/3/450

A polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was developed to identify and differentiate genotypes of Rhizoctonia solani anastomosis group 3 subgroup PT (AG-3 PT), a fungal pathogen of potato. Polymorphic co-dominant single-locus PCR-RFLP markers were identified after sequencing of clones from a genomic library and digestion with restriction enzymes. Multilocus genotypes were determined by a combination of PCR product and digestion with a specific restriction enzyme for each of seven loci. A sample of 104 isolates from one commercial field in each of five counties in eastern North Carolina was analyzed, and evidence for high levels of gene flow between populations was revealed. When data were clone-corrected and samples pooled into one single North Carolina population, random associations of alleles were found for all loci or pairs of loci, indicating random mating. However, when all genotypes were analyzed, the observed genotypic diversity deviated from panmixia and alleles within and between loci were not randomly associated. These findings support a model of population structure for R. solani AG-3 PT on potato that includes both recombination and clonality.

Chaverri, P., L. A. Castlebury, et al. (2003). "Hypocrea/Trichoderma: species with conidiophore elongations and green conidia." Mycologia **95**(6): 1100-1140.

http://www.mycologia.org/cgi/content/abstract/95/6/1100

Species of Trichoderma and Hypocrea that have green conidia and sterile or fertile elongations of their conidiophores are described or redescribed and their phylogenetic position explored. The described species include T. crassum, T. fasciculatum, T. fertile, T. hamatum, T. longipile, T. oblongisporum, T. pubescens, T. spirale, T. strictipile, T. strigosum, T. stromaticum, T. tomentosum, Hypocrea aureoviridis f. macrospora, H. ceramica. and H. semiorbis. Trichoderma fasciculatum originally was described from cultures from ascospores of an unidentified Hypocrea specimen; it is considered to be a synonym of T. strictipile. The remaining species of Trichoderma considered here have not been linked to teleomorphs, and the Trichoderma anamorphs of H. aureoviridis f. macrospora and H. semiorbis have not been named. Five new species of Hypocrea are described, viz. H. cremea, H. cuneispora, H. estonica, H. strictipilosa and H. surrotunda. The

phylogenetic relationships of these species were inferred based on partial RPB2 and EF-1{alpha} DNA sequence data and phenotypic characteristics, including teleomorph, anamorph, colony and growth rates. Trichoderma crassum was found to be a sister species to T. virens, based on molecular sequences and phenotypic data. Hypocrea surrotunda and H. cremea, H. cuneispora and T. longipile, T. fertile and T. oblongisporum, T. tomentosum and H. atrogelatinosa, and T. hamatum and T. pubescens, respectively, were found to be closely related phylogenetically, based on RPB2 and EF-1{alpha} gene genealogies. Anamorph and teleomorph phenotype, including conidiophore elongations, phialide morphology, conidial morphology, stroma anatomy and ascospore morphology are not useful predictors of relationships. Despite the shared phenotypic characters of these Trichoderma and Hypocrea species, they are distributed between two major clades of Trichoderma/Hypocrea. Redescriptions and a key to species of Hypocrea/Trichoderma with green conidia and conidiophore elongations are presented.

Coetzee, M. P. A., B. D. Wingfield, et al. (2003). "Molecular identification and phylogeny of Armillaria isolates from South America and Indo-Malaysia." Mycologia **95**(2): 285-293.

http://www.mycologia.org/cgi/content/abstract/95/2/285

Armillaria root rot is a serious disease, chiefly of woody plants, caused by many species of Armillaria that occur in temperate, tropical and subtropical regions of the world. Very little is known about Armillaria in South America and Southeast Asia, although Armillaria root rot is well known in these areas. In this study, we consider previously unidentified isolates collected from trees with symptoms of Armillaria root rot in Chile, Indonesia and Malaysia. In addition, isolates from basidiocarps resembling A. novae-zelandiae and A. limonea, originating from Chile and Argentina, respectively, were included in this study because their true identity has been uncertain. All isolates in this study were compared, based on their similarity in ITS sequences with previously sequenced Armillaria species, and their phylogenetic relationship with species from the Southern Hemisphere was considered. ITS sequence data for Armillaria also were compared with those available at GenBank. Parsimony and distance analyses were conducted to determine the phylogenetic relationships between the unknown isolates and the species that showed high ITS sequence similarity. In addition, IGS-1 sequence data were obtained for some of the species to validate the trees obtained from the ITS data set. Results of this study showed that the ITS sequences of the isolates obtained from basidiocarps resembling A. novae-zelandiae are most similar to those for this species. ITS sequences for isolates from Indonesia and Malaysia had the highest similarity to A. novae-zelandiae but were phylogenetically separated from this species. Isolates from Chile, for which basidiocarps were not found, were similar in their ITS and IGS-1 sequences to the isolate from Argentina that resembled A. limonea. These isolates, however, had the highest ITS and IGS-1 sequence similarity to authentic isolates of A. luteobubalina and were phylogenetically more closely related to this species than to A. limonea.

Cruse, M., R. Telerant, et al. (2002). "Cryptic species in Stachybotrys chartarum." Mycologia **94**(5): 814-822.

http://www.mycologia.org/cgi/content/abstract/94/5/814

Stachybotrys chartarum has received much attention as a possible cause of sick-building syndrome. Because morphological species recognition in fungi can hide diversity, we applied a phylogenetic approach to search for cryptic species. We examined 23 isolates from the San Francisco Bay Area, and another seven from around the US. Using markers we developed for three polymorphic protein coding loci (chitin synthase 1, beta-tubulin 2, and trichodiene synthase 5), we infer that two distinct phylogenetic species exist within the single described morphological species. We have found no correlation between genetic isolation and geographic distance.

Farr, D. F., L. A. Castlebury, et al. (2002). "Morphological and molecular characterization of Phomopsis vaccinii and additional isolates of Phomopsis from blueberry and cranberry in the eastern United States." Mycologia **94**(3): 494-504.

http://www.mycologia.org/cgi/content/abstract/94/3/494

Forty isolates of Phomopsis were obtained from twigs and berries of highbush blueberry, Vaccinium corymbosum, and cranberry, Vaccinium macrocarpon, isolated primarily from plants grown in the eastern United States. They were characterized using conidiomatal morphology, conidial dimensions, colony appearance and growth rate, and sequences of ITS rDNA. Based on morphological and molecular similarities, most isolates grouped together with an authentic culture of Phomopsis vaccinii Shear. This taxon is described and illustrated. However, some Phomopsis isolates from Vaccinium differed in colony and conidiomatal morphology from P. vaccinii and, based on ITS sequences, were related to isolates of Phomopsis from diverse hosts. These isolates were excluded from P. vaccinii.

Jacobs, A., M. P. A. Coetzee, et al. (2003). "Phylogenetic relationships among Phialocephala species and other ascomycetes." Mycologia **95**(4): 637-645.

http://www.mycologia.org/cgi/content/abstract/95/4/637

Phialocephala was established for species in the Leptographium complex that produce conidia from phialides at the apices of dark mononematous conidiophores. Some species previously included in Phialocephala were re-allocated to Sporendocladia because they resembled Thielaviopsis in having ring-wall-building conidial development and conidia with two attachment points that emerge in false chains. Despite this significant realignment of the genus, a great deal of morphological heterogeneity remains in Phialocephala. The objective of this study was to consider the heterogeneity among Phialocephala spp. based on comparisons of sequence data derived from the large and small subunits (LSU and SSU) of the rRNA operon of species in Phialocephala. Phialocephala dimorphospora, the type species of the genus, and P. fortinii grouped with genera of the Helotiales in phylogenetic trees generated based on the LSU and SSU datasets. Phialocephala xalapensis and P. fusca clearly are unrelated to Phialocephala sensu stricto and should represent a new genus in the Ophiostomatales. Phialocephala compacta resides with representatives of the Hypocreales, and we believe that it represents a distinct genus. Phialocephala scopiformis and P. repens are not closely related to the other Phialocephala species and group within the Dothideales. The morphological heterogeneity among species of Phialocephala clearly is reflected by phylogenetic analysis of sequence data from two conserved rRNA gene regions. Appropriate genera now need to be found to accommodate these fungi.

Jacobson, D. J., A. J. Powell, et al. (2004). "Neurospora in temperate forests of western North America." Mycologia **96**(1): 66-74.

http://www.mycologia.org/cgi/content/abstract/96/1/66

The fungal genus Neurospora has a distinguished history as a laboratory model in genetics and biochemistry. The most recent milestone in this history has been the sequencing of the genome of the best known species, N. crassa. The hope and promise of a complete genome sequence is a full understanding of the biology of the organism. Full understanding cannot be achieved,

however, in the absence of fundamental knowledge of natural history. We report that species of Neurospora, heretofore thought to occur mainly in moist tropical and subtropical regions, are common primary colonizers of trees and shrubs killed by forest fires in western North America, in regions that are often cold and dry. Surveys in 36 forest-fire sites from New Mexico to Alaska yielded more than 500 cultures, 95% of which were the rarely collected N. discreta. Initial characterization of genotypes both within a site and on a single tree showed diversity consistent with sexual reproduction of N. discreta. These discoveries fill important gaps in knowledge of the distribution of members of the genus on both large and small spatial scales and provide the framework for future studies in new regions and microhabitats. The overall result is that population biology and genetics now can be combined, placing the genus Neurospora in a unique position to expand its role in experimental biology as a useful model organism for ecology, population genetics and evolution.

Klich, M. A., J. W. Cary, et al. (2003). "Phylogenetic and morphological analysis of Aspergillus ochraceoroseus." Mycologia **95**(6): 1252-1260.

http://www.mycologia.org/cgi/content/abstract/95/6/1252

Aspergillus ochraceoroseus produces the yellow-gold conidia and other characteristics of Aspergillus subgenus Circumdati section Circumdati. However, this species produces aflatoxin, a secondary metabolite characteristic of some members of subgenus Circumdati section Flavi and sterigmatocystin, a related secondary metabolite usually associated with subgenus Nidulantes sections Nidulantes and Versicolores, as well as members of several other genera. Our morphological data support the placement of A. ochraceoroseus in subgenus Circumdati. Sequence data from A. ochraceoroseus aflatoxin and sterigmatocystin genes aflR and nor-1/stcE, as well as 5.8S ITS and beta tubulin genes, were compared to those of aspergilli in sections Circumdati, Flavi, Nidulantes and Versicolores. In the sequence comparisons, A. ochraceoroseus was related more closely to the species in subgenus Nidulantes than to species from subgenus Circumdati.

Moon, C. D., C. O. Miles, et al. (2002). "The evolutionary origins of three new Neotyphodium endophyte species from grasses indigenous to the Southern Hemisphere." Mycologia **94**(4): 694-711.

http://www.mycologia.org/cgi/content/abstract/94/4/694

Members of the genus Neotyphodium are asexual, seedborne, protective fungal endophytes of cool season grasses that have likely evolved either directly from sexual Epichloe; species, or by the interspecific hybridization of distinct lineages of Epichloe; and Neotyphodium. We investigated the evolutionary origins of Neotyphodium endophytes from several grasses that are indigenous to the Southern Hemisphere using a multiple-gene phylogenetic approach. Intron regions of the genes encoding {beta}-tubulin (tub2), translation elongation factor 1-{alpha} (tef1) and actin (act1) were amplified by polymerase chain reaction and sequenced. Phylogenetic analyses of these sequences, aligned with homologous sequences from Epichloe; spp., revealed the evolutionary origins of the Southern Hemisphere endophytes, where one lineage of apparently non-hybrid origin, and three lineages of unique interspecific hybrid origin were identified. On the basis of morphology, host range and evolutionary history, we propose three new species of Neotyphodium. Neotyphodium aotearoae was isolated from Echinopogon ovatus populations from New Zealand and Australia, and comprised a unique, apparently non-hybrid lineage within the Epichloe; species phylogeny. In contrast, an interspecific hybrid lineage was identified from two Australian Ec. ovatus populations, whose ancestry apparently involved lineages closely related to extant E. festucae and an E. typhina genotype similar to that of isolates from Poa pratensis. Endophytes infecting South African Melica racemosa and M. decumbens (dronkgras)

appeared to be hybrids of E. festucae and N. aotearoae or close relatives. The names N. australiense and N. melicicola are proposed for these two hybrid lineages, respectively. The origin of N. tembladerae, an established endophyte species from South American Poa and Festuca spp., was also investigated. Neotyphodium tembladerae appeared to be of hybrid origin, involving E. festucae and an E. typhina genotype similar to that of isolates from Poa nemoralis. The results of this study highlight the widespread occurrence of interspecific hybrid Neotyphodium lineages on a global scale, and the extent of endophyte gene-flow between the Northern and Southern Hemispheres.

Myburg, H., M. Gryzenhout, et al. (2004). "Phylogenetic relationships of Cryphonectria and Endothia species, based on DNA sequence data and morphology." Mycologia **96**(5): 990-1001.

http://www.mycologia.org/cgi/content/abstract/96/5/990

The fungal genera Endothia and Cryphonectria include some of the most important pathogens of forest trees. Despite available new technology, no comprehensive comparative study based on DNA sequence data and morphology has been done on the available isolates representing these two genera. The main objectives of this study were to assess the phylogenetic relationships among species of Cryphonectria and Endothia, for which cultures are available, and to establish a taxonomic framework based on DNA sequence and morphological data, which will aid future studies and identification of species in these and related genera. Comparisons were based on sequence variation found in the ITS region of the ribosomal RNA operon and two regions of the {beta}-tu-bulin gene. In addition, the morphology of these species was examined. The phylogenetic data indicated that Endothia and Cryphonectria reside in two distinct phylogenetic clades. Cryphonectria parasitica, C. macrospora, C. nitschkei, C. eucalypti and C. radicalis represented the Cryphonectria clade. Endothia gyrosa and E. singularis were included in the Endothia clade. An isolate representing E. viridistroma grouped outside the Endothia clade and separately from other groups. Other clades outside the one encompassing Cryphonectria were those represented by the C. cubensis isolates and fungi isolated from Elaeocarpus dentatus originating from New Zealand. These clades could be distinguished from Endothia and Cryphonectria, based on anamorph morphology, stromatal structure and ascospore septation. Cryphonectria and Endothia, therefore, appear to be paraphyletic and taxonomic relationships for these fungi need to be revised.

Poldmaa, K. (2003). "Three species of Hypomyces growing on basidiomata of Stereaceae." Mycologia **95**(5): 921-933.

http://www.mycologia.org/cgi/content/abstract/95/5/921

Of the eight species of Hypomyces that occur on basidiomata of Stereum species, only H. sympodiophorus grows exclusively on members of this genus. Morphologically similar fungi were found on species of Xylobolus, a genus closely related to Stereum. These are described as two new species of Hypomyces: H. thailandicus, collected on Xylobolus cf. illudens in Thailand; and H. xyloboli, on X. frustulatus and X. subpileatus in the eastern United States. These three species are unusual in Hypomyces because of their almost indistinguishable anamorphs. In parsimony analysis of LSU nuclear rDNA sequences, the three species growing only on Stereaceae do not form a monophyletic group but their constrained monophyly is not rejected either. A morphologically similar anamorphic species, Sibirina gamsii, included in the study, is transferred to the genus Cladobotryum.

Powell, A. J., D. J. Jacobson, et al. (2003). "Variation among natural isolates of Neurospora on small spatial scales." Mycologia **95**(5): 809-819.

http://www.mycologia.org/cgi/content/abstract/95/5/809

Although species of Neurospora are among the most studied model organisms in genetics and biochemistry, basic questions remain with respect to their ecology and population biology. In this study, we sought to clarify relationships among individuals over a small spatial scale, toward assessing both local variation and mode of colonization. Isolates of Neurospora were collected after fires in the Florida Everglades (May 1999), where abundant colonies appeared on diverse plants, including grasses and woody shrubs. Colonies were sampled in a linear fashion from two adjacent scorched sugarcane stems at one site and from a burned woody shrub at a distant second site. Species and mating types were assigned based on crossing behavior. Variation at two loci, het-c and frq, was determined by direct sequencing of PCR products. The results demonstrated substantial within- and among-species variation on a small scale, with up to three species and six different haplotypes occurring on a single stem. In total, four species and more than 10 genetically distinct individuals (haplotypes) were present across the three stems, often with multiple individuals occupying the same position. A permutation analysis revealed that individuals were not distributed randomly and that adjacent nodes on cane stems were more likely than chance to be colonized by the same haplotype. This suggests that visible eruptions of conidia on burned plants reflect substantial vegetative mycelial spread through subsurface tissues after primary colonization. Results also revealed that adjacent isolates from a single plant can possess different functional alleles at het-c, an observation meaningful in the context of the proposed role of het-c in self recognition.

Pryor, B. M. and R. L. Gilbertson (2002). "Relationships and taxonomic status of Alternaria radicina, A. carotiincultae, and A. petroselini based upon morphological, biochemical, and molecular characteristics." Mycologia **94**(1): 49-61.

http://www.mycologia.org/cgi/content/abstract/94/1/49

Alternaria radicina, A. carotiincultae, and A. petroselini are closely related pathogens of umbelliferous crops. Relationships among these fungi were determined based on growth rate, spore morphology, cultural characteristics, toxin production, and host range. Random amplified polymorphic DNA (RAPD) analysis of these species, other species of Alternaria, and closely related fungi was also performed. A. petroselini was readily differentiated from A. radicina and A. carotiincultae on the basis of spore morphology, production of microsclerotia, host range, and RAPD analysis. Alternaria radicina and A. carotiincultae were considerably more similar to each other than to A. petroselini, but could be differentiated on the basis of growth rate, spore morphology, colony morphology, and, to a limited extent, RAPD analysis. When grown on media having a high nutritional content, A. radicina produced a diffusible yellow pigment and crystals of the fungal metabolite radicinin. In contrast, A. carotiincultae produced little or no radicinin. However, when A. carotiincultae was grown on the same medium amended with radicinin, growth rate and colony and conidial morphology were more similar to those of A. radicina. These results suggest that the morphological differences between A. radicina and A. carotiincultae are due, at least in part, to radicinin production, and that these fungi are conspecific. Therefore, we propose that A. carotiincultae be considered a synonym of A. radicina.

Schmitt, I., H. T. Lumbsch, et al. (2003). "Phylogeny of the lichen genus Placopsis and its allies based on Bayesian analyses of nuclear and mitochondrial sequences." Mycologia **95**(5): 827-835.

http://www.mycologia.org/cgi/content/abstract/95/5/827

The phylogenetic relationships of the lichen genus Placopsis and related genera in the Agyriales were analyzed using molecular data. We obtained a total of 66 new sequences from the nuclear ITS, LSU and the mitochondrial SSU rDNA. Phylogenetic analyses were conducted in a Bayesian and a maximum-parsimony framework. Our analyses show that Placopsis is paraphyletic with members of Orceolina nesting within the genus. A morphological character supporting the Placopsis-Orceolina clade is the non-amyloid ascus. The section Aspiciliopsis as defined by sunken fruiting bodies is not supported, but the type species of Aspiciliopsis is more closely related to Orceolina. This clade shares apothecia with reduced amphithecia as apomorphic character. We suggest resurrecting the generic name Aspiciliopsis. Trapelia is the sister genus to Placopsis and Aspiciliopsis/Orceolina.

Smith, J. A., R. A. Blanchette, et al. (2004). "Molecular and morphological characterization of the willow rust fungus, Melampsora epitea, from arctic and temperate hosts in North America." Mycologia **96**(6): 1330-1338.

http://www.mycologia.org/cgi/content/abstract/96/6/1330

Current taxonomy places all rust fungi that occur on willow (Salix spp.) in North America in one species complex, Melampsora epitea Thum. Characteristics of M. epitea isolates from the Canadian arctic were compared to M. epitea isolates from temperate regions of North America. Sequences from internal transcribed spacer (ITS) regions of rDNA were obtained from urediniospores from rust-infected Salix leaves collected in the Canadian arctic and in Minnesota and compared. Phylogenetic analysis of nuclear ribosomal ITS regions indicated that arctic M. epitea samples were divergent from temperate M. epitea isolates, perhaps in part because all rusts examined diverged according to host species. Four urediniospore characteristics were examined: area, circularity (shape factor), major axis length and spine density. Statistically significant ($P < 0.05$) differences were observed for spine density among all host species except S. nigra and S. bebbiana. However major axis length differed between these species. These results represent the first evidence that arctic and temperate Melampsora species on Salix hosts in North America have evolved distinct molecular and morphological characters.

van Niekerk, J. M., P. W. Crous, et al. (2004). "DNA phylogeny, morphology and pathogenicity of Botryosphaeria species on grapevines." Mycologia **96**(4): 781-798.

http://www.mycologia.org/cgi/content/abstract/96/4/781

Several species of Botr yosphaeria are known to occur on grapevines, causing a wide range of disorders including bud mortality, dieback, brown wood streaking and bunch rot. In this study the 11 Botryosphaeria spp. associated with grapevines growing in various parts of the world, but primarily in South Africa, are distinguished based on morphology, DNA sequences (ITS-1, 5.8S, ITS-2 and EF1-{alpha}) and pathological data. Botryosphaeria australis, B. lutea, B. obtusa, B. parva, B. rhodina and a Diplodia sp. are confirmed from grapevines in South Africa, while Diplodia porosum, Fusicoccum viticlavatum and F. vitifusiforme are described as new. Although isolates of B. dothidea and B. stevensii are confirmed from grapevines in Portugal, neither of these species occurred in South Africa, nor were any isolates of B. ribis confirmed from grapevines. All grapevine isolates from Portugal, formerly presumed to be B. ribis, are identified as B. parva based on their EF1-{alpha} equence data. From artificial inoculations on grapevine shoots, we conclude that B. australis, B. parva, B. ribis and B. stevensii are more virulent than the other species studied. The Diplodia sp. collected from grapevine canes is morphologically similar

but phylogenetically distinct from D. sarmentorum. Diplodia sarmentorum is confirmed as anamorph of Otthia spiraeae, the type species of the genus Otthia (Botryosphaeriaceae). A culture identified as O. spiraeae clustered within Botryosphaeria and thus is regarded as probable synonym. These findings confirm earlier suggestions that the generic concept of Botryosphaeria should be expanded to include genera with septate ascospores and Diplodia anamorphs.

Nitric Oxide (3)

Andrade, M. A., M. Siles-Lucas, et al. (2004). "Echinococcus multilocularis laminated-layer components and the E14t 14-3-3 recombinant protein decrease NO production by activated rat macrophages in vitro." Nitric Oxide **10**(3): 150.

http://www.sciencedirect.com/science/article/B6WNT-4CBDC8M-1/2/ef492737c8a436451b82cca6449f9180

Echinococcus multilocularis and Echinococcus granulosus cause alveolar and cystic (unilocular) echinococcosis, respectively, in humans and animals. It is known that these parasites can affect, among other molecules, nitric oxide (NO) production by periparasitic host cells. Nevertheless, detailed dissection of parasite components specifically affecting cell NO production has not been done to date. We compare the effect of E. granulosus and E. multilocularis defined metacestode structural (laminated-layer associated) and metabolic (14-3-3 protein, potentially related with E. multilocularis metacestode tumor-like growth) components on the NO production by rat alveolar macrophages in vitro. Our results showed that none of these antigens could stimulate macrophage NO production in vitro. However, a reversed effect of some Echinococcus antigens on NO in vitro production was found when cells were previously exposed to LPS stimulation. This inhibitory effect was found when E. multilocularis laminated-layer (LL) or cyst wall (CW) soluble components from both species were used. Pre-stimulation of cells with LPS also resulted in a strong, dose-dependent reduction of NO and iNOS mRNA production after incubation of cells with the E14t protein. Thus, the E. multilocularis 14-3-3 protein appears to be one of the components accounting for the suppressive effect of the CW and LL metacestode extracts.

Ghilardi, G., M. L. Biondi, et al. (2003). "Vascular invasion in human breast cancer is correlated to T->786C polymorphism of NOS3 gene." Nitric Oxide **9**(2): 118.

http://www.sciencedirect.com/science/article/B6WNT-49RCFTH-2/2/299fc4d5e9636de6128d0125971be938

Background. Nitric oxide (NO) is a free radical known to be a major regulator of vascular tonus, to inhibit cell proliferation, induce apoptosis, and be a mediator of macrophage cytostatic and cytotoxic effects. Recently, NO synthesis has been reported to be elevated in different cancers and is expected to promote metastasis by maintaining a vasodilator tone in blood vessels in and around the tumour. Two different common genetic polymorphisms were found on endothelial NO synthase (NOS3) gene: Glu298Asp on exon 7 and T->786C in the promoter region.Purpose. To evaluate the impact of the NOS3 polymorphisms on vascular invasion and metastasis in breast cancer patients.Design. Two NOS3 gene polymorphisms (Glu298Asp and T->786C) were genotyped in 71 patients operated for breast cancer and followed for 6-30 months (median 21). A control population of 91 age and sex matched tumour-free subjects was also genotyped for the

same polymorphisms.Results. The distribution of both polymorphisms was not different between cases and controls. In patients without vascular invasion, T allele frequency was significantly lower than in patients with vascular invasion (p=0.033). At the end of the follow-up, T allele frequency was found to be less frequent in the metastasis free group than normal population (0.51 vs 0.64; p=0.047).Conclusion. Our results suggest that T allele reduction at the NOS3 promoter region may reduce vascular invasion in breast cancer and consequently reduce metastatic spread and be a favorable prognostic factor. These results need further validation in larger studies.

Zamora, R., Y. Vodovotz, et al. (2002). "A DNA microarray study of nitric oxide-induced genes in mouse hepatocytes: implications for hepatic heme oxygenase-1 expression in ischemia/reperfusion." Nitric Oxide **7**(3): 165.

http://www.sciencedirect.com/science/article/B6WNT-46XHFMS-3/2/a59898196c06b5704ea6f2cc614e3209

Nutrition Research (1)

Nakamura, M., M. Tanaka, et al. (2000). "Association between beta 3-adrenergic receptor polymorphism and a lower reduction in the ratio of visceral fat to subcutaneous fat area during weight loss in Japanese obese women." Nutrition Research **20**(1): 25.

http://www.sciencedirect.com/science/article/B6TB1-3YS2C5R-5/2/a5c77d05b78037df7da63b16db25d7f0

We investigated whether amino acid substitution of tryptophan by arginine at the residue 64 (64 Arg) of beta 3-adrenergic receptor affects on the degree of reduction in the abdominal fat distribution during a 3-month weight reduction program in either pre- or postmenopausal Japanese women. Beta 3-adrenergic receptor gene polymorphism was examined in 90 Japanese obese women by restriction-enzyme cleavage conformation. The visceral and subcutaneous fat area was measured by magnetic resonance imaging. The baseline body mass index, body weight, fat mass and abdominal subcutaneous and total fat area in 15 obese postmenopausal women with a beta 3-adrenergic receptor (64 Arg) were significantly higher than those in 25 postmenopausal obese women with a beta 3-adrenergic receptor (64 Trp/64 Trp). In contrast, no such differences were found in the 50 premenopausal obese women. After a 3-month weight reduction period, the ratio of visceral to subcutaneous fat areas tended to be lower in both pre- and post-menopausal women with a beta 3-adrenergic receptor (64 Arg), but statistically significant in only the premenopausal obese women ($p<0.05$). The absolute changes in visceral fat areas in 5 homozygotes with a beta 3-adrenegic receptor (64 Arg/64 Arg) was significantly smaller than those in 50 obese women with a beta 3-adrenegic receptor (64 Trp/64 Trp). These results thus suggest that an amino acid substitution at residue 64 of beta 3-adrenergic receptor may play an important role in the regulation of fat distribution in Japanese obese women.

Organisms Diversity & Evolution (1)

Giribet, G., G. D. Edgecombe, et al. (2004). "Is Ellipura monophyletic? A combined analysis of basal hexapod relationships with emphasis on the origin of insects." Organisms Diversity & Evolution **4**(4): 319.

http://www.sciencedirect.com/science/article/B7GJ9-4DTKXNN-1/2/b0eead5e3a1faff8900b467269411b8e

Hexapoda includes 33 commonly recognized orders, most of them insects. Ongoing controversy concerns the grouping of Protura and Collembola as a taxon Ellipura, the monophyly of Diplura, a single or multiple origins of entognathy, and the monophyly or paraphyly of the silverfish (Lepidotrichidae and Zygentoma s.s.) with respect to other dicondylous insects. Here we analyze relationships among basal hexapod orders via a cladistic analysis of sequence data for five molecular markers and 189 morphological characters in a simultaneous analysis framework using myriapod and crustacean outgroups. Using a sensitivity analysis approach and testing for stability, the most congruent parameters resolve Tricholepidion as sister group to the remaining Dicondylia, whereas most suboptimal parameter sets group Tricholepidion with Zygentoma. Stable hypotheses include the monophyly of Diplura, and a sister group relationship between Diplura and Protura, contradicting the Ellipura hypothesis. Hexapod monophyly is contradicted by an alliance between Collembola, Crustacea and Ectognatha (i.e., exclusive of Diplura and Protura) in molecular and combined analyses.

Parasitology International (5)

Hatabu, T., Y. Matsumoto, et al. (2002). "The expression system of biologically active canine interleukin-8 in Leishmania promastigotes." Parasitology International **51**(1): 63.

http://www.sciencedirect.com/science/article/B6TB7-44V20RR-1/2/c4240505ef2cd6894961cc16d41d6bca

It has been reported that Leishmania promastigotes have ability to express foreign genes on drug selectable plasmids. To investigate further abilities of the recently described expression vector, P6.5, in the transfection of Leishmania organisms (Chen D-Q, Kolli BK, Yadava N et al. Episomal expression of specific sense and antisense mRNAs in Leishmania amazonensis: modulation of gp63 levels in promastigotes and their infection of macrophages in vitro. Infect Immun 2000;68:80-86), the constructed expression vector, which contains canine interleukin-8 (cIL-8) coding cDNA, was introduced by electroporation to promastigotes of four species of the genus Leishmania: Leishmania amazonensis, L. equatorensis, L. donovani and L. infantum. Extrachromosomal DNAs and total RNAs from the transfected promastigotes were subjected to polymerase chain reaction (PCR) and reverse transcriptase-PCR, respectively, using cIL-8 gene specific primers, and a predicted product of 330 bp was detected. Western blot analysis using a mouse monoclonal antibody raised against cIL-8 demonstrated the successful expression of cIL-8 in the transfectants and culture supernatants. Culture supernatants of the transfected L. amazonensis and L. equatorensis promastigotes showed a high chemotactic activity to both dog and mouse polymorphonuclear leukocytes. These results indicate that Leishmania promastigotes transfected with the expression vector P6.5 containing cIL-8 cDNA are capable of producing

biologically active cIL-8. The Leishmania expression system using the P6.5 vector might be a useful alternative for the production of biologically active recombinant cytokines.

Robinson, B. S., P. T. Monis, et al. (2004). "Detection and significance of the potentially pathogenic amoeboflagellate Naegleria italica in Australia." Parasitology International **53**(1): 23.

http://www.sciencedirect.com/science/article/B6TB7-4BRJH86-3/2/d1a8c12abb30a5a026376dcc12dfd80d

Thermophilic amoeboflagellates in the genus Naegleria include both virulent and benign species. One of the less studied species, N. italica, has not been detected in the environment since the first reports from Italy in the 1980s; its virulence is known only from infection of laboratory mice. Two recent strains from recreational water in Western Australia (AWQC NG960, NG961) were tentatively identified as N. italica from the characteristic mobilities of seven isozymes. Sequences of the 5.8S rRNA gene and its flanking ITS aligned with a 380+bp length of the published sequence for N. italica with 98% identity. Differences from the type strain were confined to ITS2. Shorter alignments (Naegleria species, corresponding to conserved regions of the 5.8S gene and ITS. Unlike the European type strain of N. italica, the Australian isolates failed to infect laboratory mice intranasally, confirming that infectivity of this species is variable and often lower than in N. fowleri.

Safitri, I., A. Jalloh, et al. (2003). "Sequence diversity in the amino-terminal region of the malaria-vaccine candidate serine repeat antigen in natural Plasmodium falciparum populations." Parasitology International **52**(2): 117.

http://www.sciencedirect.com/science/article/B6TB7-47T8SF7-1/2/cf103bd509b085de7321772035787d14

The amino-terminal region of the serine repeat antigen (SERA) of Plasmodium falciparum is a major malaria-vaccine candidate. Variation in this molecule is essentially dimorphic and alleles may be grouped into the types FCR3, K1 and Honduras1. The Honduras1-type is thought to be the product of homologous recombination between FCR3 and K1 alleles. Here we have examined patterns of sequence diversity in exon II of SERA gene, which encodes most of the amino-terminal region of the antigen, in wild P. falciparum isolates from Indonesia (n=60), Myanmar (n=10) and Thailand (n=14). Among the Indonesian isolates the FCR-3 type predominated (56/60), twenty of which we characterized as novel alleles. A new K1-type allele was also found. In Myanmar, however, all isolates displayed K1-type SERA sequences, which included one new allele. The Honduras1-type was not detected in both countries. In contrast, the 14 isolates from Thailand displayed all three allelic types, with one new Honduras1-type and three new K1-type alleles. On examining the global distribution of SERA alleles by combining previously published sequence data with our results, the FCR3-type alleles predominated in Indonesia, Brazil, and Solomon Islands, but were not found in wild isolates from Myanmar and Africa. Brazil was the only area where K1-type alleles were not found. The distribution of Honduras1-type alleles seems to be mostly restricted to parasite populations from Vietnam, Thailand and Africa. In the allelic families FCR3 and K1, most diversity resulted from variation in sequence and number of octamer repeat units and of allotypes encoding the stretch of serine residues. Sequence analysis indicated that both insertions and deletions of repetitive motifs (creating variation within dimorphic allelic families) and homologous recombination between alleles belonging to different allelic families (creating Honduras1-type alleles) play a role in generating new SERA alleles. Since repeat motifs in the amino-terminal region of SERA contain epitopes recognized by parasite-inhibitory antibodies, sequence variation in exon II may represent one of the parasite's immune-evasion strategies.

Sakihama, N., T. Matsuo, et al. (2004). "Relative frequencies of polymorphisms of variation in Block 2 repeats and 5' recombinant types of Plasmodium falciparum msp1 alleles." Parasitology International **53**(1): 59.

http://www.sciencedirect.com/science/article/B6TB7-4BFVSG0-2/2/0f823b2de04a4a987069bb9efc318a66

The mechanisms producing the genetic polymorphism at Plasmodium falciparum merozoite surface antigen-1 locus (pfmsp1) include the insertion and deletion of the different type of dimorphic Block 2 9-nucleotide repeat units as well as the intragenic recombination. To study relative occurrence frequencies of these two distinct mechanisms, we have developed a sensitive PCR strategy to identify both 5' recombinant types and the number of Block 2 repeats from the same sample. This method can specifically detect the target 5' recombinant type (Blocks 2-6) at the sensitivity of 1-4 copies of the pfmsp1. Applying the new method to field isolates from the Solomon Islands enabled us to identify six different 5' recombinant types and variation in Block 2 repeat number in three of them, thus distinguishing 10 different alleles. Distribution of these alleles in local three villages in the study area suggests that frequencies of variation in the number of Block 2 9-bp repeats and recombination events within Blocks 2-6 are mutually independent and the frequency of repeat variation is relatively high as compared to that of recombination events at the pfmsp1 locus in P. falciparum populations from the Solomon Islands.

Stunzenas, V., J. R. Cryan, et al. (2004). "Comparison of rDNA sequences from colchicine treated and untreated sporocysts of Phyllodistomum folium and Bucephalus polymorphus (Digenea)." Parasitology International **53**(3): 223.

http://www.sciencedirect.com/science/article/B6TB7-4CCCP61-3/2/5765c3cfe9f0562838df3c77f36b5883

The most frequently used antimitotic agent in cytogenetic studies is colchicine. We investigated whether the initial treatment of trematodes for karyological analysis with colchicine would have mutagenic or degradational effect on rDNA sequences. Dreissena polymorpha is the intermediate host of Phyllodistomum folium and Bucephalus polymorphus, and the sporocyst stage of these trematode species develop, respectively, in the gills and gonads of this mussel. Sporocysts of P. folium and B. polymorphus were obtained from D. polymorpha collected from waterbodies in Belarus and in Lithuania. 5.8S and 28S rDNA genes, ITS1 and ITS2 of P. folium and B. polymorphus were sequenced and compared, and no nucleotide sequence differences between colchicine treated and untreated trematodes were found. Based on these results, we conclude that colchicine treatment for 3-5 h has no mutagenic or degradational effect on rDNA sequences. During the course of this investigation, two genetically different P. folium samples were noted in Belarus.

Pesticide Biochemistry and Physiology (1)

Zhang, A., J. B. Dunn, et al. (1999). "An Efficient Strategy for Validation of a Point Mutation Associated with Acetylcholinesterase Sensitivity to Azinphosmethyl in Colorado Potato Beetle." Pesticide

Biochemistry and Physiology **65**(1): 25.

http://www.sciencedirect.com/science/article/B6WP8-45FCPYC-R/2/f1116bb4a60412504ab3a05c2ea1cf77

An A to G point mutation that results in a serine to glycine amino acid change (S291G) in the acetylcholinesterase (AChE, EC 3.1.1.7) gene was identified previously as associated with azinphosmethyl resistance in Colorado potato beetle due to target site insensitivity. To efficiently validate the detection process of the S291G mutation and base the DNA diagnostic method on direct determination of nucleic acid sequence, a single-stranded conformational polymorphism (SSCP) protocol and a minisequencing reaction were developed. SSCP protocols using a 163-bp DNA template that spans the mutation resulted in an easy, rapid, cheap, and rugged DNA-based diagnostic method, which was capable of separating azinphosmethyl-susceptible and - resistant beetles. For minisequencing, PCR-amplified and biotinylated DNA templates from both susceptible and resistant beetles, which contain the mutation site, were bound to streptavidin-coated microplate strips. Minisequencing was accomplished with a detection primer that annealed adjacent to the point mutation, digoxigenin-labeled dATP, or alternatively, digoxigenin-labeled dUTP and AmpliTaq polymerase. The sequencing reaction added a digoxigenin-labeled dATP only when matched to the biotinylated DNA template (dATP and 3'.GGTCA.5'). Digoxigenin-labeled DNA was detected using peroxidase-conjugated digoxigenin antibodies and quantitated as optical density (OD) at 450 nm in a microplate reader. The OD readings obtained with digoxigenin-labeled dATP in the presence of susceptible AChE DNA template was 0.319 +/- 0.05, which was significantly higher than that obtained in the presence of the azinphosmethyl-resistant template (0.031 +/- 0.018) ($P < 0.001$). These highly significant results agree well with the susceptibility of AChE from individual insects as judged by AChE inhibition by azinphosmethyl-oxon and further support the contention that A to G point mutation, which occurs only in AChE gene of azinphosmethyl-resistant beetles, is responsible for enzyme insensitivity. Compared with SSCP, the minisequencing reaction provides a direct means to validate this specific point mutation. Coupling minisequencing with the ease and durability of SSCP will allow us to determine the presence or absence of the S291G mutation in an efficient and unambiguous manner. As such, similar approaches could be used to validate point mutations in any resistant strain of insect.

Phytochemistry (7)

Finardi-Filho, F., T. E. Mirkov, et al. (1996). "A putative precursor protein in the evolution of the bean [alpha]-amylase inhibitor." Phytochemistry **43**(1): 57.

http://www.sciencedirect.com/science/article/B6TH7-3V9CVG6-1T/2/53949212e245f1aca75e3f9fe5bc37dc

Seeds of the common bean Phaseolus vulgaris and the tepary bean (P. acutifolius) contain a family of plant defence proteins that includes phytohaemagglutinin (PHA), arcelin and [alpha]-amylase inhibitor ([alpha]AI). These homologous proteins differ by the absence of short loops at the surface of the protein and by the presence of a proteolytic processing site (Asn77) that allows [alpha]AI to be post-translationally cleaved and activated. We now report the derived amino acid sequence of two amylase inhibitor-like (AIL) proteins that are not proteolytically processed, although they have the typical processing site. One protein is from the common bean, and the other from the tepary bean. On a dendrogram, these proteins are grouped with [alpha]AIs rather

than with the arcelins or lectins. [alpha]AI differs from AIL primarily by the deletion of a 15-amino-acid segment from the middle of the AIL sequence. When [alpha]AI is expressed in tobacco, it is proteolytically processed to form an active molecule. However, AIL sequences are not processed. We suggest that the AIL proteins may be an intermediate in the evolution of an active [alpha]AI.

Kreft, S., M. Ravnikar, et al. (1997). "Jasmonic acid inducible aspartic proteinase inhibitors from potato." Phytochemistry **44**(6): 1001.

http://www.sciencedirect.com/science/article/B6TH7-3S9M8WM-1J/2/fd9b343d53e750a11c1e709936fb5d9d

A new cDNA clone coding for an aspartic proteinase inhibitor homologue was isolated from a potato tuber cDNA library. Southern blot analysis was used to study the structural diversity of the aspartic proteinase inhibitor gene family in several species of the Solanaceae. The existence of sequence-homologous genes was confirmed in the genomic DNA of different potato cultivars (Solanum tuberosum L. cv. Desiree, Pentland Squire and Igor), tomato (Lycopersicon esculentum Mill.). aubergine (S. melongena L.) and a wild type of bittersweet (S. dulcamara L.). Northern blot hybridization of total RNA, isolated from leaves under non-stress conditions, of different solanaceous species and of potato tubers showed that the gene transcripts encoding aspartic proteinase inhibitors occur mainly in potato tubers. The presence of several cathepsin D inhibitor isoforms has been detected at the protein level. At least four isoforms were isolated by affinity chromatography on cathepsin D-Sepharose and characterized. Additinally, exogenous treatment of potato plantlets by jasmonic acid (JA) over a wide range of concentrations (0-100 [mu]M) was performed in a stem node culture in vitro. We demonstrated that the expression of aspartic proteinase inhibitor mRNA was drastically induced in potato shoots at concentrations of 50-100 [mu]M JA.

Kumar, A. and B. E. Ellis (2003). "A family of polyketide synthase genes expressed in ripening Rubus fruits." Phytochemistry **62**(3): 513.

http://www.sciencedirect.com/science/article/B6TH7-481DXX0-X/2/4c20bdcd94481d1a39828bc00cf49cc6

Quality traits of raspberry fruits such as aroma and color derive in part from the polyketide derivatives, benzalacetone and dihydrochalcone, respectively. The formation of these metabolites during fruit ripening is the result of the activity of polyketide synthases (PKS), benzalcetone synthase and chalcone synthase (CHS), during fruit development. To gain an understanding of the regulation of these multiple PKSs during fruit ripening, we have characterized the repertoire of Rubus PKS genes and studied their expression patterns during fruit ripening. Using a PCR-based homology search, a family of ten PKS genes (Ripks1-10) sharing 82-98% nucleotide sequence identity was identified in the Rubus idaeus genome. Low stringency screening of a ripening fruit-specific cDNA library, identified three groups of PKS cDNAs. Group 1 and 2 cDNAs were also represented in the PCR amplified products, while group 3 represented a new class of Rubus PKS gene. The Rubus PKS gene-family thus consists of at least eleven members. The three cDNAs exhibit distinct tissue-specific and developmentally regulated patterns of expression. RiPKS5 has high constitutive levels of expression in all organs, including developing flowers and fruits, while RiPKS6 and RiPKS11 expression is consistent with developmental and tissue-specific regulation in various organs. The recombinant proteins encoded by the three RiPKS cDNAs showed a typical CHS-type PKS activity. While phylogenetic analysis placed the three Rubus PKSs in one cluster, suggesting a recent duplication event, their distinct expression patterns suggest that their regulation, and thus function(s), has evolved independently of the structural genes themselves.

McKegney, G. R., S. L. Butland, et al. (1996). "Expression of poplar phenylalanine ammonia-lyase in insect cell cultures." Phytochemistry **41**(5): 1259.

http://www.sciencedirect.com/science/article/B6TH7-3TKMD7F-1B/2/3a2d2dce8cb4583764ab20cd19d40e61

A cDNA encoding one of the phenylalanine ammonia-lyase genes from Populus trichocarpa x deltoides was inserted into a baculovirus expression vector and the PAL protein was successfully expressed in insect cell cultures. High levels of active holoenzyme were obtained that could be purified in a single chromatographic step. Site-directed mutagenesis and expression of the mutant enzyme confirmed that conversion of the putative active site serine202 residue to alanine is sufficient to destroy the catalytic activity of PAL.

Pelt, J. L., W. A. Downes, et al. (2003). "Flavanone 3-hydroxylase expression in Citrus paradisi and Petunia hybrida seedlings." Phytochemistry **64**(2): 435.

http://www.sciencedirect.com/science/article/B6TH7-494C2F2-6/2/c1f4ad19511098aa39ad8149d50aa562

Petunia hybrida and Citrus paradisi have significantly different flavonoid accumulation patterns. Petunia sp. tend to accumulate flavonol glycosides and anthocyanins while Citrus paradisi is known for its accumulation of flavanone diglycosides. One possible point of regulation of flavanone metabolism is flavanone 3-hydroxylase (F3H) expression. To test whether this is a key factor in the different flavanone usage by Petunia hybrida and Citrus paradisi, F3H mRNA expression in seedlings of different developmental stages was measured using semi-quantitative RT-PCR. Primers were designed to conserved regions of F3H and used to amplify an approximately 350 bp segment for quantitation by PhosphorImaging. Primary leaves of 32 day old grapefruit seedlings and a grapefruit flower bud had the highest levels of F3H mRNA expression. Petunia seedlings had much lower levels of F3H mRNA expression relative to grapefruit. The highest expression in petunia was in primary leaves and roots of 65 day old seedlings. These results indicate that preferential use of naringenin for production of high levels of flavanone glycosides in young grapefruit leaves cannot be attributed to decreased F3H mRNA expression.

Prosser, I., A. L. Phillips, et al. (2002). "(+)-(10R)-Germacrene A synthase from goldenrod, Solidago canadensis; cDNA isolation, bacterial expression and functional analysis." Phytochemistry **60**(7): 691.

http://www.sciencedirect.com/science/article/B6TH7-460WG1W-3/2/afbc4fb74a5b9e383f99c6dbe9f1345c

Rogelj, B., T. Popovic, et al. (1998). "Chelidocystatin, a novel phytocystatin from Chelidonium majus." Phytochemistry **49**(6): 1645.

http://www.sciencedirect.com/science/article/B6TH7-44D42G6-W/2/ebe152c446997c65b09d5c0dc4ca76d4

Greater celandine (Chelidonium majus L.) has traditional uses in European and Chinese herbal

medicine. In the plant sap significant inhibitory activity against papain was observed. A cysteine proteinase inhibitor, named chelidocystatin, was isolated from the plant using papain Sepharose affinity chromatography followed by gel filtration and ion-exchange chromatography. Chelidocystatin showed a Mr of 10 000 on SDS-PAGE with the pI of 9.3, and was a strong inhibitor of cathepsin L (Ki=5.6 x 10-11 M), papain (Ki=1.1 x 10-10 M) and cathepsin H (Ki=7.5 x 10-9 M). The complete amino acid sequence of the protein was obtained with N-terminal sequencing and sequencing of the peptides after digestion of the protein. Moreover, a major part of the sequence was verified by molecular cloning. The conserved glycine residue at the N-terminal region and the QVVAG motif, which are both believed to be involved in the inhibitory activity, indicate that it is a member of the cystatin superfamily. The amino acid sequence of chelidocystatin shows a high degree of homology with cysteine proteinase inhibitors belonging to the phytocystatin group, especially with the recently described carrot and sunflower phytocystatins with which it shares 57% and 54% homology, respectively.

Phytomedicine (1)

Seidlova-Wuttke, D., O. Hesse, et al. (2004). "Belamcanda chinensis and the thereof purified tectorigenin have selective estrogen receptor modulator activities." Phytomedicine **11**(5): 392.

http://www.sciencedirect.com/science/article/B7GVW-4CRPN61-4/2/1af9f35083976de9add124cb5144338c

Belamcanda chinensis (BC) belongs to the family of iridaceae and the isoflavone tectorigenin has been isolated from the rhizome of this plant. Whether this isoflavone has estrogenic, possibly selective estrogen receptor modulator activities and if so, whether they are mediated via the estrogen receptor [alpha] or [beta] is unknown at present. Therefore, we performed binding studies with recombinant human ER[alpha] and ER[beta] to show that tectorigenin binds to both receptor subtypes. In ER[alpha]-expressing MCF7 and ER[beta]-expressing MDA-MB231 reporter gene transfected cells tectorigenin causes transactivation. When given intravenously to ovariectomized (ovx) rats, it inhibits pulsatile pituitary LH secretion. In postmenopausal women estrogen-unopposed LH pulses correlate with hot flushes. Therefore, suppression of pulsatile LH secretion may be beneficial in women suffering from hot flushes. Upon chronic application to ovx rats a BC extract containing 5% Belamcanda at a daily dose of 33 mg or 130 mg of the extract had no effect on uterine weight or on estrogen-regulated uterine gene expression while estrogenic effects in the bone, on bone mineral density of the metaphysis of the tibia could be established. Hence, tectorigenin may have antiosteoporotic effects also in postmenopausal women. Immunohistochemical staining of proliferating cell nuclear antigen--a proliferation marker--in the mammary gland did not indicate a mammotrophic effect of the tectorigenin-containing BC extract at both tested doses. In summary, tectorigenin or the B. chinensis extract containing tectorigenin had a strong hypothalamotropic and osteotropic effect but no effect in the uterus or the mammary gland. Therefore, tectorigenin may be in the future a clinically useful selective estrogen receptor modulator.

PLANT CELL (12)

Chakravarthy, S., R. P. Tuori, et al. (2003). "The Tomato Transcription Factor Pti4 Regulates Defense-Related Gene Expression via GCC Box and Non-GCC Box cis Elements." PLANT CELL **15**(12): 3033-3050.

http://www.plantcell.org/cgi/content/abstract/15/12/3033

The tomato transcription factor Pti4, an ethylene-responsive factor (ERF), interacts physically with the disease resistance protein Pto and binds the GCC box cis element that is present in the promoters of many pathogenesis-related (PR) genes. We reported previously that Arabidopsis plants expressing Pti4 constitutively express several GCC box-containing PR genes and show reduced disease symptoms compared with wild-type plants after inoculation with Pseudomonas syringae pv tomato or Erysiphe orontii. To gain insight into how genome-wide gene expression is affected by Pti4, we used serial analysis of gene expression (SAGE) to compare transcripts in wild-type and Pti4-expressing Arabidopsis plants. SAGE provided quantitative measurements of >20,000 transcripts and identified the 50 most highly expressed genes in Arabidopsis vegetative tissues. Comparison of the profiles from wild-type and Pti4-expressing Arabidopsis plants revealed 78 differentially abundant transcripts encoding defense-related proteins, protein kinases, ribosomal proteins, transporters, and two transcription factors (TFs). Many of the genes identified were expressed differentially in wild-type Arabidopsis during infection by Pseudomonas syringae pv tomato, supporting a role for them in defense-related processes. Unexpectedly, the promoters of most Pti4-regulated genes did not have a GCC box. Chromatin immunoprecipitation experiments confirmed that Pti4 binds in vivo to promoters lacking this cis element. Potential binding sites for ERF, MYB, and GBF TFs were present in statistically significantly increased numbers in promoters regulated by Pti4. Thus, Pti4 appears to regulate gene expression directly by binding the GCC box and possibly a non-GCC box element and indirectly by either activating the expression of TF genes or interacting physically with other TFs.

Cheong, Y. H., K.-N. Kim, et al. (2003). "CBL1, a Calcium Sensor That Differentially Regulates Salt, Drought, and Cold Responses in Arabidopsis." PLANT CELL **15**(8): 1833-1845.

http://www.plantcell.org/cgi/content/abstract/15/8/1833

Although calcium is a critical component in the signal transduction pathways that lead to stress gene expression in higher plants, little is known about the molecular mechanism underlying calcium function. It is believed that cellular calcium changes are perceived by sensor molecules, including calcium binding proteins. The calcineurin B-like (CBL) protein family represents a unique group of calcium sensors in plants. A member of the family, CBL1, is highly inducible by multiple stress signals, implicating CBL1 in stress response pathways. When the CBL1 protein level was increased in transgenic Arabidopsis plants, it altered the stress response pathways in these plants. Although drought-induced gene expression was enhanced, gene induction by cold was inhibited. In addition, CBL1-overexpressing plants showed enhanced tolerance to salt and drought but reduced tolerance to freezing. By contrast, cbl1 null mutant plants showed enhanced cold induction and reduced drought induction of stress genes. The mutant plants displayed less tolerance to salt and drought but enhanced tolerance to freezing. These studies suggest that CBL1 functions as a positive regulator of salt and drought responses and a negative regulator of cold response in plants.

Gupta, R., J. T. L. Ting, et al. (2002). "A Tumor Suppressor Homolog, AtPTEN1, Is Essential for Pollen Development in Arabidopsis." PLANT CELL **14**(10): 2495-2507.

http://www.plantcell.org/cgi/content/abstract/14/10/2495

Although it is well known that Tyr phosphatases play a critical role in signal transduction in animal cells, little is understood of the functional significance of Tyr phosphatases in higher plants. Here, we describe the functional analysis of an Arabidopsis gene (AtPTEN1) that encodes a Tyr phosphatase closely related to PTEN, a tumor suppressor in animals. The recombinant AtPTEN1 protein, like its homologs in animals, is an active phosphatase that dephosphorylates phosphotyrosine and phosphatidylinositol substrates. RNA gel blot analysis and examination of promoter-reporter constructs in transgenic Arabidopsis plants revealed that the AtPTEN1 gene is expressed exclusively in pollen grains during the late stage of development. Suppression of AtPTEN1 gene expression by RNA interference caused pollen cell death after mitosis. We conclude that AtPTEN1 is a pollen-specific phosphatase and is essential for pollen development.

Hoffmann, L., S. Besseau, et al. (2004). "Silencing of Hydroxycinnamoyl-Coenzyme A Shikimate/Quinate Hydroxycinnamoyltransferase Affects Phenylpropanoid Biosynthesis." PLANT CELL **16**(6): 1446-1465.

http://www.plantcell.org/cgi/content/abstract/16/6/1446

The hydroxyl group in the 3-position of the phenylpropanoid compounds is introduced at the level of coumarate shikimate/quinate esters, whose synthesis implicates an acyltransferase activity. Specific antibodies raised against the recombinant tobacco (Nicotiana tabacum) acyltransferase revealed the accumulation of the enzyme in stem vascular tissues of tobacco, in accordance with a putative role in lignification. For functional analysis, the acyltransferase gene was silenced in Arabidopsis thaliana and N. benthamiana by RNA-mediated posttranscriptional gene silencing. In Arabidopsis, gene silencing resulted in a dwarf phenotype and changes in lignin composition as indicated by histochemical staining. An in-depth study of silenced N. benthamiana plants by immunological, histochemical, and chemical methods revealed the impact of acyltransferase silencing on soluble phenylpropanoids and lignin content and composition. In particular, a decrease in syringyl units and an increase in p-hydroxyphenyl units were recorded. Enzyme immunolocalization by confocal microscopy showed a correlation between enzyme accumulation levels and lignin composition in vascular cells. These results demonstrate the function of the acyltransferase in phenylpropanoid biosynthesis.

Kim, K.-N., Y. H. Cheong, et al. (2003). "CIPK3, a Calcium Sensor-Associated Protein Kinase That Regulates Abscisic Acid and Cold Signal Transduction in Arabidopsis." PLANT CELL **15**(2): 411-423.

http://www.plantcell.org/cgi/content/abstract/15/2/411

Plants respond to environmental stress by activating "stress genes." The plant hormone abscisic acid (ABA) plays an important role in stress-responsive gene expression. Although Ca2+ serves as a common second messenger in signaling stress and ABA, little is known about the molecular basis of Ca2+ action in these pathways. Here, we show that CIPK3, a Ser/Thr protein kinase that associates with a calcineurin B-like calcium sensor, regulates ABA response during seed germination and ABA- and stress-induced gene expression in Arabidopsis. The expression of the CIPK3 gene itself is responsive to ABA and stress conditions, including cold, high salt, wounding, and drought. Disruption of CIPK3 altered the expression pattern of a number of stress gene markers in response to ABA, cold, and high salt. However, drought-induced gene expression was not altered in the cipk3 mutant plants, suggesting that CIPK3 regulates select pathways in response to abiotic stress and ABA. These results identify CIPK3 as a molecular link between

stress- and ABA-induced calcium signal and gene expression in plant cells. Because the cold signaling pathway is largely independent of endogenous ABA production, CIPK3 represents a cross-talk "node" between the ABA-dependent and ABA-independent pathways in stress responses.

Milioni, D., P.-E. Sado, et al. (2002). "Early Gene Expression Associated with the Commitment and Differentiation of a Plant Tracheary Element Is Revealed by cDNA-Amplified Fragment Length Polymorphism Analysis." PLANT CELL **14**(11): 2813-2824.

http://www.plantcell.org/cgi/content/abstract/14/11/2813

Isolated mesophyll cells from Zinnia elegans are induced by auxin and cytokinin to form tracheary elements (TEs) in vitro with high synchrony. To reveal the changing patterns of gene expression during the 48 h of transdifferentiation from meso-phyll to TE cell fate, we used a cDNA-amplified fragment length polymorphism approach to generate expression profiles of >30,000 cDNA fragments. Transcriptional changes of 652 cDNA fragments were observed, of which 304 have no previously described function or sequence identity. Sixty-eight genes were upregulated within 30 min of induction and represent key candidates for the processes that underlie the early stages of commitment and differentiation to a TE cell fate.

Millar, A. A. and F. Gubler (2005). "The Arabidopsis GAMYB-Like Genes, MYB33 and MYB65, Are MicroRNA-Regulated Genes That Redundantly Facilitate Anther Development." PLANT CELL **17**(3): 705-721.

http://www.plantcell.org/cgi/content/abstract/17/3/705

The functions of the vast majority of genes encoding R2R3 MYB domain proteins remain unknown. The closely related MYB33 and MYB65 genes of Arabidopsis thaliana have high sequence similarity to the barley (Hordeum vulgare) GAMYB gene. T-DNA insertional mutants were isolated for both genes, and a myb33 myb65 double mutant was defective in anther development. In myb33 myb65 anthers, the tapetum undergoes hypertrophy at the pollen mother cell stage, resulting in premeiotic abortion of pollen development. However, myb33 myb65 sterility was conditional, where fertility increased both under higher light or lower temperature conditions. Thus, MYB33/MYB65 facilitate, but are not essential for, anther development. Neither single mutant displayed a phenotype, implying that MYB33 and MYB65 are functionally redundant. Consistent with functional redundancy, promoter-{beta}-glucuronidase (GUS) fusions of MYB33 and MYB65 gave identical expression patterns in flowers (sepals, style, receptacle, anther filaments, and connective but not in anthers themselves), shoot apices, and root tips. By contrast, expression of a MYB33:GUS translational fusion in flowers was solely in young anthers (consistent with the male sterile phenotype), and no staining was seen in shoot meristems or root tips. A microRNA target sequence is present in the MYB genes, and mutating this sequence in the MYB33:GUS fusion results in an expanded expression pattern, in tissues similar to that observed in the promoter-GUS lines, implying that the microRNA target sequence is restricting MYB33 expression. Arabidopsis transformed with MYB33 containing the mutated microRNA target had dramatic pleiotrophic developmental defects, suggesting that restricting MYB33 expression, especially in the shoot apices, is essential for proper plant development.

Palaisa, K. A., M. Morgante, et al. (2003). "Contrasting Effects of Selection on Sequence Diversity and Linkage Disequilibrium at Two Phytoene Synthase Loci." PLANT CELL **15**(8): 1795-1806.

http://www.plantcell.org/cgi/content/abstract/15/8/1795

We investigated the effects of human selection for yellow endosperm color, representing increased carotenoid content, on two maize genes, the Y1 phytoene synthase and PSY2, a putative second phytoene synthase. Multiple polymorphic sites were identified at Y1 and PSY2 in 75 white and yellow maize inbred lines. Many polymorphic sites showed strong association with the endosperm color phenotype at Y1, but no detectable association was found at PSY2. Nucleotide diversity was equivalent for whites and yellows at PSY2 but was 19-fold less in yellows than in whites at Y1, consistent with the white ancestral state of the gene. The strong sequence haplotype conservation within yellows at Y1 and a significant, negative Tajima's D both verified positive selection for yellow endosperm. We propose that two independent gain-of-function events associated with insertions into the promoter of the Y1 gene and upregulation of expression in endosperm have been incorporated into yellow maize.

Rose, J. K. C., K.-S. Ham, et al. (2002). "Molecular Cloning and Characterization of Glucanase Inhibitor Proteins: Coevolution of a Counterdefense Mechanism by Plant Pathogens." PLANT CELL **14**(6): 1329-1345.

http://www.plantcell.org/cgi/content/abstract/14/6/1329

A characteristic plant response to microbial attack is the production of endo-{beta}-1,3-glucanases, which are thought to play an important role in plant defense, either directly, through the degradation of {beta}-1,3/1,6-glucans in the pathogen cell wall, or indirectly, by releasing oligosaccharide elicitors that induce additional plant defenses. We report the sequencing and characterization of a class of proteins, termed glucanase inhibitor proteins (GIPs), that are secreted by the oomycete Phytophthora sojae, a pathogen of soybean, and that specifically inhibit the endoglucanase activity of their plant host. GIPs are homologous with the trypsin class of Ser proteases but are proteolytically nonfunctional because one or more residues of the essential catalytic triad is absent. However, specific structural features are conserved that are characteristic of protein-protein interactions, suggesting a mechanism of action that has not been described previously in plant pathogen studies. We also report the identification of two soybean endoglucanases: EGaseA, which acts as a high-affinity ligand for GIP1; and EGaseB, with which GIP1 does not show any association. In vitro, GIP1 inhibits the EGaseA-mediated release of elicitor-active glucan oligosaccharides from P. sojae cell walls. Furthermore, GIPs and soybean endoglucanases interact in vivo during pathogenesis in soybean roots. GIPs represent a novel counterdefensive weapon used by plant pathogens to suppress a plant defense response and potentially function as important pathogenicity determinants.

Snowden, K. C., A. J. Simkin, et al. (2005). "The Decreased apical dominance1/Petunia hybrida CAROTENOID CLEAVAGE DIOXYGENASE8 Gene Affects Branch Production and Plays a Role in Leaf Senescence, Root Growth, and Flower Development." PLANT CELL **17**(3): 746-759.

http://www.plantcell.org/cgi/content/abstract/17/3/746

Carotenoids and carotenoid cleavage products play an important and integral role in plant development. The Decreased apical dominance1 (Dad1)/PhCCD8 gene of petunia (Petunia hybrida) encodes a hypothetical carotenoid cleavage dioxygenase (CCD) and ortholog of the MORE AXILLARY GROWTH4 (MAX4)/AtCCD8 gene. The dad1-1 mutant allele was inactivated by insertion of an unusual transposon (Dad-one transposon), and the dad1-3 allele is a revertant allele of dad1-1. Consistent with its role in producing a graft-transmissible compound that can alter branching, the Dad1/PhCCD8 gene is expressed in root and shoot tissue. This expression is

upregulated in the stems of the dad1-1, dad2, and dad3 increased branching mutants, indicating feedback regulation of the gene in this tissue. However, this feedback regulation does not affect the root expression of Dad1/PhCCD8. Overexpression of Dad1/PhCCD8 in the dad1-1 mutant complemented the mutant phenotype, and RNA interference in the wild type resulted in an increased branching phenotype. Other differences in phenotype associated with the loss of Dad1/PhCCD8 function included altered timing of axillary meristem development, delayed leaf senescence, smaller flowers, reduced internode length, and reduced root growth. These data indicate that the substrate(s) and/or product(s) of the Dad1/PhCCD8 enzyme are mobile signal molecules with diverse roles in plant development.

Tuteja, J. H., S. J. Clough, et al. (2004). "Tissue-Specific Gene Silencing Mediated by a Naturally Occurring Chalcone Synthase Gene Cluster in Glycine max." PLANT CELL **16**(4): 819-835.

http://www.plantcell.org/cgi/content/abstract/16/4/819

Chalcone synthase, a key regulatory enzyme in the flavonoid pathway, constitutes an eight-member gene family in Glycine max (soybean). Three of the chalcone synthase (CHS) gene family members are arranged as inverted repeats in a 10-kb region, corresponding to the I locus (inhibitor). Spontaneous mutations of a dominant allele (I or ii) to a recessive allele (i) have been shown to delete promoter sequences, paradoxically increasing total CHS transcript levels and resulting in black seed coats. However, it is not known which of the gene family members contribute toward pigmentation and how this locus affects CHS expression in other tissues. We investigated the unusual nature of the I locus using four pairs of isogenic lines differing with respect to alleles of the I locus. RNA gel blots using a generic open reading frame CHS probe detected similar CHS transcript levels in stems, roots, leaves, young pods, and cotyledons of the yellow and black isolines but not in the seed coats, which is consistent with the dominant I and ii alleles mediating CHS gene silencing in a tissue-specific manner. Using real-time RT-PCR, a variable pattern of expression of CHS genes in different tissues was demonstrated. However, increase in pigmentation in the black seed coats was associated with release of the silencing effect specifically on CHS7/CHS8, which occurred at all stages of seed coat development. These expression changes were linked to structural changes taking place at the I locus, shown to encompass a much wider region of at least 27 kb, comprising two identical 10.91-kb stretches of CHS gene duplications. The suppressive effect of this 27-kb I locus in a specific tissue of the G. max plant represents a unique endogenous gene silencing mechanism.

Yang, G., Y.-H. Lee, et al. (2005). "A Two-Edged Role for the Transposable Element Kiddo in the rice ubiquitin2 Promoter." PLANT CELL **17**(5): 1559-1568.

http://www.plantcell.org/cgi/content/abstract/17/5/1559

Miniature inverted repeat transposable elements (MITEs) are thought to be a driving force for genome evolution. Although numerous MITEs are found associated with genes, little is known about their function in gene regulation. Whereas the rice ubiquitin2 (rubq2) promoter in rice (Oryza sativa) line IR24 contains two nested MITEs (Kiddo and MDM1), that in line T309 has lost Kiddo, providing an opportunity to understand the role of MITEs in promoter function. No difference in endogenous rubq2 transcript levels between T309 and IR24 was evident using RT-PCR. However, promoter analysis using both transient and stably transformed calli revealed that Kiddo contributed some 20% of the total expression. Bisulfite genomic sequencing of the rubq2 promoters revealed specific DNA methylation at both symmetric and asymmetric cytosine residues on the MITE sequences, possibly induced by low levels of homologous transcripts. When methylation of the MITEs was blocked by 5-azacytidine treatment, a threefold increase in the endogenous rubq2 transcript level was detected in IR24 compared with that in T309.

Together with the observed MITE methylation pattern, the detection of low levels of transcripts, but not small RNAs, corresponding to Kiddo and MDM1 suggested that RNA-dependent DNA methylation is induced by MITE transcripts. We conclude that, although Kiddo enhances transcription from the rubq2 promoter, this effect is mitigated by sequence-specific epigenetic modification.

Plant Cell Physiol. (9)

Kazama, Y., A. Koizumi, et al. (2005). "Expression of the Floral B-Function Gene SLM2 in Female Flowers of Silene latifolia Infected with the Smut Fungus Microbotryum violaceum." Plant Cell Physiol.: pci080.

http://pcp.oupjournals.org/cgi/content/abstract/pci080v1

Silene latifolia is a dioecious plant in which sex is determined by X and Y chromosomes. Expression of the B-function gene SLM2, an ortholog of PISTILLATA (PI) in Arabidopsis, was examined by in situ hybridization. SLM2 was not expressed in suppressed stamens of female flowers, but was expressed in developing stamens of smut-infected female flowers. These results indicate that the control of SLM2 is independent of the presence of the Y chromosome. Smut-infected females provide a useful system for clarifying the relationship between the B-function gene and the sex determination factor.

Matsunaga, S., W. Uchida, et al. (2004). "Sex-Specific Cell Division during Development of Unisexual Flowers in the Dioecious Plant Silene latifolia." Plant Cell Physiol. **45**(6): 795-802.

http://pcp.oupjournals.org/cgi/content/abstract/45/6/795

We analyzed cell division patterns during the differentiation of unisexual flowers of the dioecious plant Silene latifolia using in situ hybridization with histone H4 and cyclin A1 genes. The gene expression patterns indicated that the activation of cell divisions in whorls 3 and 4 was reversed in young male and female flower buds. During maturation of flower buds, a remarkable reduction in cell division activity occurred in the male gynoecium primordium and female stamen primordia. Our analyses showed that differential activation and reduction of cell division strongly correlated with sex-specific promotion and cessation in the sex differentiation of unisexual flowers.

Matsuzaki, M., M. Abe, et al. (2003). "An Abundant Periplasmic Protein of the Denitrifying Phototroph Rhodobacter sphaeroides f. sp. denitrificans is PstS, a Component of an ABC Phosphate Transport System." Plant Cell Physiol. **44**(2): 212-216.

http://pcp.oupjournals.org/cgi/content/abstract/44/2/212

To understand a physiological role of an abundant 34-kDa periplasmic protein in the denitrifying phototroph Rhodobacter sphaeroides f. sp. denitrificans grown in a medium containing malate as the carbon source, the gene for the protein was isolated. The deduced amino acid sequence of the protein had a sequence similarity of 66.2% to that of PstS from Sinorhizobium meliloti. The

downstream sequence of the Rhodobacter pstS contained five genes similar to pstCAB and phoUB, and its upstream sequence contained a putative regulatory sequence that is analogous to the Pho box involved in phosphate-limitation-induced gene expression in Escherichia coli. Both the amount of the PstS and the pstS promoter-driven expression of lacZ activity increased about two-fold in response to phosphate limitation. This is the first isolation of pst genes encoding proteins of an ABC phosphate transporter system from phototrophic bacteria.

Scutt, C. P., T. Jenkins, et al. (2002). "Male Specific Genes from Dioecious White Campion Identified by Fluorescent Differential Display." Plant Cell Physiol. **43**(5): 563-572.

http://pcp.oupjournals.org/cgi/content/abstract/43/5/563

Fluorescent differential display (FDD) has been used to screen for cDNAs that are differentially up-regulated in male flowers of the dioecious plant Silene latifolia in which an X/Y chromosome system of sex determination operates. To adapt FDD to the cloning of large numbers of differential cDNAs, a novel method of confirming the differential expression of these has been devised. FDD gels were Southern electro-blotted and probed with mixtures of individual cDNA clones derived from different FDD product ligation reactions. These Southern blots were then stripped and re-probed with further mixtures of individual cloned FDD products to identify the maximum number of recombinant clones carrying the true differential amplification products. Of 135 differential bands identified by FDD, 56 differential amplification products were confirmed; these represent 23 unique differentially expressed genes as determined by virtual Northern analysis and two genes expressed at or below the level of detection by virtual Northern analysis. These two low expressed genes show bands of hybridization on genomic Southern blots that are specific to male plants, indicating that they are derived from, or closely related to, Y chromosome genes.

Sonoda, Y., A. Ikeda, et al. (2003). "Distinct Expression and Function of Three Ammonium Transporter Genes (OsAMT1;1 - 1;3) in Rice." Plant Cell Physiol. **44**(7): 726-734.

http://pcp.oupjournals.org/cgi/content/abstract/44/7/726

To study the regulation of ammonium uptake into rice roots, three ammonium transporter genes (OsAMT1;1, 1;2 and 1;3; Oryza sativa ammonium transporter) were isolated and examined. OsAMT1s belong to AMT1 family, containing 11 putative transmembrane-spanning domains. Southern blot analysis and screening of the rice genome database confirmed that with OsAMT1;1-1;3 the complete AMT1 family of rice had been isolated. Heterologous expression of OsAMT1s in the yeast Saccharomyces cerevisiae mutant 31019b showed that all three OsAMT1s exhibit ammonium transport activity. Northern blot analysis showed a distinct expression pattern for the three genes; more constitutive expression in shoots and roots for OsAMT1;1, root-specific and ammonium-inducible expression for OsAMT1;2, and root-specific and nitrogen-derepressible expression for OsAMT1;3. In situ mRNA detection revealed that OsAMT1;2 is expressed in the central cylinder and cell surface of root tips. This gene expression analysis revealed a distinct nitrogen-dependent regulation for AMTs in rice, differing from that in tomato or Arabidopsis.

Sonoda, Y., A. Ikeda, et al. (2003). "Feedback Regulation of the Ammonium Transporter Gene Family AMT1 by Glutamine in Rice." Plant Cell Physiol. **44**(12): 1396-1402.

http://pcp.oupjournals.org/cgi/content/abstract/44/12/1396

The three members of the rice OsAMT1 gene family of ammonium transporters show distinct expression patterns; constitutive and ammonium-promoted expression in shoots and roots for OsAMT1;1; root-specific and ammonium-inducible expression for OsAMT1;2; root-specific and nitrogen-repressible expression for OsAMT1;3 [Sonoda et al. (2003), Plant Cell Physiol. 44: 726]. To clarify the feedback mechanisms, and to identify regulatory factors of the OsAMT1 genes, the accumulation of the three mRNAs and its dependence on endogenous nitrogen compounds (as quantified by capillary electrophoresis) was studied. Ammonium application to roots following a period of nitrogen starvation induced accumulation of OsAMT1;1 and OsAMT1;2 mRNA, but a decrease of OsAMT1;3 mRNA levels. The expression patterns of the three genes showed good correlation (positive in OsAMT1;1 and OsAMT1;2, negative in OsAMT1;3) with the root tissue contents of glutamine but not of ammonium. The ammonium effects on OsAMT1 expression were prevented by methionine sulfoximine, an inhibitor of glutamine synthetase. Moreover, glutamine had the same effect on transcriptional regulation of OsAMT1 genes as ammonium, indicating that glutamine rather than ammonium controls the expression of ammonium transporter genes in rice. These results imply that rice possesses unique mechanisms of adaptation to variable nitrogen sources in the soil.

Sookmark, U., V. Pujade-Renaud, et al. (2002). "Characterization of Polypeptides Accumulated in the Latex Cytosol of Rubber Trees Affected by the Tapping Panel Dryness Syndrome." Plant Cell Physiol. **43**(11): 1323-1333.

http://pcp.oupjournals.org/cgi/content/abstract/43/11/1323

The tapping panel dryness (TPD) syndrome of rubber is characterized by the reduction or ultimately total cessation of latex flow upon tapping, due to physiological disorders in the bark tissue. The protein pattern in the cytoplasm from healthy and TPD tree latex cells was compared by electrophoresis. Two polypeptides (P15 and P22) of 15 and 22 kDa, respectively, were found to accumulate in the cytosol of the TPD-affected trees, whereas a 29 kDa polypeptide (P29) appeared de novo. P15 and P22 were identified as REF (Hev b1) and SRPP (Hev b3), respectively, two proteins proposed to be involved in rubber biosynthesis. P29 appeared to be a new member of the patatin-like protein family. Specific molecular probes were designed for a detailed characterization of REF and SRPP gene expression and RFLP mapping. This allowed the demonstration that REF and SRPP display very similar expression profiles. They are highly over-expressed by the tapping-induced metabolic activation, although not by wounding per se, or ethylene or ABA. In addition to this similarity in gene expression, they were found to share one common locus in the genome. No significant difference in REF and SRPP gene expression was observed between healthy and TPD trees, indicating that their TPD-related accumulation in the cytosol was not transcriptionally regulated. Western blot analysis demonstrated that osmotic lysis of the sedimentable organelles (lutoids) in vitro caused the release of REF and SRPP from the rubber particle membrane into the cytosol. A mechanism of cellular delocalization as a consequence of the lutoids instability is proposed to explain REF and SRPP accumulation in the cytosol of TPD trees.

Takahama, K., M. Matsuoka, et al. (2004). "High-Frequency Gene Replacement in Cyanobacteria Using a Heterologous rps12 Gene." Plant Cell Physiol. **45**(3): 333-339.

http://pcp.oupjournals.org/cgi/content/abstract/45/3/333

Multiple targeted gene replacements are often required for functional analyses of cyanobacterial genomes. For this purpose, we previously devised a simple genetic method, termed rps12-mediated gene replacement, in a cyanobacterium Synechococcus elongatus PCC 7942 for construction of mutants free from drug resistance markers. Here, we improved the method by

employing a heterologous rps12 gene encoding a ribosomal protein S12 from Synechocystis sp. PCC 6803. Dominant streptomycin-sensitive phenotype of the Synechocystis rps12 gene was manifested only when it was expressed under the strong promoter of psbAI gene in S. elongatus PCC 7942 bearing a streptomycin-resistant rps12 allele. Transformation of the rps12 heteroallelic strains with non-replicating template plasmids permitted the selection of recombinants with gene replacement at frequencies up to 50% among streptomycin-resistant progeny.

Tezuka, T., K. Onosato, et al. (2004). "Development of Q-chromosome-specific DNA Markers in Tobacco and Their Use for Identification of a Tobacco Monosomic Line." Plant Cell Physiol. **45**(12): 1863-1869.

http://pcp.oupjournals.org/cgi/content/abstract/45/12/1863

We developed seven Q-chromosome-specific DNA markers in Nicotiana tabacum by random amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR) analysis using two hybrid lines, and we were able to identify tobacco monosomic plants among F1 progeny derived from the cross N. tabacum Haplo-Q x N. tabacum cv. Samsun NN using Q-chromosome-specific DNA markers. Based on the results, we discuss the roles of the Q chromosome in embryo sac development and embryogenesis. Here, we propose a new method for identifying DNA markers for a particular chromosome in the genus Nicotiana.

Plant Physiology (26)

Ayre, B. G., F. Keller, et al. (2003). "Symplastic Continuity between Companion Cells and the Translocation Stream: Long-Distance Transport Is Controlled by Retention and Retrieval Mechanisms in the Phloem." Plant Physiology **131**(4): 1518-1528.

http://www.plantphysiol.org/cgi/content/abstract/131/4/1518

Substantial symplastic continuity appears to exist between companion cells (CCs) and sieve elements of the phloem, which suggests that small solutes within the CC are subject to indiscriminate long-distance transport via the translocation stream. To test this hypothesis, the distributions of exotic and endogenous solutes synthesized in the CCs of minor veins were studied. Octopine, a charged molecule derived from arginine and pyruvate, was efficiently transported through the phloem but was also transferred in substantial amounts to the apoplast, and presumably other non-phloem compartments. The disaccharide galactinol also accumulated in non-phloem compartments, but long-distance transport was limited. Conversely, sucrose, raffinose, and especially stachyose demonstrated reduced accumulation and efficient transport out of the leaf. We conclude that small metabolites in the cytosol of CCs do enter the translocation stream indiscriminately but are also subject to distributive forces, such as nonselective and carrier-mediated membrane transport and symplastic dispersal, that may effectively clear a compound from the phloem or retain it for long-distance transport. A model is proposed in which the transport of oligosaccharides is an adaptive strategy to improve photoassimilate retention, and consequently translocation efficiency, in the phloem.

Bago, B., P. E. Pfeffer, et al. (2003). "Carbon Export from Arbuscular Mycorrhizal Roots Involves the Translocation of Carbohydrate as well as Lipid." Plant Physiology **131**(3): 1496-1507.

http://www.plantphysiol.org/cgi/content/abstract/131/3/1496

Arbuscular mycorrhizal (AM) fungi take up photosynthetically fixed carbon from plant roots and translocate it to their external mycelium. Previous experiments have shown that fungal lipid synthesized from carbohydrate in the root is one form of exported carbon. In this study, an analysis of the labeling in storage and structural carbohydrates after 13C1 glucose was provided to AM roots shows that this is not the only pathway for the flow of carbon from the intraradical to the extraradical mycelium (ERM). Labeling patterns in glycogen, chitin, and trehalose during the development of the symbiosis are consistent with a significant flux of exported glycogen. The identification, among expressed genes, of putative sequences for glycogen synthase, glycogen branching enzyme, chitin synthase, and for the first enzyme in chitin synthesis (glutamine fructose-6-phosphate aminotransferase) is reported. The results of quantifying glycogen synthase gene expression within mycorrhizal roots, germinating spores, and ERM are consistent with labeling observations using 13C-labeled acetate and glycerol, both of which indicate that glycogen is synthesized by the fungus in germinating spores and during symbiosis. Implications of the labeling analyses and gene sequences for the regulation of carbohydrate metabolism are discussed, and a 4-fold role for glycogen in the AM symbiosis is proposed: sequestration of hexose taken from the host, long-term storage in spores, translocation from intraradical mycelium to ERM, and buffering of intracellular hexose levels throughout the life cycle.

Bago, B., W. Zipfel, et al. (2002). "Translocation and Utilization of Fungal Storage Lipid in the Arbuscular Mycorrhizal Symbiosis." Plant Physiology **128**(1): 108-124.

http://www.plantphysiol.org/cgi/content/abstract/128/1/108

The arbuscular mycorrhizal (AM) symbiosis is responsible for huge fluxes of photosynthetically fixed carbon from plants to the soil. Carbon is transferred from the plant to the fungus as hexose, but the main form of carbon stored by the mycobiont at all stages of its life cycle is triacylglycerol. Previous isotopic labeling experiments showed that the fungus exports this storage lipid from the intraradical mycelium (IRM) to the extraradical mycelium (ERM). Here, in vivo multiphoton microscopy was used to observe the movement of lipid bodies through the fungal colony and to determine their sizes, distribution, and velocities. The distribution of lipid bodies along fungal hyphae suggests that they are progressively consumed as they move toward growing tips. We report the isolation and measurements of expression of an AM fungal expressed sequence tag that encodes a putative acyl-coenzyme A dehydrogenase; its deduced amino acid sequence suggests that it may function in the anabolic flux of carbon from lipid to carbohydrate. Time-lapse image sequences show lipid bodies moving in both directions along hyphae and nuclear magnetic resonance analysis of labeling patterns after supplying 13C-labeled glycerol to either extraradical hyphae or colonized roots shows that there is indeed significant bidirectional translocation between IRM and ERM. We conclude that large amounts of lipid are translocated within the AM fungal colony and that, whereas net movement is from the IRM to the ERM, there is also substantial recirculation throughout the fungus.

Busov, V. B., R. Meilan, et al. (2003). "Activation Tagging of a Dominant Gibberellin Catabolism Gene (GA 2-oxidase) from Poplar That Regulates Tree Stature." Plant Physiology **132**(3): 1283-1291.

http://www.plantphysiol.org/cgi/content/abstract/132/3/1283

We identified a dwarf transgenic hybrid poplar (Populus tremula x Populus alba) after screening of 627 independent activation-tagged transgenic lines in tissue culture, greenhouse, and field environments. The cause of the phenotype was a hyperactivated gene encoding GA 2-oxidase (GA2ox), the major gibberellin (GA) catabolic enzyme in plants. The mutation resulted from insertion of a strong transcriptional enhancer near the transcription start site. Overexpression of the poplar GA2ox gene (PtaGA2ox1) caused hyperaccumulation of mRNA transcripts, quantitative shifts in the spectrum of GAs, and similarity in phenotype to transgenic poplars that overexpress a bean (Phaseolus coccineus) GA2ox gene. The poplar PtaGA2ox1 sequence was most closely related to PsGA2ox2 from pea (Pisum sativum) and two poorly known GA2oxs from Arabidopsis (AtGA2ox4 and AtGA2ox5). The dwarf phenotype was reversible through gibberellic acid application to the shoot apex. Transgenic approaches to producing semidwarf trees for use in arboriculture, horticulture, and forestry could have significant economic and environmental benefits, including altered fiber and fruit production, greater ease of management, and reduced risk of spread in wild populations.

Choi, D.-W., E. M. Rodriguez, et al. (2002). "Barley Cbf3 Gene Identification, Expression Pattern, and Map Location." Plant Physiology **129**(4): 1781-1787.

http://www.plantphysiol.org/cgi/content/abstract/129/4/1781

Although cold and drought adaptation in cereals and other plants involve the induction of a large number of genes, inheritance studies in Triticeae (wheat [Triticum aestivum], barley [Hordeum vulgare], and rye [Secale cereale]) have revealed only a few major loci for frost or drought tolerance that are consistent across multiple genetic backgrounds and environments. One might imagine that these loci could encode highly conserved regulatory factors that have global effects on gene expression; therefore, genes encoding central regulators identified in other plants might be orthologs of these Triticeae stress tolerance genes. The CBF/DREB1 regulators, identified originally in Arabidopsis as key components of cold and drought regulation, merit this consideration. We constructed barley cDNA libraries, screened these libraries and a barley bacterial artificial chromosome library using rice (Oryza sativa) and barley Cbf probes, found orthologs of Arabidopsis CBF/DREB1 genes, and examined the expression and genetic map location of the barley Cbf3 gene, HvCbf3. HvCbf3 was induced by a chilling treatment. HvCbf3 is located on barley chromosome 5H between markers WG364b and saflp58 on the barley cv Dicktoo x barley cv Morex genetic linkage map. This position is some 40 to 50 cM proximal to the winter hardiness quantitative trait locus that includes the Vrn-1H gene, but may coincide with the wheat 5A Rcg1 locus, which governs the threshold temperature at which cor genes are induced. From this, it remains possible that HvCbf3 is the basis of a minor quantitative trait locus in some genetic backgrounds, though that possibility remains to be thoroughly explored.

D'Ovidio, R., A. Raiola, et al. (2004). "Characterization of the Complex Locus of Bean Encoding Polygalacturonase-Inhibiting Proteins Reveals Subfunctionalization for Defense against Fungi and Insects." Plant Physiology **135**(4): 2424-2435.

http://www.plantphysiol.org/cgi/content/abstract/135/4/2424

Polygalacturonase-inhibiting proteins (PGIPs) are extracellular plant inhibitors of fungal endopolygalacturonases (PGs) that belong to the superfamily of Leu-rich repeat proteins. We have characterized the full complement of pgip genes in the bean (Phaseolus vulgaris) genotype BAT93. This comprises four clustered members that span a 50-kb region and, based on their similarity, form two pairs (Pvpgip1/Pvpgip2 and Pvpgip3/Pvpgip4). Characterization of the encoded products revealed both partial redundancy and subfunctionalization against fungal-derived PGs. Notably, the pair PvPGIP3/PvPGIP4 also inhibited PGs of two mirid bugs (Lygus

rugulipennis and Adelphocoris lineolatus). Characterization of Pvpgip genes of Pinto bean showed variations limited to single synonymous substitutions or small deletions. A three-amino acid deletion encompassing a residue previously identified as crucial for recognition of PG of Fusarium moniliforme was responsible for the inability of BAT93 PvPGIP2 to inhibit this enzyme. Consistent with the large variations observed in the promoter sequences, reverse transcription-PCR expression analysis revealed that the different family members differentially respond to elicitors, wounding, and salicylic acid. We conclude that both biochemical and regulatory redundancy and subfunctionalization of pgip genes are important for the adaptation of plants to pathogenic fungi and phytophagous insects.

Davis, E. M., K. L. Ringer, et al. (2005). "Monoterpene Metabolism. Cloning, Expression, and Characterization of Menthone Reductases from Peppermint." Plant Physiology **137**(3): 873-881.

http://www.plantphysiol.org/cgi/content/abstract/137/3/873

(-)-Menthone is the predominant monoterpene produced in the essential oil of maturing peppermint (Mentha x piperita) leaves during the filling of epidermal oil glands. This early biosynthetic process is followed by a second, later oil maturation program (approximately coincident with flower initiation) in which the C3-carbonyl of menthone is reduced to yield (-)-(3R)-menthol and (+)-(3S)-neomenthol by two distinct NADPH-dependent ketoreductases. An activity-based in situ screen, by expression in Escherichia coli of 23 putative redox enzymes from an immature peppermint oil gland expressed sequence tag library, was used to isolate a cDNA encoding the latter menthone:(+)-(3S)-neomenthol reductase. Reverse transcription-PCR amplification and RACE were used to acquire the former menthone:(-)-(3R)-menthol reductase directly from mRNA isolated from the oil gland secretory cells of mature leaves. The deduced amino acid sequences of these two reductases share 73% identity, provide no apparent subcellular targeting information, and predict inclusion in the short-chain dehydrogenase/reductase family of enzymes. The menthone:(+)-(3S)-neomenthol reductase cDNA encodes a 35,722-D protein, and the recombinant enzyme yields 94% (+)-(3S)-neomenthol and 6% (-)-(3R)-menthol from (-)-menthone as substrate, and 86% (+)-(3S)-isomenthol and 14% (+)-(3R)-neoisomenthol from (+)-isomenthone as substrate, has a pH optimum of 9.3, and Km values of 674 {micro}M, > 1 mM, and 10 {micro}M for menthone, isomenthone, and NADPH, respectively, with a kcat of 0.06 s-1. The recombinant menthone:(-)-(3R)-menthol reductase has a deduced size of 34,070 D and converts (-)-menthone to 95% (-)-(3R)-menthol and 5% (+)-(3S)-neomenthol, and (+)-isomenthone to 87% (+)-(3R)-neoisomenthol and 13% (+)-(3S)-isomenthol, displays optimum activity at neutral pH, and has Km values of 3.0 {micro}M, 41 {micro}M, and 0.12 {micro}M for menthone, isomenthone, and NADPH, respectively, with a kcat of 0.6 s-1. The respective activities of these menthone reductases account for all of the menthol isomers found in the essential oil of peppermint. Biotechnological exploitation of these genes could lead to improved production yields of (-)-menthol, the principal and characteristic flavor component of peppermint.

Dias, A. P., E. L. Braun, et al. (2003). "Recently Duplicated Maize R2R3 Myb Genes Provide Evidence for Distinct Mechanisms of Evolutionary Divergence after Duplication." Plant Physiology **131**(2): 610-620.

http://www.plantphysiol.org/cgi/content/abstract/131/2/610

R2R3 Myb genes are widely distributed in the higher plants and comprise one of the largest known families of regulatory proteins. Here, we provide an evolutionary framework that helps explain the origin of the plant-specific R2R3 Myb genes from widely distributed R1R2R3 Myb genes, through a series of well-established steps. To understand the routes of sequence

divergence that followed Myb gene duplication, we supplemented the information available on recently duplicated maize (Zea mays) R2R3 Myb genes (C1/Pl1 and P1/P2) by cloning and characterizing ZmMyb-IF35 and ZmMyb-IF25. These two genes correspond to the recently expanded P-to-A group of maize R2R3 Myb genes. Although the origins of C1/Pl1 and ZmMyb-IF35/ZmMyb-IF25 are associated with the segmental allotetraploid origin of the maize genome, other gene duplication events also shaped the P-to-A clade. Our analyses indicate that some recently duplicated Myb gene pairs display substantial differences in the numbers of synonymous substitutions that have accumulated in the conserved MYB domain and the divergent C-terminal regions. Thus, differences in the accumulation of substitutions during evolution can explain in part the rapid divergence of C-terminal regions for these proteins in some cases. Contrary to previous studies, we show that the divergent C termini of these R2R3 MYB proteins are subject to purifying selection. Our results provide an in-depth analysis of the sequence divergence for some recently duplicated R2R3 Myb genes, yielding important information on general patterns of evolution for this large family of plant regulatory genes.

Downie, B., S. Gurusinghe, et al. (2003). "Expression of a GALACTINOL SYNTHASE Gene in Tomato Seeds Is Up-Regulated before Maturation Desiccation and Again after Imbibition whenever Radicle Protrusion Is Prevented." Plant Physiology **131**(3): 1347-1359.

http://www.plantphysiol.org/cgi/content/abstract/131/3/1347

Raffinose family oligosaccharides (RFOs) have been implicated in mitigating the effects of environmental stresses on plants. In seeds, proposed roles for RFOs include protecting cellular integrity during desiccation and/or imbibition, extending longevity in the dehydrated state, and providing substrates for energy generation during germination. A gene encoding galactinol synthase (GOLS), the first committed enzyme in the biosynthesis of RFOs, was cloned from tomato (Lycopersicon esculentum Mill. cv Moneymaker) seeds, and its expression was characterized in tomato seeds and seedlings. GOLS (LeGOLS-1) mRNA accumulated in developing tomato seeds concomitant with maximum dry weight deposition and the acquisition of desiccation tolerance. LeGOLS-1 mRNA was present in mature, desiccated seeds but declined within 8 h of imbibition in wild-type seeds. However, LeGOLS-1 mRNA accumulated again in imbibed seeds prevented from completing germination by dormancy or water deficit. Gibberellin-deficient (gib-1) seeds maintained LeGOLS-1 mRNA amounts after imbibition unless supplied with gibberellin, whereas abscisic acid (ABA) did not prevent the loss of LeGOLS-1 mRNA from wild-type seeds. The presence of LeGOLS-1 mRNA in ABA-deficient (sitiens) tomato seeds indicated that wild-type amounts of ABA are not necessary for its accumulation during seed development. In all cases, LeGOLS-1 mRNA was most prevalent in the radicle tip. LeGOLS-1 mRNA accumulation was induced by dehydration but not by cold in germinating seeds, whereas both stresses induced LeGOLS-1 mRNA accumulation in seedling leaves. The physiological implications of LeGOLS-1 expression patterns in seeds and leaves are discussed in light of the hypothesized role of RFOs in plant stress tolerance.

Johnson, P. E., N. J. Patron, et al. (2003). "A Low-Starch Barley Mutant, Riso 16, Lacking the Cytosolic Small Subunit of ADP-Glucose Pyrophosphorylase, Reveals the Importance of the Cytosolic Isoform and the Identity of the Plastidial Small Subunit." Plant Physiology **131**(2): 684-696.

http://www.plantphysiol.org/cgi/content/abstract/131/2/684

To provide information on the roles of the different forms of ADP-glucose pyrophosphorylase (AGPase) in barley (Hordeum vulgare) endosperm and the nature of the genes encoding their subunits, a mutant of barley, Riso 16, lacking cytosolic AGPase activity in the endosperm was identified. The mutation specifically abolishes the small subunit of the cytosolic AGPase and is

attributable to a large deletion within the coding region of a previously characterized small subunit gene that we have called Hv.AGP.S.1. The plastidial AGPase activity in the mutant is unaffected. This shows that the cytosolic and plastidial small subunits of AGPase are encoded by separate genes. We purified the plastidial AGPase protein and, using amino acid sequence information, we identified the novel small subunit gene that encodes this protein. Studies of the Riso 16 mutant revealed the following. First, the reduced starch content of the mutant showed that a cytosolic AGPase is required to achieve the normal rate of starch synthesis. Second, the mutant makes both A- and B-type starch granules, showing that the cytosolic AGPase is not necessary for the synthesis of these two granule types. Third, analysis of the phylogenetic relationships between the various small subunit proteins both within and between species, suggest that the cytosolic AGPase single small subunit gene probably evolved from a leaf single small subunit gene.

Kovalchuk, I., V. Abramov, et al. (2004). "Molecular Aspects of Plant Adaptation to Life in the Chernobyl Zone." Plant Physiology **135**(1): 357-363.

http://www.plantphysiol.org/cgi/content/abstract/135/1/357

With each passing year since the Chernobyl accident of 1986, more questions arise about the potential for organisms to adapt to radiation exposure. Often this is thought to be attributed to somatic and germline mutation rates in various organisms. We analyzed the adaptability of native Arabidopsis plants collected from areas with different levels of contamination around the Chernobyl nuclear power plant from 1986 to 1992. Notably, progeny of Chernobyl plants resisted higher concentrations of the mutagens Rose Bengal and methyl methane sulfonate. We analyzed the possible molecular mechanisms of their resistance to mutagens and found a more than 10-fold lower frequency of extrachromosomal homologous recombination, significant differences in the expression of radical scavenging (CAT1 and FSD3) and DNA-repair (RAD1 and RAD51-like) genes upon exposure to mutagens (Rose Bengal and x-rays), and a higher level of global genome methylation. This data suggests that adaptation to ionizing radiation is a complex process involving epigenetic regulation of gene expression and genome stabilization that improves plants' resistance to environmental mutagens.

Liu, J., B. Cong, et al. (2003). "Generation and Analysis of an Artificial Gene Dosage Series in Tomato to Study the Mechanisms by Which the Cloned Quantitative Trait Locus fw2.2 Controls Fruit Size." Plant Physiology **132**(1): 292-299.

http://www.plantphysiol.org/cgi/content/abstract/132/1/292

It has been proposed that fw2.2 encodes a negative fruit-growth regulator that underlies natural fruit-size variation in tomato (Lycopersicon spp.) via heterochronic allelic variation of fw2.2 expression, rather than by variation in the structural protein itself. To further test the negative regulator and the transcriptional control hypotheses, a gene dosage series was constructed, which produced a wider range of fw2.2 transcript accumulation than can be found in natural tomato populations. Fruit developmental analyses revealed that fw2.2 transcript levels were highly correlated (negatively) with fruit mass, supporting the negative regulator and transcriptional regulation hypotheses. Further, the effect of fw2.2 on fruit mass was mediated by repressing three- and two-dimensional cell division in placental and pericarp tissues, respectively. Finally, fw2.2 had little effect on fertility and seed size/number, indicating that fruit size effects of fw2.2 are due largely to expression in the maternal tissues of developing fruit and not mediated through fertility or seed-setting-related processes.

Madlung, A., R. W. Masuelli, et al. (2002). "Remodeling of DNA Methylation and Phenotypic and Transcriptional Changes in Synthetic Arabidopsis Allotetraploids." Plant Physiology **129**(2): 733-746.

http://www.plantphysiol.org/cgi/content/abstract/129/2/733

The joining of different genomes in allotetraploids played a major role in plant evolution, but the molecular implications of this event are poorly understood. In synthetic allotetraploids of Arabidopsis and Cardaminopsis arenosa, we previously demonstrated the occurrence of frequent gene silencing. To explore the involvement of epigenetic phenomena, we investigated the occurrence and effects of DNA methylation changes. Changes in DNA methylation patterns were more frequent in synthetic allotetraploids than in the parents. Treatment with 5-aza-2'-deoxycytidine, an inhibitor of DNA methyltransferase, resulted in the development of altered morphologies in the synthetic allotetraploids, but not in the parents. We profiled mRNAs in control and 5-aza-2'-deoxycytidine-treated parents and allotetraploids by amplified fragment length polymorphism-cDNA. We show that DNA demethylation induced and repressed two different transcriptomes. Our results are consistent with the hypothesis that synthetic allotetraploids have compromised mechanisms of epigenetic gene regulation.

Mena, M., F. J. Cejudo, et al. (2002). "A Role for the DOF Transcription Factor BPBF in the Regulation of Gibberellin-Responsive Genes in Barley Aleurone." Plant Physiology **130**(1): 111-119.

http://www.plantphysiol.org/cgi/content/abstract/130/1/111

Functional analyses of a number of hydrolase gene promoters, induced by gibberellin (GA) in aleurone cells following germination, have identified a GA-responsive complex as a tripartite element containing a pyrimidine box motif 5'-CCTTTT-3'. We describe here that BPBF, a barley (Hordeum vulgare) transcription factor of the DOF (DNA-Binding with One Finger) class, previously shown to be an activator of reserve protein encoding genes during development, also has a role in the control of hydrolase genes following seed germination. Northern-blot, reverse transcriptase-polymerase chain reaction, and in situ hybridization analyses evidenced that the transcripts of the BPBF-encoding gene (Pbf), besides being present during endosperm development, are also expressed in aleurone cells of germinated seeds where they are induced by GA, an effect counteracted by abscisic acid. Electrophoretic mobility shift assays have shown that the BPBF protein binds specifically to the pyrimidine box motif in vitro within the different sequence contexts that naturally occur in the promoters of genes encoding a cathepsin B-like protease (Al21) and a low-isoelectric point [alpha]-amylase (Amy2/32b), both induced in the aleurone layers in response to GA. In transient expression experiments, BPBF repressed transcription of the Al21 promoter in GA-treated barley aleurone layers and reverted the GAMYB-mediated activation of this protease promoter.

Morishige, D. T., K. L. Childs, et al. (2002). "Targeted Analysis of Orthologous Phytochrome A Regions of the Sorghum, Maize, and Rice Genomes using Comparative Gene-Island Sequencing." Plant Physiology **130**(4): 1614-1625.

http://www.plantphysiol.org/cgi/content/abstract/130/4/1614

A "gene-island" sequencing strategy has been developed that expedites the targeted acquisition of orthologous gene sequences from related species for comparative genome analysis. A 152-kb bacterial artificial chromosome (BAC) clone from sorghum (Sorghum bicolor) encoding phytochrome A (PHYA) was fully sequenced, revealing 16 open reading frames with a gene

density similar to many regions of the rice (Oryza sativa) genome. The sequences of genes in the orthologous region of the maize (Zea mays) and rice genomes were obtained using the gene-island sequencing method. BAC clones containing the orthologous maize and rice PHYA genes were identified, sheared, subcloned, and probed with the sorghum PHYA-containing BAC DNA. Sequence analysis revealed that approximately 75% of the cross-hybridizing subclones contained sequences orthologous to those within the sorghum PHYA BAC and less than 25% contained repetitive and/or BAC vector DNA sequences. The complete sequence of four genes, including up to 1 kb of their promoter regions, was identified in the maize PHYA BAC. Nine orthologous gene sequences were identified in the rice PHYA BAC. Sequence comparison of the orthologous sorghum and maize genes aided in the identification of exons and conserved regulatory sequences flanking each open reading frame. Within genomic regions where micro-colinearity of genes is absolutely conserved, gene-island sequencing is a particularly useful tool for comparative analysis of genomes between related species.

Moy, M., H. M. Li, et al. (2002). "Endophytic Fungal beta -1,6-Glucanase Expression in the Infected Host Grass." Plant Physiology **130**(3): 1298-1308.

http://www.plantphysiol.org/cgi/content/abstract/130/3/1298

Mutualistic fungal endophytes infect many grass species and often confer benefits to the hosts such as reduced herbivory by insects and animals. The physiological interactions between the endophytes and their hosts have not been well characterized. Fungal-secreted proteins are likely to be important components of the interaction. In the interaction between Poa ampla and the endophyte Neotyphodium sp., a fungal [beta]-1,6-glucanase is secreted into the apoplast, and activity of the enzyme is detectable in endophyte-infected plants. Sequence analysis indicates the [beta]-1,6-glucanase is homologous to enzymes secreted by the mycoparasitic fungi Trichoderma harzianum and Trichoderma virens. DNA gel-blot analysis indicated the [beta]-1,6-glucanase was encoded by a single gene. As a secreted protein, the [beta]-1,6-glucanase may have a nutritional role for the fungus. In culture, [beta]-1,6-glucanase activity was induced in the presence of [beta]-1,6-glucans. From RNA gel blots, similar [beta]-1,6-glucanases were expressed in tall fescue (Festuca arundinacea Schreb.) and Chewings fescue (Festuca rubra L. subsp. fallax [Thuill] Nyman) infected with the endophyte species Neotyphodium coenophialum and Epichloe festucae, respectively.

Nocito, F. F., L. Pirovano, et al. (2002). "Cadmium-Induced Sulfate Uptake in Maize Roots." Plant Physiology **129**(4): 1872-1879.

http://www.plantphysiol.org/cgi/content/abstract/129/4/1872

The effect of cadmium (Cd) on high-affinity sulfate transport of maize (Zea mays) roots was studied and related to the changes in the levels of sulfate and nonprotein thiols during Cd-induced phytochelatin (PC) biosynthesis. Ten micromolar CdCl2 in the nutrient solution induced a 100% increase in sulfate uptake by roots. This was not observed either for potassium or phosphate uptake, suggesting a specific effect of Cd2+ on sulfate transport. The higher sulfate uptake was not dependent on a change in the proton motive force that energizes it. In fact, in Cd-treated plants, the transmembrane electric potential difference of root cortical cells was only slightly more negative than in the controls, the external pH did not change, and the activity of the plasma membrane H+-ATPase did not increase. Kinetics analysis showed that in the range of the high-affinity sulfate transport systems, 10 to 250 {micro}M, Cd exposure did not influence the Km value (about 20 {micro}M), whereas it doubled the Vmax value with respect to the control. Northern-blot analysis showed that Cd-induced sulfate uptake was related to a higher level of mRNA encoding for a putative high-affinity sulfate transporter in roots. Cd-induced sulfate uptake

was associated to both a decrease in the contents of sulfate and glutathione and synthesis of a large amount of PCs. These results suggest that Cd-induced sulfate uptake depends on a pretranslational regulation of the high-affinity sulfate transporter gene and that this response is necessary for sustaining the higher sulfur demand during PC biosynthesis.

Osman, A., B. Jordan, et al. (2003). "Genetic Diversity of Eurycoma longifolia Inferred from Single Nucleotide Polymorphisms." Plant Physiology **131**(3): 1294-1301.

http://www.plantphysiol.org/cgi/content/abstract/131/3/1294

Eurycoma longifolia Jack. is a treelet that grows in the forests of Southeast Asia and is widely used throughout the region because of its reported medicinal properties. Widespread harvesting of wild-grown trees has led to rapid thinning of natural populations, causing a potential decrease in genetic diversity among E. longifolia. Suitable genetic markers would be very useful for propagation and breeding programs to support conservation of this species, although no such markers currently exist. To meet this need, we have applied a genome complexity reduction strategy to identify a series of single nucleotide polymorphisms (SNPs) within the genomes of several E. longifolia accessions. We have found that the occurrence of these SNPs reflects the geographic origins of individual plants and can distinguish different natural populations. This work demonstrates the rapid development of molecular genetic markers in species for which little or no genomic sequence information is available. The SNP markers that we have developed in this study will also be useful for identifying genetic fingerprints that correlate with other properties of E. longifolia, such as high regenerability or the appearance of bioactive metabolites.

Ozga, J. A., J. Yu, et al. (2003). "Pollination-, Development-, and Auxin-Specific Regulation of Gibberellin 3beta -Hydroxylase Gene Expression in Pea Fruit and Seeds." Plant Physiology **131**(3): 1137-1146.

http://www.plantphysiol.org/cgi/content/abstract/131/3/1137

To understand further how pollination, seeds, auxin (4-chloroindole-3-acetic acid [4-Cl-IAA]), and gibberellins (GAs) regulate GA biosynthesis in pea (Pisum sativum) fruit, we studied expression of the gene PsGA3ox1 that codes for the enzyme that converts GA20 to biologically active GA1 using real-time reverse transcription-polymerase chain reaction analysis. PsGA3ox1 mRNA levels were minimally detectable in prepollinated pericarps and ovules ([-]2 d after anthesis [DAA]), increased dramatically after pollination (0 DAA), then decreased by 1 DAA. Seed PsGA3ox1 mRNA levels increased at 4 DAA and again 8 to 12 DAA, when seed development was rapid. Pericarp PsGA3ox1 mRNA levels peaked coincidentally with rapid pod diameter expansion (6-10 DAA) to accommodate the growing seeds. The effects of seeds and hormones on the expression of pericarp PsGA3ox1 were investigated over a 24-h treatment period. Pericarp PsGA3ox1 mRNA levels gradually increased from 2 to 3 DAA when seeds were present; however, when the seeds were removed, the pericarp transcript levels dramatically declined. When 2-DAA deseeded pericarps were treated with 4-Cl-IAA, PsGA3ox1 mRNA levels peaked 4 h after hormone treatment (270-fold increase), then decreased. PsGA3ox1 mRNA levels in deseeded pericarps treated with indole-3-acetic acid or GA3 were the same or lower than deseeded controls. These data show that PsGA3ox1 is expressed and developmentally regulated in pea pericarps and seeds. These data also show that pericarp PsGA3ox1 expression is hormonally regulated and suggest that the conversion of GA20 to GA1 occurs in the pericarp and is regulated by the presence of seeds and 4-Cl-IAA for fruit growth.

Perez-Perez, J. M., M. R. Ponce, et al. (2004). "The ULTRACURVATA2 Gene of Arabidopsis Encodes an FK506-Binding Protein Involved in Auxin and Brassinosteroid Signaling." Plant Physiology **134**(1): 101-117.

http://www.plantphysiol.org/cgi/content/abstract/134/1/101

The dwarf ucu (ultracurvata) mutants of Arabidopsis display vegetative leaves that are spirally rolled downwards and show reduced expansion along the longitudinal axis. We have previously determined that the UCU1 gene encodes a SHAGGY/GSK3-like kinase that participates in the signaling pathways of auxins and brassinosteroids. Here, we describe four recessive alleles of the UCU2 gene, whose homozygotes display helical rotation of several organs in addition to other phenotypic traits shared with ucu1 mutants. Following a map-based strategy, we identified the UCU2 gene, which was found to encode a peptidyl-prolyl cis/trans-isomerase of the FK506-binding protein family, whose homologs in metazoans are involved in cell signaling and protein trafficking. Physiological and double mutant analyses suggest that UCU2 is required for growth and development and participates in auxin and brassinosteroid signaling.

Perrin, R. M., Z. Jia, et al. (2003). "Analysis of Xyloglucan Fucosylation in Arabidopsis." Plant Physiology **132**(2): 768-778.

http://www.plantphysiol.org/cgi/content/abstract/132/2/768

Xyloglucan (XyG) is a load-bearing primary wall component in dicotyledonous and non-graminaceous monocotyledonous plants. XyG fucosyltransferase (FUTase), encoded by the Arabidopsis gene AtFUT1, directs addition of fucose (Fuc) residues to terminal galactose residues on XyG side chains. Reverse transcription-polymerase chain reaction and analysis of promoter-{beta}-glucuronidase transgenic plants indicated highest expression of AtFUT1 in the upper portion of elongating inflorescence stems of Arabidopsis. XyG FUTase activity was highest in Golgi vesicles prepared from growing Arabidopsis tissues and low in those isolated from mature tissues. There was no discernible correlation between the Fuc contents of XyG oligosaccharides derived from different Arabidopsis organs and the level of AtFUT1 expression in the organs. Thus, organ-specific variations in AtFUT1 expression and enzyme activity probably reflect differential rates of cell wall biosynthesis, rather than differences in levels of XyG fucosylation. The effects of manipulating AtFUT1 expression were examined using an Arabidopsis mutant (atfut1) containing a T-DNA insertion in the AtFUT1 locus and transgenic plants with strong constitutive expression of AtFUT1. No Fuc was detected in XyG derived from leaves or roots of atfut1. Plants overexpressing AtFUT1 had higher XyG FUTase activity than wild-type plants, but the XyG oligosaccharides derived from the transgenic and wild-type plants contained comparable amounts of Fuc, indicating that suitable acceptor substrates are limiting. Galactosyl residues had slightly higher levels of O-acetylation in XyG from plants that overexpressed AtFUT1 than in XyG from wild-type plants. O-Acetylation of galactose residues was considerably reduced in Fuc-deficient mutants (atfut1, mur1, and mur2) that synthesize XyG containing little or no Fuc. These results suggest that fucosylated XyG is a suitable substrate for at least one O-acetyltransferase in Arabidopsis.

Simkin, A. J., B. A. Underwood, et al. (2004). "Circadian Regulation of the PhCCD1 Carotenoid Cleavage Dioxygenase Controls Emission of {beta}-Ionone, a Fragrance Volatile of Petunia Flowers." Plant Physiology **136**(3): 3504-3514.

http://www.plantphysiol.org/cgi/content/abstract/136/3/3504

Carotenoids are thought to be the precursors of terpenoid volatile compounds that contribute to flavor and aroma. One such volatile, {beta}-ionone, is important to fragrance in many flowers, including petunia (Petunia hybrida). However, little is known about the factors regulating its synthesis in vivo. The petunia genome contains a gene encoding a 9,10(9',10') carotenoid cleavage dioxygenase, PhCCD1. The PhCCD1 is 94% identical to LeCCD1A, an enzyme responsible for formation of{beta} -ionone in tomato (Lycopersicon esculentum; Simkin AJ, Schwartz SH, Auldridge M, Taylor MG, Klee HJ [2004] Plant J [in press]). Reduction of PhCCD1 transcript levels in transgenic plants led to a 58% to 76% decrease in {beta}-ionone synthesis in the corollas of selected petunia lines, indicating a significant role for this enzyme in volatile synthesis. Quantitative reverse transcription-PCR analysis revealed that PhCCD1 is highly expressed in corollas and leaves, where it constitutes approximately 0.04% and 0.02% of total RNA, respectively. PhCCD1 is light-inducible and exhibits a circadian rhythm in both leaves and flowers.{beta} -Ionone emission by flowers occurred principally during daylight hours, paralleling PhCCD1 expression in corollas. The results indicate that PhCCD1 activity and{beta} -ionone emission are likely regulated at the level of transcript.

Springer, N. M., O. N. Danilevskaya, et al. (2002). "Sequence Relationships, Conserved Domains, and Expression Patterns for Maize Homologs of the Polycomb Group Genes E(z), esc, and E(Pc)." Plant Physiology **128**(4): 1332-1345.

http://www.plantphysiol.org/cgi/content/abstract/128/4/1332

Polycomb group (PcG) proteins play an important role in developmental and epigenetic regulation of gene expression in fruit fly (Drosophila melanogaster) and mammals. Recent evidence has shown that Arabidopsis homologs of PcG proteins are also important for the regulation of plant development. The objective of this study was to characterize the PcG homologs in maize (Zea mays). The 11 cloned PcG proteins from fruit fly and the Enhancer of zeste [E(z)], extra sex combs (esc), and Enhancer of Polycomb [E(Pc)] homologs from Arabidopsis were used as queries to perform TBLASTN searches against the public maize expressed sequence tag database and the Pioneer Hi-Bred database. Maize homologs were found for E(z), esc, and E(Pc), but not for Polycomb, pleiohomeotic, Posterior sex combs, Polycomblike, Additional sex combs, Sex combs on midleg, polyhometoic, or multi sex combs. Transcripts of the three maize Enhancer of zeste-like genes, Mez1, Mez2, and Mez3, were detected in all tissues tested, and the Mez2 transcript is alternatively spliced in a tissue-dependent pattern. Zea mays fertilization independent endosperm1 (ZmFie1) expression was limited to developing embryos and endosperms, whereas ZmFie2 expression was found throughout plant development. The conservation of E(z) and esc homologs across kingdoms indicates that these genes likely play a conserved role in repressing gene expression.

Tagmount, A., A. Berken, et al. (2002). "An Essential Role of S-Adenosyl-L-Methionine:L-Methionine S-Methyltransferase in Selenium Volatilization by Plants. Methylation of Selenomethionine to Selenium-Methyl-L-Selenium- Methionine, the Precursor of Volatile Selenium." Plant Physiology **130**(2): 847-856.

http://www.plantphysiol.org/cgi/content/abstract/130/2/847

Selenium (Se) phytovolatilization, the process by which plants metabolize various inorganic or organic species of Se (e.g. selenate, selenite, and Se-methionine [Met]) into gaseous Se forms (e.g. dimethylselenide), is a potentially important means of removing Se from contaminated environments. Before attempting to genetically enhance the efficiency of Se phytovolatilization, it is essential to elucidate the enzymatic pathway involved and to identify its rate-limiting steps. The present research tested the hypothesis that S-adenosyl-L-Met:L-Met S-methyltransferase (MMT)

is the enzyme responsible for the methylation of Se-Met to Se-methyl Se-Met (SeMM). To this end, we identified and characterized an Arabidopsis T-DNA mutant knockout for MMT. The lack of MMT in the Arabidopsis T-DNA mutant plant resulted in an almost complete loss in its capacity for Se volatilization. Using chemical complementation with SeMM, the presumed enzymatic product of MMT, we restored the capacity of the MMT mutant to produce volatile Se. Overexpressing MMT from Arabidopsis in Escherichia coli, which is not known to have MMT activity, produced up to 10 times more volatile Se than the untransformed strain when both were supplied with Se-Met. Thus, our results provide in vivo evidence that MMT is the key enzyme catalyzing the methylation of Se-Met to SeMM.

Townsend, B. J., A. Poole, et al. (2005). "Antisense Suppression of a (+)-{delta}-Cadinene Synthase Gene in Cotton Prevents the Induction of This Defense Response Gene during Bacterial Blight Infection But Not Its Constitutive Expression." Plant Physiology: pp.104.056010.

http://www.plantphysiol.org/cgi/content/abstract/pp.104.056010v1

In cotton (Gossypium hirsutum) the enzyme (+)-{delta}-cadinene synthase (CDNS) catalyzes the first committed step in the biosynthesis of cadinane-type sesquiterpenes, such as gossypol, that provide constitutive and inducible protection against pests and diseases. A cotton cDNA clone encoding CDNS (cdn1-C4) was isolated from developing embryos and functionally characterized. Southern analysis showed that CDNS genes belong to a large multigene family, of which five genomic clones were studied, including three pseudogenes and one gene that may represent another subfamily of CDNS. CDNS expression was shown to be induced in cotton infected with either the bacterial blight or verticillium wilt pathogens. Constructs for the constitutive or seed-specific antisense suppression of cdn1-C4 were introduced into cotton by Agrobacterium-mediated transformation. Gossypol levels were not reduced in the seeds of transformants with either construct, nor was the induction of CDNS expression affected in stems of the constitutive antisense plants infected with Verticillium dahliae Kleb. However, the induction of CDNS mRNA and protein in response to bacterial blight infection of cotyledons was completely blocked in the constitutive antisense plants. These results suggest that cdn1-C4 may be involved specifically in the bacterial blight response and that the CDNS multigene family comprises a complex set of genes differing in their temporal and spatial regulation and responsible for different branches of the cotton sesquiterpene pathway.

Xie, D.-Y., L. A. Jackson, et al. (2004). "Molecular and Biochemical Analysis of Two cDNA Clones Encoding Dihydroflavonol-4-Reductase from Medicago truncatula." Plant Physiology **134**(3): 979-994.

http://www.plantphysiol.org/cgi/content/abstract/134/3/979

Dihydroflavonol-4-reductase (DFR; EC1.1.1.219) catalyzes a key step late in the biosynthesis of anthocyanins, condensed tannins (proanthocyanidins), and other flavonoids important to plant survival and human nutrition. Two DFR cDNA clones (MtDFR1 and MtDFR2) were isolated from the model legume Medicago truncatula cv Jemalong. Both clones were functionally expressed in Escherichia coli, confirming that both encode active DFR proteins that readily reduce taxifolin (dihydroquercetin) to leucocyanidin. M. truncatula leaf anthocyanins were shown to be cyanidin-glucoside derivatives, and the seed coat proanthocyanidins are known catechin and epicatechin derivatives, all biosynthesized from leucocyanidin. Despite high amino acid similarity (79% identical), the recombinant DFR proteins exhibited differing pH and temperature profiles and differing relative substrate preferences. Although no pelargonidin derivatives were identified in M. truncatula, MtDFR1 readily reduced dihydrokaempferol, consistent with the presence of an asparagine residue at a location known to determine substrate specificity in other DFRs, whereas

MtDFR2 contained an aspartate residue at the same site and was only marginally active on dihydrokaempferol. Both recombinant DFR proteins very efficiently reduced 5-deoxydihydroflavonol substrates fustin and dihydrorobinetin, substances not previously reported as constituents of M. truncatula. Transcript accumulation for both genes was highest in young seeds and flowers, consistent with accumulation of condensed tannins and leucoanthocyanidins in these tissues. MtDFR1 transcript levels in developing leaves closely paralleled leaf anthocyanin accumulation. Overexpression of MtDFR1 in transgenic tobacco (Nicotiana tabacum) resulted in visible increases in anthocyanin accumulation in flowers, whereas MtDFR2 did not. The data reveal unexpected properties and differences in two DFR proteins from a single species.

Plant Physiology and Biochemistry (3)

Bassett, E. V., B. Y. Bouchet, et al. (2003). "PALA-mediated pyrimidine starvation increases expression of aspartate transcarbamoylase (pyrB) in Arabidopsis seedlings." Plant Physiology and Biochemistry **41**(8): 695.

http://www.sciencedirect.com/science/article/B6VRD-494P6F5-3/2/d45a1dd5d210dbe7754cc8e3a6ac42ee

Aspartate transcarbamoylase (ATCase; EC 2.1.3.2) catalyzes the committed step in the de novo synthesis of pyrimidine nucleotides. We investigated the effects of N-(phosphonacetyl)-L-aspartate (PALA), a transition-state analog inhibitor of ATCase, on seedling growth and development, RNA and soluble protein contents, ATCase activity and enzyme protein levels, and pyrB gene expression in Arabidopsis thaliana L. cv. "Columbia". In vitro, PALA was a potent inhibitor of ATCase, with an apparent Ki = 22 nM. After 5 d of treatment with 1 mM PALA, seedlings exhibited delayed germination, inhibition of cotyledon expansion, leaf development and root growth, and general chlorosis. Total RNA contents of these seedlings were decreased by 81% and total soluble protein contents decreased by 74%, compared with untreated control plants. Levels of pyrB mRNA increased about tenfold in PALA-treated plants, while ATCase activity and enzyme protein levels increased twofold. Plants grown on media containing a lower (0.1 mM) concentration of PALA did not exhibit significant inhibition of growth until after 9 d of treatment, but had markedly reduced RNA contents (40% of controls) and elevated pyrB mRNA levels (fourfold increase) after 12 d of treatment.

Hewitt, M. M., J. M. Carr, et al. "Effects of phosphate limitation on expression of genes involved in pyrimidine synthesis and salvaging in Arabidopsis." Plant Physiology and Biochemistry **In Press, Corrected Proof** http://www.sciencedirect.com/science/article/B6VRD-4FG2TY7-1/2/616a67e37527e20048f687cb1614b013

Arabidopsis seedlings grown for 14 d without phosphate (P) exhibited stunted growth and other visible symptoms associated with P deficiency. RNA contents in shoots decreased nearly 90%, relative to controls. In shoots, expression of Pht1;2, encoding an inducible high-affinity phosphate transporter, increased threefold, compared with controls, and served as a molecular marker for P limitation. Transcript levels for five enzymes (aspartate transcarbamoylase, ATCase, EC 2.1.3.2; carbamoyl phosphate synthetase, CPSase, EC 6.3.5.5); UMP synthase, EC 2.4.1.10, EC 4.1.1.23; uracil phosphoribosyltransferase, UPRTase, EC 2.4.2.9; UMP kinase, EC 2.7.1.14)

increased 2-10-fold in response to P starvation in shoots. These enzymes, which utilize phosphorylated intermediates at putative regulated steps in de novo synthesis and salvaging pathways leading to UMP and pyrimidine nucleotide formation, appear to be coordinately regulated, at the level of gene expression. This response may facilitate pyrimidine nucleotide synthesis under P limitation in this plant. Expression of P-dependent and P-independent phosphoribosyl pyrophosphate (PRPP) synthases (PRS2 and PRS3, respectively) which provide PRPP, the phosphoribosyl donor in UMP synthesis via both de novo and salvaging pathways, was differentially regulated in response to P limitation. PRS2 mRNA levels increased twofold in roots and shoots of P-starved plants, while PRS3 was constitutively-expressed. PRS3 may play a novel role in providing PRPP to cellular metabolism under low P availability.

Mbeguie-A-Mbeguie, D., B. Gouble, et al. (2002). "Two expansin cDNAs from Prunus armeniaca expressed during fruit ripening are differently regulated by ethylene." Plant Physiology and Biochemistry **40**(5): 445.

http://www.sciencedirect.com/science/article/B6VRD-45SGTMS-9/2/bfc247c54fc786e02df88e66577a416d

Little is known about gene expression during fruit ripening of apricot (Prunus armeniaca L. cv. Bergeron), especially for enzymes involved in cell wall modifications. A partial cDNA clone encoding a protein homologous to expansin was isolated from a ripe apricot fruit cDNA library. This clone was used to isolate two full-length expansin cDNAs, Pa-Exp1 (accession no. U93167) and Pa-Exp2 (accession no. AF038815) from the same cDNA library. The predicted polypeptides encoded by these two cDNAs are different and belong to the [alpha]-expansin family; Pa-Exp1 and Pa-Exp2 are two different members of a multigene family. These two clones are mostly expressed in fruit, during its ripening. Pa-Exp1 mRNA accumulated abundantly at the half-ripe stage of fruit development and decreased thereafter. Pa-Exp2 mRNA level increased from the immature-green stage to the half-ripe stage where it peaked before declining. During the ripening process, Pa-Exp1 and Pa-Exp2 gene expression appeared to be positively correlated with fruit size. Post-harvest treatments by air, ethylene, and 1-methyl cyclopropene led us to conclude that Pa-Exp1 appears to be developmentally down-regulated by ethylene while Pa-Exp2 is not affected. The relationship between Pa-Exp1, Pa-Exp2 and the softening process is also discussed.

Plant Science (34)

Cary, J. W., K. Rajasekaran, et al. (2000). "Transgenic expression of a gene encoding a synthetic antimicrobial peptide results in inhibition of fungal growth in vitro and in planta." Plant Science **154**(2): 171.

http://www.sciencedirect.com/science/article/B6TBH-3YVDPXV-8/2/fb1276ab533b29cbe55de6153f127eed

Transgenic tobacco plants producing the synthetic antimicrobial peptide D4E1, encoded by a gene under the control of an enhanced cauliflower mosaic virus 35S RNA promoter, were obtained by Agrobacterium-mediated transformation. Successful transformation was demonstrated by PCR and Southern hybridization analysis of tobacco DNAs. Expression of the

synthetic D4E1 gene was shown by RT-PCR of tobacco mRNA. Crude protein extracts from leaf tissue of transformed plants significantly reduced the number of fungal colonies arising from germinating conidia of Aspergillus flavus and Verticillium dahliae by up to 75 and 99%, respectively, compared to extracts from plants transformed with pBI121. Compared to negative controls, tobacco plants expressing the D4E1 gene showed greater levels of disease resistance in planta to the fungal pathogen, Colletotrichum destructivum, which causes anthracnose.

Cordeiro, G. M., R. Casu, et al. (2001). "Microsatellite markers from sugarcane (Saccharum spp.) ESTs cross transferable to erianthus and sorghum." Plant Science **160**(6): 1115.

http://www.sciencedirect.com/science/article/B6TBH-42Y11N6-4/2/7b54ff298e2d75249705568a0eba2e4e

Analysis of a sugarcane (Saccharum spp.) EST (expressed sequence tag) library of 8678 sequences revealed approximately 250 microsatellite or simple sequence repeats (SSRs) sequences. A diversity of dinucleotide and trinucleotide SSR repeat motifs were present although most were of the (CGG)n trinucleotide motif. Primer sets were designed for 35 sequences and tested on five sugarcane genotypes. Twenty-one primer pairs produced a PCR product and 17 pairs were polymorphic. Primer pairs that produced polymorphisms were mainly located in the coding sequence with only a single pair located within the 5' untranslated region. No primer pairs producing a polymorphic product were found in the 3' untranslated region. The level of polymorphism (PIC value) in cultivars detected by these SSRs was low in sugarcane (0.23). However, a subset of these markers showed a significantly higher level of polymorphism when applied to progenitor and related genera (Erianthus sp. and Sorghum sp.). By contrast, SSRs isolated from sugarcane genomic libraries amplify more readily, show high levels of polymorphism within sugarcane with a higher PIC value (0.72) but do not transfer to related species or genera well.

Corniquel, B. and L. Mercier (1994). "Date palm (Phoenix dactylifera L.) cultivar identification by RFLP and RAPD." Plant Science **101**(2): 163.

http://www.sciencedirect.com/science/article/B6TBH-47T1J5B-22/2/a5a2f1479ed52be9b36883b2bcf158ce

An RFLP analysis of five date palm (Phoenix dactylifera L.) elite cultivars (cvs. Barhee, Deglet Nour, Khalassa, Khadrawy, and Medjool) has been performed on offshoot leaves surrounding the shootips used to initiate tissue culture. Total DNA digested by EcoRI was hybridized with cDNA probes randomly selected from a cDNA library constructed from highly organogenic calli of cv. Boustammi Noire, and with a heterologous 1.7-kb nuclear rDNA fragment, amplified during a polymerase chain reaction (PCR) of jojoba genomic DNA. Discrimination among the five cultivars was easily made with cDNA probe 1, which was highly polymorphic. A polymorphismm among cultivars was also observed by amplification with random primers of total DNA extracted from offshoot leaves. Preliminary attempts made to asses the extent of variability at the DNA level as a result of tissue culture, are also reported. With the availability of probes such as cDNA 1, the use of RFLP for rapid and reliable cultivar identification, and screening of cultivated populations with economically important traits in date palm growing countries is now conceivable.

De Melis, L. E., P. H. Whiteman, et al. (1999). "Isolation and characterisation of a cDNA clone encoding cinnamyl alcohol dehydrogenase in Eucalyptus globulus Labill." Plant Science **143**(2): 173.

http://www.sciencedirect.com/science/article/B6TBH-3WNMGG3-6/2/dfaf96ca331e10c084ffb812f4c1f5a5

Cinnamyl alcohol dehydrogenase (CAD) (EC 1.1.1.195) catalyses the final step in lignin precursor synthesis reducing the cinnamyl aldehydes (para-coumaryl, coniferyl and sinapyl aldehydes) to the corresponding alcohols in the presence of NADPH. In this paper, we report the molecular cloning and characterisation of a Eucalyptus globulus genomic fragment encoding CAD2, and the corresponding full-length cDNA isolated from young stem material. This was achieved using the polymerase chain reaction-based method known as rapid amplification of cDNA ends, with oligonucleotide primers corresponding to regions of homology between CAD-encoding sequences from other eucalypt species. The identity of the clones was inferred by sequence data comparison and the cDNA sequence (1423 bp) was found to encode a protein of 356 amino acid residues. The CAD2 transcript was most abundant in stem, followed by root and midrib tissues, which corresponds with the role of lignin in water retention in plants and in providing mechanical support. Low level expression was also observed in leaf tissue. Southern blot analysis revealed a single CAD gene in this species with the presence of possibly different allelic representations.

Defilippi, B. G., A. A. Kader, et al. (2005). "Apple aroma: alcohol acyltransferase, a rate limiting step for ester biosynthesis, is regulated by ethylene." Plant Science **168**(5): 1199.

http://www.sciencedirect.com/science/article/B6TBH-4F7H2S6-1/2/8e4a8412a1d3c27c2b051cbce2f4179b

The role of ethylene in aroma biosynthesis of apple fruits was investigated using transgenic 'Greensleeves' apple trees suppressed for ACC-oxidase or ACC-synthase enzyme activity, and an ethylene action inhibitor (1-methycyclopropene, 1-MCP). In the transgenic lines and 1-MCP treated fruit, reductions higher than 90% in ethylene biosynthesis and respiration rates were observed in apples held at 20 [deg]C for 14 days. We observed a major reduction in ester production in the ethylene-suppressed lines and in the 1-MCP treated fruit, with only slight differences in the levels of alcohol and aldehyde volatiles under these conditions. The activity of alcohol acyl-CoA transferase (AAT), a key enzyme in ester biosynthesis, showed an ethylene dependent pattern of regulation. Additionally, gene expression levels of specifically an AAT clone were highly regulated by ethylene. In contrast, activity and expression levels of alcohol dehydrogenase (ADH) were not affected by changes in the levels of endogenous ethylene. These results suggest that ethylene is involved in ester biosynthesis in apple via regulation of AAT.

Hatanaka, T., H. Sano, et al. (1999). "Molecular cloning and characterization of coffee cDNA encoding spermidine synthase." Plant Science **140**(2): 161.

http://www.sciencedirect.com/science/article/B6TBH-3VM6K37-6/2/bafbe4abce7b600308bfff3e2c2b0f2b

A cDNA for spermidine synthase (SPDS), which converts putrescine to spermidine using decarboxylated S-adenosylmethionine as a co-factor, has been isolated from Coffea arabica. When the SPDS cDNA is expressed in an SPDS-deficient E. coli mutant, the recombinant protein shows high SPDS activity. The C. arabica SPDS possesses the co-factor binding motifs which have been proposed for S-adenosylmethionine, and its amino acid sequence is similar to other plant SPDSs. The SPDS transcripts have been observed in roots, green stems, old and young leaves, and accumulated to a higher level in rapid growing tissues, such as green stems and younger leaves, compared to old leaves. In callus tissues, it has been expressed in all stages, even though callus growth is very slow during somatic embryogenesis.

Hilliou, F., P. Christou, et al. (1999). "Development of an efficient transformation system for Catharanthus roseus cell cultures using particle bombardment." Plant Science **140**(2): 179.

http://www.sciencedirect.com/science/article/B6TBH-3VM6K37-8/2/30406d15fdee413bea45160328175ae6

We have developed an efficient direct DNA transfer procedure for the facile engineering of Catharanthus roseus cell cultures. Particle bombardment of callus derived from leaf material permitted rapid selection and establishment of transgenic cell lines. Transgenic callus were recovered at a frequency of between 60-80% of total callus bombarded with a single plasmid. Bombardment using two separate plasmids resulted in a 25-60% frequency of transgenic callus recovered, up to 90% containing both input plasmids. Between 10-20 g FW of transgenic material was produced within 3 months of bombardment, providing sufficient material for molecular and biochemical analyses. We developed two complementary systems allowing selection on either hygromycin or kanamycin to permit re-transformation using plasmids carrying additional genes of interest. Use of leaf tissue as explant for transformation avoids time-consuming and labor intensive procedures involving suspension cultures. We provide molecular data on integration and expression of selected and non selected transgenes in a number of transgenic callus lines. Transgene integration events for co-transformed plasmids were relatively simple, occurring at one or two sites in the genome for most of the lines we analysed. Molecular analysis of callus resulting from co-transformation experiments using two different plasmids revealed that in nine of 10 putative transgenic lines we selected for analysis both plasmids had integrated into the genome. RNA gel-blot analysis and histochemical staining showed that an unselected transgene, gusA, was expressed in seven of the ten lines we analysed.

Hurkman, W. J., K. F. McCue, et al. (2003). "Effect of temperature on expression of genes encoding enzymes for starch biosynthesis in developing wheat endosperm." Plant Science **164**(5): 873.

http://www.sciencedirect.com/science/article/B6TBH-482YHVX-3/2/0c32d03d378277c95b71b22f21a554fe

The effect of high temperature on starch accumulation, starch granule populations, and expression of genes encoding key enzymes for starch biosynthesis was examined during grain development in wheat (Triticum aestivum L. cv. Butte 86). High temperature applied from anthesis to maturity reduced the duration of starch accumulation. Starch accumulation ceased approximately 6 days earlier for grain produced under a 37/17 [deg]C (day/night) regimen and 21 days earlier under a 37/28 [deg]C (day/night) regimen than for grain produced under a 24/17 [deg]C (day/night) regimen. Compared to the 24/17 [deg]C regimen, starch content was approximately 19% less for mature grain produced under the 37/17 [deg]C regimen and 58% less under the 37/28 [deg]C regimen. Based on relative volume, the smaller type B starch granules were the predominant class in mature grain produced under the 24/17 and 37/17 [deg]C regimens, whereas the larger type A granules were predominant in grain produced under the 37/28 [deg]C regimen. Under the 24/17 [deg]C regimen, steady state transcript levels for ADP-glucose pyrophosphorylase, starch synthases I, II, and III, granule-bound starch synthase, and starch branching enzymes I and II were highest from 12-16 days post-anthesis (dpa). Under the 37/17 [deg]C regimen, steady state levels of these transcripts followed the same temporal pattern, but were substantially lower. Under the 37/28 [deg]C regimen, transcript levels peaked earlier, at 7 dpa. The high temperature regimens reduced the relative levels of transcripts for starch synthase more than the other starch biosynthetic enzymes.

Ichimura, K., T. Mizoguchi, et al. (1997). "ATMRK1, an Arabidopsis protein kinase related to mammal mixed-lineage kinases and Raf protein kinases." Plant Science **130**(2): 171.

http://www.sciencedirect.com/science/article/B6TBH-3S7WH76-N/2/da97b1e10563e30e0dcb900cbbd47bc6

We isolated a cDNA clone (ATMRK1) with sequence similarity to mammal mixed-lineage kinase homologues and Raf protein kinase homologues in the catalytic domain from Arabidopsis thaliana using the polymerase chain reaction (PCR). The ATMRK1 cDNA encodes a 391-amino acid polypeptide containing all 11 conserved regions of the catalytic domains of protein kinases. The catalytic domain of the putative ATMRK1 protein has sequence similarity with those of protein kinases that belong to mammalian mixed-lineage kinases, MLK-2 (33%) and c-Raf-1 protein kinase (26%). The ATMRK1 protein has the highest homology with plant protein kinases, Glycine max PK6 (36%), Arabidopsis protein kinases ATN1 (36%) and CTR1 (34%). CTR1 encodes Raf family protein kinase and negatively regulates ethylene signal transduction. The phylogenetic tree shows that the plant protein kinases ATMRK1, GmPK6 and ATN1 are classified into the same cluster close to mixed-lineage and Raf kinases. DNA gel blot analysis and a search of the plant expressed sequence tag (EST) database showed the existence of several ATMRK1-related genes in the Arabidopsis genome. RNA gel blot analysis revealed that transcripts of ATMRK1 are detected in all tissues. The highest level of its expression was obtained from root tissues.

Joshi, C. P. and H. T. Nguyen (1993). "RAPD (random amplified polymorphic DNA) analysis based intervarietal genetic relationships among hexaploid wheats." Plant Science **93**(1-2): 95.

http://www.sciencedirect.com/science/article/B6TBH-47T25KD-7K/2/23ce5273c1c4fb1c26b436a9c3600b85

The main objective of this study was to assess the extent of genetic diversity detected by RAPD (random amplified polymorphic DNA) technique among 15 varieties of common bread wheat (Triticum aestivum L.). The slow development of genetic linkage maps of wheat using conventional molecular marker strategies is attributed to the limited number of RFLPs (restriction fragment length polymorphisms) between wheat genotypes. Recently, RAPDs have been observed between closely related genotypes in several other species. We have used a set of 40 single arbitrary primers (10-mers) for the PCR (polymerase chain reaction) -mediated amplification of random genomic DNA fragments from wheats. Eighty percent of the primers yielded distinct electrophoretic profiles which could be scored. Out of 109 amplified fragments, 71 (65%) were polymorphic in these wheat cultivars. These results have assisted in the development of a dendrogram suggesting genetic relationships among these genotypes. Moreover, most of the spring and winter wheats were clustered together in this dendrogram based on Jaccard's coefficients. These results will be useful in the identification of suitable parents for the development of a mapping population for tagging agronomically important traits in wheat.

Khoshnoodi, J., C. T. Larsson, et al. (1998). "Differential accumulation of Arabidopsis thaliana Sbe2.1 and Sbe2.2 transcripts in response to light." Plant Science **135**(2): 183.

http://www.sciencedirect.com/science/article/B6TBH-3TBCD05-7/2/892397b16d1a1d767a34f97b77f95dd5

We have isolated two closely related genes, Sbe2.1 and Sbe2.2, encoding isoform II of starch branching enzyme (SBE) from an Arabidopsis thaliana genomic library. Although a partial cDNA clone encoding the isoform I of the potato SBE was used as probe, no clones corresponding to

this isoform were found in the Arabidopsis library. Sbe2.1 was completely and Sbe2.2 partially sequenced. PCR-screening of a large number of individual plants using gene specific primers, revealed that both genes were independently present in the genome of A. thaliana. The Sbe2.1 gene consisted of 18 exons interrupted by 17 introns and the open reading frame comprised 2574 bp encoding a protein with a calculated molecular mass of 97660 Da. The promoter region of both Sbe2 genes contained potential sucrose responsive elements (SURE1 and SURE2). In addition, a potential light responsive element (G-box) was present in the Sbe2.1 promoter. Northern blot analysis using gene specific probes, showed a differential accumulation of transcripts from the Sbe2 genes as a response to light. While the level of Sbe2.1 transcripts increased and decreased during light and dark, respectively, those transcribed from the Sbe2.2 gene did not change significantly. However, transcripts from both Sbe2 genes accumulated strongly when plants were incubated in light and with exogenous supply of either glucose, fructose or sucrose. No significant changes in Sbe2 transcript levels were detected in plants incubated with sorbitol under conditions of dark or light, indicating that the expression of Sbe2 genes is stimulated by specific carbohydrate signals.

Kobayashi, S., M. Ishimaru, et al. (2001). "Comparison of UDP-glucose:flavonoid 3-O-glucosyltransferase (UFGT) gene sequences between white grapes (Vitis vinifera) and their sports with red skin." Plant Science **160**(3): 543.

http://www.sciencedirect.com/science/article/B6TBH-428FKNV-M/2/ef09a5e05f3f4091415cb5bd95ff51df

The expression of the UDP-glucose:flavonoid 3-O-glucosyltransferase (UFGT) gene has been shown to be critical for anthocyanin biosynthesis in the grape berry. Using white cultivars and bud sports with red skin, we examined the expression of seven anthocyanin biosynthetic genes including the UFGT gene and compared the coding/promoter sequences of the UFGT gene. Northern blot analysis showed that the seven anthocyanin biosynthetic genes were expressed coordinately at higher levels in the red-skin sports than in the white-skin progenitors of the sports. It was especially notable that UFGT gene expression was detected only in the red-skin sports and Kyoho. However, there were no differences in either coding or promoter sequences between Italia (Vitis vinifera) and its red-skin sport Ruby Okuyama, or between Muscat of Alexandria (V. vinifera) and the red-skin sport Flame Muscat. From these findings, the phenotypic change from white to red in the sports is thought to be the result of a mutation in a regulatory gene controlling the expression of UFGT.

Kosugi, Y., K. Shibuya, et al. (2000). "Expression of genes responsible for ethylene production and wilting are differently regulated in carnation (Dianthus caryophyllus L.) petals." Plant Science **158**(1-2): 139.

http://www.sciencedirect.com/science/article/B6TBH-416BXRB-G/2/fc44de4583e5d592cafcc2d067ce565c

Carnation petals exhibit autocatalytic ethylene production and wilting during senescence. The autocatalytic ethylene production is caused by the expression of 1-aminocyclopropane-1-carboxylate (ACC) synthase and ACC oxidase genes, whereas the wilting of petals is related to the expression of the cysteine proteinase (CPase) gene. So far, it has been believed that the ethylene production and wilting are regulated in concert in senescing carnation petals, since the two events occurred closely in parallel with time. In the present study, we investigated the expression of these genes in petals of a transgenic carnation harboring a sense ACC oxidase transgene and in petals of carnation flowers treated with 1,1-dimethyl-4-(phenylsulfonyl)semicarbazide (DPSS). In petals of the transgenic carnation flowers, treatment

with exogenous ethylene caused accumulation of the transcript for CPase and in-rolling (wilting), whereas it caused no or little accumulation of the transcripts for ACC oxidase and ACC synthase and negligible ethylene production. In petals of the flowers treated with DPSS, the transcripts for ACC synthase and ACC oxidase were accumulated, but no significant change in the level of the transcript for CPase was observed. These results suggest that the expression of ACC synthase and ACC oxidase genes, which leads to ethylene production, is differentially regulated from the expression of CPase, which leads to wilting, in carnation petals.

Kozik, A., R. Heidstra, et al. (1995). "Pea lines carrying syml or sym2 can be nodulated by Rhizobium strains containing nodX; sym1 and sym2 are allelic." Plant Science **108**(1): 41.

http://www.sciencedirect.com/science/article/B6TBH-3XVH3PR-1K/2/59e3a545611e1fca527aee57179717f3

In wild pea varieties two genes, sym1 and sym2, have been identified that cause resistance to European Rhizobium leguminosarum bv. viciae (Rlv) strains. The sym2 gene has previously been studied in some detail and it was shown that the additional nodulation gene nodX is sufficient to overcome the sym2 controlled nodulation resistance. Here we characterize the sym1 gene. We show that the resistance conferred by sym1 can be overcome by the introduction of nodX in European Rlv strains, indicating that sym1 just as sym2 is involved in Nod factor recognition. Both sym1 and sym2 display a recessive or dominant nature depending on the Rlv strain used for inoculation. Furthermore, introgression lines containing either sym1 or sym2 are able to form nodules with Rlv strain 248 at 26[deg]C, but not at 18[deg]C, indicating that both sym1 and sym2 have a temperature sensitive nature. sym2 was mapped on the pea RFLP map. We found that sym1 maps in the same region of chromosome 1 as sym2. By crossing sym1 and sym2 containing introgression lines we demonstrate that sym1 and sym2 are allelic.

Kyo, M., S. Hattori, et al. (2003). "Cloning and characterization of cDNAs associated with the embryogenic dedifferentiation of tobacco immature pollen grains." Plant Science **164**(6): 1057.

http://www.sciencedirect.com/science/article/B6TBH-486BPW1-1/2/9586493384abadd08376051557f1e280

We conducted differential screening to obtain cDNAs showing that gene expression is highly associated with the transformation from immature pollen to embryogenic cell, so-called embryogenic dedifferentiation of pollen, in a Nicotiana tabacum pollen culture system and analyzed their expression and sequences. Seventy-seven cDNA clones were independently isolated and distinguished into 16 groups based on their sequences. The groups were further categorized into two classes, Class I and II, based on the gene expression pattern of the representative clone of each group under various pollen culture conditions arranged for examining the coincidence with the dedifferentiation. The 13 groups in Class I showed prominent expression under the conditions allowing or facilitating pollen dedifferentiation and the expression level increased earlier than A-type cyclin genes, but they were not markedly expressed in the cell populations rich in S-phase cells, i.e. young anthers with pollen mother cells, BY-2 cells at the growth phase and early phase embryos derived from immature pollen. The other three groups in Class II encoded homologs to H1 histone, H2A histone and minichromosome maintenance (MCM) protein, respectively. The level of their transcripts increased during dedifferentiation, but it was also high in anthers containing pollen mother cells and in the proliferating BY-2 cells indicating that their expression is coincident with the S phase but not with dedifferentiation. These findings suggest that pollen dedifferentiation is a complex process accompanied with the reentrance of cell cycle and unknown events probably caused by specific expression of many genes, at least, listed in Class I. These genes should be used as reliable markers and important

clues for further studies on the molecular mechanism of dedifferentiation.

Kyo, M., N. Yamaji, et al. (2002). "Isolation of cDNAs coding for NtEPb1-b3, marker proteins for pollen dedifferentiation in a tobacco pollen culture system." Plant Science **163**(5): 1055.

http://www.sciencedirect.com/science/article/B6TBH-46P1KGF-4/2/f7d0b3aeec79e0c10a2b75f7bbe0d41c

Several phosphoproteins, Nicotiana tabacum L. embryogenic pollen-abundant phosphoproteins (NtEPs), characteristically appear in the dedifferentiation process from immature pollen grains to embryogenic cells in a pollen culture system. Among NtEPs we focused our attention on three proteins (NtEPb1-b3) which showed the highest correlation with the dedifferentiation and possessed different pI values and similar molecular weights (ca. 22 kDa). Using probes designed from the N-terminal amino acid sequence common to the three, we isolated 14 clones of cDNA belonging to three similar sequences which probably correspond to NtEPb1-b3. The predicted amino acid sequences showed moderate homology to NtEPc, several type-1 copper-binding glycoproteins and a kind of early nodulin. The level of the transcripts for NtEPbs is highly associated with the pollen dedifferentiation but not with pollen maturation nor with cell division accompanied by meiosis or proliferation of BY-2 cells. Such an expression manner was distinguished from that of a gene coding for A-type cyclin (Ntcyc 25), indicating that NtEPb genes are not under cell cycle control. These results suggest that there exist genes related to an unknown event other than the reentrance of cell cycle in the dedifferentiation process of immature pollen that may be important for acquisition of embryogenic competence.

Larsen, K. (2004). "Cloning and characterization of a ryegrass (Lolium perenne) gene encoding cinnamoyl-CoA reductase (CCR)." Plant Science **166**(3): 569.

http://www.sciencedirect.com/science/article/B6TBH-4B0NVHH-5/2/68f2c3f8d40f09f4ed69dcf70a6b5f70

Cinnamoyl-CoA reductase (CCR; EC 1.2.1.44) catalyzes the reduction of cinnamoyl acid-CoA esters into their corresponding aldehydes, the first step in the biosynthesis of lignin. The synthesis of lignin monomers is strictly associated with the activity of this enzyme which occurs in significant concentrations in lignin producing tissues. A 1207 bp cDNA (LpCCR) encoding a polypeptide of 344 amino acids with a predicted molecular mass of 37.4 kDa was isolated from a ryegrass (Lolium perenne) stem cDNA library. The identity of the LpCCR was established by comparison of the deduced polypeptide sequence with other isolated plant CCR enzymes. A motif, NWYCY, representing a putative CCR active site is conserved in the encoded LpCCR amino acid sequence. The encoded polypeptide exhibits sequence similarity to CCRs from different plants, the highest identities (87 and 86%, respectively) being to CCRs from Festuca arundicinea and Hordeum vulgare. Phylogenetic analysis shows that LpCCR and CCR from other monocotyledons form a group distinct from dicot CCRs. Genomic Southern blot hybridization demonstrated that LpCCR probably is represented as a single-copy gene in the ryegrass genome. The isolation of a single genomic CCR clone (gLpCCR) from a ryegrass leaf library further supports this observation. The gLpCCR contains four introns, the same number found in the Eucalyptus gunnii gCCR. Computer analysis of a 1333 bp 5'-flanking region of gLpCCR suggests the presence of binding sites for a MYB transcription factor and a binding site for a P-box factor of the Dof class transcription factors. In agreement with the involvement of CCR in lignification, the ryegrass CCR mRNA was detected in stem tissue only. The isolation and characterization of gLpCCR provides tools for genetically modifying plants with reduced lignin content thereby increasing forage digestibility.

Margis-Pinheiro, M., J. Marivet, et al. (1994). "Bean class IV chitinase gene: structure, developmental expression and induction by heat stress." Plant Science **98**(2): 163.

http://www.sciencedirect.com/science/article/B6TBH-47STS3K-YW/2/a2fdacb78ca6184b4849c5b9c05e27f7

A P4-chitinase genomic sequence was isolated from a bean (Phaseolus vulgaris) genomic library using a P4-ch cDNA. The complete sequence of the P4-ch gene was determined. Primer extension analysis allowed the identification of the transcriptional start site located 79 bp upstream of the translational initiation codon. The gene is interrupted by an intervening sequence. In the 5' upstream region, a TATT box occurs in place of a TATA at position -33, while a typical CAAT box is found at position -33 with respect to the transcription initiation site. Gibberellic acid, heat shock and salicylic acid regulatory responsive sequences were also identified. Transient expression of chimeric genes in tobacco protoplasts indicated that all the elements required for expression of the coding sequences are present within the first 600 bp of P4-ch 5' flanking DNA. Various stress conditions such as wounding, salicylic acid and NaCl treatments, heat and cold stress have been applied to the plants. Whereas wounding, NaCl treatment and cold stress are ineffective, transcription of P4-mRNAs is induced upon salicylic acid treatment and, suprisingly, in response to heat stress. P4-chitinase is induced during germination and seems to be constitutively expressed in roots of mature plants.

Mentewab, A., V. Cardoza, et al. "Genomic analysis of the response of Arabidopsis thaliana to trinitrotoluene as revealed by cDNA microarrays." Plant Science **In Press, Corrected Proof** http://www.sciencedirect.com/science/article/B6TBH-4DW3JY0-1/2/ad430c2e39557a93de1ddbb155e370cc

2,4,6-trinitrotoluene (TNT) is a nitro-substituted xenobiotic explosive that is toxic to plants and animals. Plants absorb and metabolize TNT, but the pathways are uncertain and plant responses at the molecular level have not been adequately characterized. We analyzed gene expression in response to relatively long-term exposure to TNT at low and high concentration in Arabidopsis through the use of cDNA microarrays. Arabidopsis seedlings were grown on media containing 1 and 10 [mu]M TNT, concentrations that were empirically determined by plant growth analysis. Microarray analysis revealed that a total of 52 genes were significantly upregulated, and 47 genes were downregulated in response to TNT at a 1.7-fold cut-off for differential gene expression. A substantial number of these genes have predicted functions in cell defense and detoxification. Conserved motifs were discovered in the promoter region of co-regulated genes, some of which are potentially novel cis-regulatory elements. With follow-up real time RT-PCR, we confirmed findings from the microarray experiments and examined the regulation of selected genes to two other xenobiotic substances: the explosive RDX and thiodiglycol. Results showed that increased transcription of At5g61600 encoding for a DNA-binding protein in shoots is specific to TNT and increased transcription of At5g42530 encoding for an unknown protein in shoots is specific to both TNT and RDX.

Narita, Y., H. Taguchi, et al. (2004). "Characterization of the salt-inducible methionine synthase from barley leaves." Plant Science **167**(5): 1009.

http://www.sciencedirect.com/science/article/B6TBH-4CTJ43C-1/2/7a466f1f9b68e509dd8c2466a4188053

We have cloned a cDNA encoding a putative methionine synthase from barley leaves by differential display. The full-length cDNA contained an open reading frame (2295 bp) encoding a deduced 765 amino acid polypeptide without any typical signal sequence, suggesting that it is localized in the cytosol. Expression of this gene was induced by salt stress in barley leaves. The gene expression was induced by light, but more strongly induced under salt, drought and cold stresses and by treatment with ABA or H2O2. A complementation test using a yeast mutant lacking the ability to synthesize methionine showed that this gene complements the yeast mutant under both non-stress and high-salinity (1 M NaCl) conditions. Although plant methionine synthase is known to be induced at the level of mRNA but not at that of protein, we found that the protein level of methionine synthase is also significantly increased in barley leaves under salt stress.

Patel, N., V. Cardoza, et al. (2004). "Differential gene expression of Chlamydomonas reinhardtii in response to 2,4,6-trinitrotoluene (TNT) using microarray analysis." Plant Science **167**(5): 1109.

http://www.sciencedirect.com/science/article/B6TBH-4CS4GX0-1/2/53c527190482e38b2b203f9bc981cf7c

The exposure of Chlamydomonas reinhardtii to environmental stress, such as that caused by the explosive 2,4,6-trinitrotoluene (TNT) can alter its gene expression. Expression analysis was conducted using a microarray composed of 3079 Chlamydomonas ESTs to characterize the broad range of responses of gene expression exposed to this common ordnance compound. TNT treatment conditions were determined by growth analysis of Chlamydomonas in 0-5 [mu]g/mL TNT. One and 3 [mu]g/mL were used for microarray analysis since 1 [mu]g/mL of TNT did not decrease the cell count after 7 days of treatment, whereas 3 [mu]g/mL of TNT was the maximum TNT concentration that allowed growth, respectively. Transcriptional profiling revealed that approximately 158 responsive genes were differentially expressed representing several functional categories. Genes responsible for photosynthesis, energy metabolism and oxidative stress were upregulated in the presence of TNT, while the expression of cell wall related genes were downregulated. Several unidentified genes were also affected. The microarray results were validated using real-time RT-PCR for a subset of genes. Information from the microarray analysis can be used to engineer algae-based sensors to signal TNT exposure in addition to potential explosives cleanup applications.

Pugliesi, C., M. G. Biasini, et al. (1993). "Genetic transformation by Agrobacterium tumefaciens in the interspecific hybrid Helianthus annuus x Helianthus tuberosus." Plant Science **93**(1-2): 105.

http://www.sciencedirect.com/science/article/B6TBH-47T25KD-7M/2/d6f8f3a88bd5308ff8c26fa973f8cdcc

A genetic transformation method has been developed in the interspecific hybrid Helianthus annuus x Helianthus tuberosus using Agrobacterium tumefaciens. Leaf explants of a clone with an efficient tissue culture regeneration capacity were inoculated with A. tumefaciens carrying a disarmed Ti plasmid containing the Cauliflower Mosaic Virus (CaMV) 35S-GUS fusion gene with the nopaline sinthase (NOS) neomycin phosphotransferase II (NPT II) gene. On selection medium containing 25 mg/l of kanamycin, the inoculated leaf explants formed meristematic centers with buds and embryo-like structures that successively developed into putative transformed shoots, when transferred onto medium without growth regulators. Under suitable conditions, three days of cocultivation on medium containing BAP and NAA, the highest transformation frequency was 5.4%. Histochemical staining for the [beta]-glucuronidase (GUS) activity provided evidence for transformation in different tissues and organs of transgenic plants. Integration of foreign DNA into genomic H. annuus x H. tuberosus DNA was demonstrated by

Southern analysis.

Redinbaugh, M. G. and W. H. Campbell (1998). "Nitrate regulation of the oxidative pentose phosphate pathway in maize (Zea mays L.) root plastids: induction of 6-phosphogluconate dehydrogenase activity, protein and transcript levels." Plant Science **134**(2): 129.

http://www.sciencedirect.com/science/article/B6TBH-3T3JCKY-2/2/79c7634a8b1e32511839801123c233db

We examined the effect of nitrate on the expression of the NADPH producing enzymes of the oxidative pentose phosphate pathway, glucose-6-phosphate dehydrogenase (G6PDH) and 6-phosphogluconate dehydrogenase (6PGDH) in maize seedlings (Zea mays L. W64A x A182E). In extracts of 5 day old maize roots and leaves treated with 10 mM KNO3, G6PDH and 6PGDH activities increased by 44 and 53%, respectively, relative to untreated roots. In isolated plastids from KNO3 treated roots, G6PDH and 6PGDH specific activities were more than 25- and 12-fold higher than in the untreated control. Western blot analysis showed higher levels of 6PGDH protein in root plastid extracts from KNO3 treated plants. The data suggest that KNO3 specifically affects the plastidic forms of G6PDH and 6PGDH. Three classes of 6PGDH cDNA were identified in maize roots. Of these, one cDNA hybridized with a transcript that accumulated rapidly and transiently in response to low concentrations of external nitrate. The accumulation of this transcript was not affected by pretreating plants with 50 [mu]g/ml cycloheximide, which was previously shown to inhibit cytoplasmic protein synthesis in maize roots by more than 85% (Gowri et al., Plant Mol. Biol. 26 (1998) 679). Neither NH4+ nor K+ treatment affected transcript accumulation. The data indicate coordinated regulation of genes and enzymes required for NO3- assimilation and NADPH production in root plastids.

Rogers, H. J. and H. C. Parkes (1999). "Direct PCR amplification from leaf discs." Plant Science **143**(2): 183.

http://www.sciencedirect.com/science/article/B6TBH-3WNMGG3-7/2/4568b3f4b4ec1646a3844bcb23943cc0

PCR amplification from leaf tissue has become an integral part of many plant molecular biology applications including screening for transformants, plant breeding, and molecular ecology. We have adapted an existing method to produce a simple protocol for the amplification of single or multicopy genes directly from leaf discs, particularly useful when screening large numbers of individuals.

Scarabel, L., N. Carraro, et al. (2004). "Molecular basis and genetic characterisation of evolved resistance to ALS-inhibitors in Papaver rhoeas." Plant Science **166**(3): 703.

http://www.sciencedirect.com/science/article/B6TBH-4B5J8VT-1/2/28e76dabc2436328b034602602bbef8b

ALS gene from susceptible and field-selected Papaver rhoeas populations resistant to ALS-inhibitor herbicides was studied. The full-length cDNA and genomic sequence coding for acetolactate synthase (ALS) of a susceptible population of poppy was cloned and sequenced. Some peculiarities in poppy ALS gene were identified aligning the sequences in GenBank. Southern analysis using a 800 bp fragment of ALS showed that poppy possesses a single copy of

the gene. Partial ALS genomic DNAs from nine poppy populations resistant to ALS-inhibitor herbicides and four susceptible populations, collected in central and southern Italy where resistance to ALS-inhibitors is increasing due to repeated use of sulfonylureas, were amplified and sequenced. Comparison of the coding sequences identified three independent point mutations leading to different amino acid substitutions in the deduced polypeptide sequence. The three point mutations, all at proline 197 (CCT) (based on Arabidopsis numbering) in the conserved domain A of the gene, included a change of Pro to His (CAT), to Thr (ACT) or to Ser (TCT). These mutations cause similar cross-resistance patterns. Analysis of the progeny of two crosses between resistant (R) and susceptible (S) biotypes indicated that resistance is inherited as a dominant monogenic trait, although seed dormancy may interfere with a correct segregation of R and S biotypes in the progeny. These are the first mutations completely characterised in poppy ALS gene that confer resistance to ALS-inhibitor herbicides.

Shih, C.-Y. T., J. Wu, et al. (2001). "Purification of an osmotin-like protein from the seeds of Benincasa hispida and cloning of the gene encoding this protein." Plant Science **160**(5): 817.

http://www.sciencedirect.com/science/article/B6TBH-42RDSMC-5/2/29807e91989e4045bed6d25da78d1b04

A pathogenesis-related (PR) protein was purified from the seeds of Benincasa hispida, which is a medicinal plant and a member of the Cucurbitaceae family. Purification was achieved by using a procedure consisting of an acid treatment step followed by two chromatography steps. The protein is a basic protein with molecular mass of ~28 kDa. The sequences of the N-terminal 30 amino acids and four peptides generated from protease digestion were determined. These sequences indicated that the protein is an osmotin-like protein (OLP). Osmotin and OLPs are members of the thaumatin-like, PR-5 family of the PR proteins. A genomic clone of the gene encoding the protein was isolated and sequenced. The predicted protein has a signal peptide of 18 amino acids, and the mature protein has a molecular mass of 24.8 kDa with an isoelectric point of 7.67. The protein has 17 cysteine residues, of which 16 appear in the same positions as those appear in the sweet-tasting protein thaumatin and several other thaumatin-like proteins. Southern hybridization analysis indicated that the gene encoding the protein is a single copy gene. A computer-generated, three-dimensional model of the protein is presented.

Skadsen, R. W., P. Sathish, et al. (2000). "Expression of thaumatin-like permatin PR-5 genes switches from the ovary wall to the aleurone in developing barley and oat seeds." Plant Science **156**(1): 11.

http://www.sciencedirect.com/science/article/B6TBH-40SFG52-2/2/79c88f4e8cab3cc994806ef42be88677

Permatins are antifungal thaumatin-like proteins (TLPs) of the PR-5 family of pathogenesis-related proteins. They occur in many cereals, but little is known of their expression and roles. Permatin cDNA clones were produced and used to study expression in developing barley and oat seeds. Actin and CDC48 mRNAs declined rapidly following inoculation of barley spikes with Fusarium graminearum. Despite this, permatin mRNA levels remained constant or increased slightly. Studies of permatin gene expression in healthy plants revealed that developing barley and oat seeds accumulate permatin mRNA in an unusual bimodal pattern. Permatin mRNA and protein are highly abundant around the time of pollination and then decrease rapidly to near-zero. A second peak occurs in the doughy stage of development. Antibody and DNA probe hybridization studies showed that expression initially occurs in the ovary wall and then switches to the aleurone and ventral furrow of developing seeds, reaching a peak in the doughy stage. Small amounts of permatin mRNAs also occur in certain vegetative tissues. The barley and oat

permatin sequences provided sufficient comparisons between cereal TLPs to suggest that deletions or additions in specific elements could have led to the divergence of leaf- and seed-specific TLPs.

Suzuki, S. and J. N. Burnell (2003). "The pck1 promoter from Urochloa panicoides (a C4 plant) directs expression differently in rice (a C3 plant) and maize (a C4 plant)." Plant Science **165**(3): 603.

http://www.sciencedirect.com/science/article/B6TBH-48YVMWT-1/2/6d959a1ac460304850eea1f25d1e3ace

A chimeric gene using [beta]-glucuronidase (GUS) as a reporter gene under the control of a 1.3 kb 5'-flanking region of pck1 (involved in C4 photosynthesis in Urochloa panicoides) was introduced into rice and maize. GUS activity was detected in leaf blades, leaf sheaths and roots of transgenic rice plants and was detected at high levels in leaf blades and at low levels in leaf sheaths and roots of transgenic maize plants. The pck1 promoter drove the expression of GUS activity in transgenic maize following 6 h of illumination. In contrast, GUS activity was not induced in transgenic rice even after 24 h illumination. Histochemical analysis revealed that GUS staining was localized to bundle sheath cells and vascular bundles of both rice and maize transformants and GUS activity in bundle sheath cells of transgenic maize was induced by light. These results suggest that the 1.3 kb pck1 promoter contains cis-acting elements for preferential and abundant expression in bundle sheath cells of the leaf blade with light dependence in maize but rice lacks some trans-acting elements required for the expression controlled by pck1.

Taylor, M. A. J., M. Al-sheikh, et al. (1999). "cDNA cloning and expression of Carica papaya prochymopapain isoforms in Escherichia coli." Plant Science **145**(1): 41.

http://www.sciencedirect.com/science/article/B6TBH-3WYHT1T-5/2/d1a563f4bd1fcae7377101283b226c47

Chymopapain is one of the four known cysteine proteinases found in the latex of Carica papaya. DNA sequencing of clones derived from a leaf cDNA library identified five cDNA types coding for precursor chymopapains. All of these isoforms have a free cysteine residue at position 117, characteristic of chymopapain. Two of the isoforms possess a further free cysteine residue, which is not likely to be involved in disulphide bonds or the active site apparatus. Another amino acid substitution found in two of the isoforms at position 133 is predicted to lie in the S2 subsite of the substrate binding cleft. One of the prochymopapain isoforms was expressed in Escherichia coli. Protein was expressed as insoluble inclusion body material. This protein was solubilised, refolded and autocatalytically cleaved to yield mature chymopapain that had comparable kinetic constants to authentic native enzyme.

Tregear, J. W., S. Jouannic, et al. (1996). "An unusual protein kinase displaying characteristics of both the serine/threonine and tyrosine families is encoded by the Arabidopsis thaliana gene ATN1." Plant Science **117**(1-2): 107.

http://www.sciencedirect.com/science/article/B6TBH-3YVVC77-C/2/efb2909bff1c0314c263a18d3d5ac236

A cDNA clone, ATN1, coding for a novel protein kinase, was isolated from an Arabidopsis thaliana inflorescence cDNA library. The deduced ATN1 protein sequence of 356 amino acid

residues contains all the invariant hallmarks of the protein kinase superfamily. The ATN1 protein is however unusual in that it contains not only amino acid motifs characteristic of the protein serine/threonine kinase family, but also residues typical of protein tyrosine kinases. The ATN1 protein is not closely related to any other protein kinases characterised to date in either plants or other organisms. A database search revealed only 6 characterised protein kinases which shared greater than 30% identities with ATN1 (ranging from 33.5-41.4%) in their catalytic domains; each of the related sequences has mixed serine/threonine and tyrosine kinase affinities. The N-terminal region of ATN1 displays and N-myristoylation motif similar to those found in a number of other protein kinases. Although ATN1 does not belong to an established plant protein kinase subfamily, it is evolutionarily closest to a group which includes kinases involved in transmembrane signalling (the 'receptor-like kinases'). The ATN1 gene forms part of a small multigene family and is expressed in all organs of the plant studied.

Ullanat, R. and C. Jayabaskaran (2002). "Distinct light-, cytokinin- and tissue-specific regulation of calcium dependent protein kinase gene expression in cucumber (Cucumis sativus)." Plant Science **162**(1): 153.

http://www.sciencedirect.com/science/article/B6TBH-44HXD9P-J/2/99a5e9fed5dc37724a74cb135fe0c641

In plants, calcium dependent protein kinases (CDPKs) constitute a unique family of enzymes that is characterized by a C-terminal calmodulin (CaM)-like domain. In this study, we have cloned four partial CDPK cDNAs (CsCDPK1-4) from cucumber by reverse transcription polymerase chain reaction (RT-PCR) using degenerate oligonucleotide primers designed based on conserved regions of the other known CDPKs. Transcript levels of one of the CDPK messengers viz. CsCDPK3 were measured in intact, etiolated, excised cotyledons, hypocotyls and roots following treatments with light or phytohormones (cytokinin/auxin) using a recently evolved high-sensitivity quantitative RT-PCR method (TaqMan analysis). The highest transcript levels of CsCDPK3 were detected in hypocotyls followed by roots and cotyledons. Exposure to light was found to have a down-regulatory effect on CsCDPK3 transcript levels in excised hypocotyls and roots unlike in excised cotyledons where light was found to exert an up-regulatory effect. Treatment with benzyladenine (cytokinin) up-regulated CsCDPK3 transcript levels in cotyledons as opposed to a down-regulatory effect in roots and did not seem to have a significant effect on CsCDPK3 transcript levels in hypocotyls. On the other hand, 2,4-dichlorophenoxyacetic acid (2,4-D) (auxin) treatments did not cause any significant changes in CsCDPK3 transcript levels in hypocotyls, cotyledons or roots. Thus, our results show that light and cytokinin differentially regulate CsCDPK3 transcript levels in a tissue-specific manner.

Wang, Z.-Y., Y. Ge, et al. (2005). "Development of highly tissue culture responsive lines of Lolium temulentum by anther culture." Plant Science **168**(1): 203.

http://www.sciencedirect.com/science/article/B6TBH-4D5X79R-2/2/395fed77aa2deb23a6b063b3d664ad5e

Most of the important forage and turf grasses are outbreeders, require vernalization to flower, and in some cases are polyploid. With the development of gene sequencing information in grasses, there is an urgent need for a model system to perform large-scale functional analysis of candidate genes. We propose to use Lolium temulentum L. (Darnel ryegrass) as a model system for genetic manipulation studies in forage and turf grasses because L. temulentum has the following advantages: self-fertile, short life cycle (11 weeks), diploid, easy to grow, and closely related with other major grass species. In order to improve tissue culture response of L. temulentum, two relatively responsive lines were crossed and putative hybrid seeds obtained. Analysis of the F1

plants by SSR markers confirmed that the F1 plants were true hybrids. Anthers were dissected from F2 plants of the cross, 3.0% of the cultured anthers responded with the formation of calluses. Green haploid plants were recovered from 48.9% of the anther-derived calluses. Seeds were harvested from doubled haploid plants and mature embryos were used as explants for comparing tissue culture responses with other lines. Besides anther culture, F2 seeds of the cross were subjected to a cycle of selection for callus formation and plant regeneration, and F4 seeds were obtained from the regenerated plants. Comparison of tissue culture response of different lines revealed that the doubled haploid plants had a much higher frequency of embryogenic callus formation than that of the parental lines and the F4 seeds. This is the first report on the generation of green haploid and doubled haploid plants in L. temulentum. The technique was successfully used for the rapid production of homozygous diploid plants that are highly tissue culture responsive. The anther culture-derived new L. temulentum lines could be valuable material for functional test of genes in grasses.

Xu, J., T. Lange, et al. (2002). "Cloning and characterization of a cDNA encoding a multifunctional gibberellin 20-oxidase from perennial ryegrass (Lolium perenne L.)." Plant Science **163**(1): 147.

http://www.sciencedirect.com/science/article/B6TBH-45WGGVV-1/2/9832d08fc4ff9e0ac12d61138e61344e

Using degenerated oligonucleotide primers derived from the gibberellin (GA) 20-oxidase sequence from pumpkin (Cucurbita maxima) and Arabidopsis thaliana, an internal fragment of 525 bp was amplified by nested polymerase chain reaction from cDNA of immature inflorescence of perennial ryegrass (Lolium perenne L.). The full-length ryegrass GA 20-oxidase genomic DNA sequence was isolated by genome walking. The deduced full-length cDNA clone (Lp20ox) of perennial ryegrass immature inflorescence was isolated by polymerase chain reaction. Sequence comparison reveals that the putative amino acid sequence shares 57.1% homology with pumpkin (C. maxima) and 92.3% homology with wheat (Triticum aestivum). Expression of Lp20ox in Escherichia coli catalyzed the conversion of GA12- to GA9 and GA53- to GA20, indicating a highly specific GA 20-oxidase activity. Southern blot analysis suggests the presence of two copies of GA 20-oxidase in the ryegrass genome. Lp20ox mRNA accumulated in germinating seeds, expanding leaves, inflorescence, actively growing shoots and internodes, while it could not be detected in developing ovaries and immature seeds.

Zhao, T.-Y., R. B. Meeley, et al. (2003). "Aberrant processing of a Maize GALACTINOL SYNTHASE transcript is caused by heat stress." Plant Science **165**(1): 245.

http://www.sciencedirect.com/science/article/B6TBH-48J4M0N-1/2/024c9b4bdae48fdae532bb07612271b4

Summary: GALACTINOL SYNTHASE constitutes a highly homologous, small gene family in maize. ZmGOLS2 cDNA probe detects full-length ZmGOLS transcript in dehydration stressed-, and a smaller transcript (ST) in heat stressed-germinating seeds and callus cells. The ST can be detected in seeds imbibed at temperatures above 30 [deg]C, attaining greatest abundance at 40 [deg]C. At 45 [deg]C, the ST is no longer detected and the full-length transcript is again prevalent. Northern blot analysis of poly(A) selected mRNA indicates that the ST is polyadenylated. The ST can be detected by antisense but not by sense RNA probes. Only the five-prime-third of the ZmGOLS2 cDNA is homologous to the ST. However, ribonuclease protection assays (RPA) using a probe to the 5' portion of ZmGOLS2, led to the conclusion that only ZmGOLS3-, not ZmGOLS2-transcript, is present in heat stressed seeds. The small transcript detected by ZmGOLS2 probe is not derived from ZmGOLS2 but from an unknown, highly homologous gene. Using 3' RACE, a full-length and a short ZmGOLS3 cDNA were cloned. Sequencing revealed that the short

ZmGOLS3 transcript is a fusion of the 5'- and 3'-UTR regions of ZmGOLS3. Comparison with the gene sequence revealed that there are no typical intron-exon junction structures around the deleted fragment of ZmGOLS3. Instead, a five base pair, GC-rich sequence delineates the deletion sites used to form the ZmGOLS3 short transcript. Southern blot analysis using maize genomic DNA as a template confirmed that the aberrant ZmGOLS3 transcript was produced due to aberrant RNA processing and is not due to transcription of a ZmGOLS3 pseudogene.

Postharvest Biology and Technology (3)

Boysen, M. E., S. Bjorneholm, et al. (2000). "Effect of the biocontrol yeast Pichia anomala on interactions between Penicillium roqueforti, Penicillium carneum, and Penicillium paneum in moist grain under restricted air supply." Postharvest Biology and Technology **19**(2): 173.

http://www.sciencedirect.com/science/article/B6TBJ-407GJ3B-8/2/8498a668b9436fd414c336501776274c

Penicillium roqueforti was recently reclassified into the three species P. roqueforti, Penicillium carneum, and Penicillium paneum based on differences in ribosomal DNA sequences and secondary metabolites, e.g. mycotoxins. This is the first report on interaction between these closely related mould species under stress conditions. The yeast Pichia anomala (J121) inhibits growth of P. roqueforti in grain stored in malfunctioning airtight storage systems. The ability of P. anomala to inhibit all three species of the P. roqueforti group was examined in separate experiments as well as the competition between the three mould species when co-cultured with or without the yeast in non-sterile wheat grain (aw 0.95) under restricted air supply. Mould growth was analysed by dilution plating after 14 days and the individual colonies identified by random amplified polymorphic DNA (RAPD) fingerprinting. When co-culturing the P. roqueforti group in wheat without P. anomala all three species were able to grow to the same extent. Also, when co-culturing all species of the P. roqueforti group together with P. anomala, the growth response of the three species was very similar. At yeast levels of 104 CFU g-1 grain a pronounced inhibition was observed and at 105 CFU g-1 grain a fungicidal effect was detected, indicating a potentiated effect of P. anomala when co-culturing the three mould species.

Narumi, T., Y. Kanno, et al. (2005). "Cloning of a cDNA encoding an ethylene receptor (DG-ERS1) from chrysanthemum and comparison of its mRNA level in ethylene-sensitive and -insensitive cultivars." Postharvest Biology and Technology **36**(1): 21.

http://www.sciencedirect.com/science/article/B6TBJ-4FG899S-1/2/b3d3f06e934f3144d24284b14c539946

A cDNA encoding a putative ethylene receptor (DG-ERS1) was isolated from chrysanthemum [Dendranthema grandiflorum (Ramat.) Kitamura] using a combination of reverse transcription PCR (RT-PCR), cDNA library screening and 5'-RACE techniques. The cDNA (2427 bp) contained an open reading frame of 1920 bp coding for 640 amino acids. The predicted DG-ERS1 protein has an amino-terminal ethylene sensor domain and a histidine kinase domain, but lacks a receiver domain. The DG-ERS1 protein has 72, 70 and 69% similarity to Arabidopsis ERS1, tomato Never ripe (NR) and carnation DC-ERS2, respectively. Real time PCR analysis revealed that DG-ERS1 mRNA was present in a large amount in ligulate corollas (hereafter, petals for

short) and mature leaves of an ethylene-sensitive cultivar 'Seiko-no-makoto' at the full-opening stage of the flower, and the amount decreased with time or in response to a 12-h ethylene treatment. In an ethylene-insensitive cultivar 'Iwa-no-hakusen', the amount of DG-ERS1 mRNA in petals was one-fourth and that in mature leaves was only one-twentieth of the amount in 'Seiko-no-makoto' at the full-opening stage, and its amount in both tissues scarcely changed with time or in response to a 12-h ethylene treatment. These findings suggest the involvement of DG-ERS1 in the perception of ethylene in cut chrysanthemum plants, especially in those of 'Seiko-no-makoto' cultivar.

Nishikawa, F., M. Kato, et al. (2003). "Two ascorbate peroxidases from broccoli: identification, expression and characterization of their recombinant proteins." Postharvest Biology and Technology **27**(2): 147.

http://www.sciencedirect.com/science/article/B6TBJ-46Y5881-2/2/6725fa548dc14d77ba35bfe949858af0

Two distinct clones having high nucleotide identity to the sequences encoding ascorbate peroxidase (APX) were isolated from broccoli (Brassica oleracea L. var. italica). Deduced amino acid sequences of both cDNAs, BO-APX 1 (accession number AB078599) and BO-APX 2 (accession number AB078600), shared identity of 92.8% and there was more than 80% identity between BO-APXs and other plant cytosolic APXs at the protein level. Gene expression and protein levels of BO-APX 1 and BO-APX 2 were investigated in various parts of broccoli after harvest. Transcript levels of BO-APX 2 gradually increased in florets, while those of BO-APX 1 decreased in florets after harvest. BO-APX 1 and BO-APX 2 were expressed in Escherichia coli as a fusion protein with glutathione S-transferase (GST) and purified to homogeneity by glutathione sepharose 4B column chromatography. Both proteins of BO-APX 1 and BO-APX 2 appeared as a single major band on SDS-PAGE corresponding to a mass of 25 kDa and reacted with polyclonal antibodies raised against recombinant BO-APX 1. Both enzymes showed high specificities for ascorbate and hydrogen peroxide. The km values of recombinant BO-APX 1 and BO-APX 2 for ascorbate were 395 and 526 [mu]M and those for hydrogen peroxide were 15 and 7 [mu]M, respectively. The role of APX was discussed in relation to ascorbate breakdown in broccoli florets during senescence.

Preventive Veterinary Medicine (1)

Milian-Suazo, F., V. Banda-Ruiz, et al. (2002). "Genotyping of Mycobacterium bovis by geographic location within Mexico." Preventive Veterinary Medicine **55**(4): 255.

http://www.sciencedirect.com/science/article/B6TBK-45S9CHT-1/2/f32a690a77e9483b44dc84637601db16

The spacer oligonucleotide typing (spoligotyping) method was used to differentiate 62 Mycobacterium bovis isolates obtained from tissues with macroscopic lesions typical of tuberculosis in dairy cattle from different regions of Mexico. Our purpose was to see if a strain from one region was genetically different from those of other regions (with the long-term aim of doing molecular trace back of isolates obtained in the laboratory). Results from the genetic analysis indicate that M. bovis isolates cannot be grouped by geographic location due to a wide

range of genetic types involved in dairy cattle infections. Isolates even from the same herd showed different spoligotypes but some isolates from different region had similar genetic patterns. Genetic typing without epidemiologic information does not seem to be a plausible method to trace back animals to source of origin to detect and eliminate sources of infection.

Reproductive Toxicology (1)

Spencer, F., L. Chi, et al. (2001). "A mechanistic assessment of 1,3-butadiene diepoxide-induced inhibition of uterine deciduoma proliferation in pseudopregnant rats." Reproductive Toxicology **15**(3): 253.

http://www.sciencedirect.com/science/article/B6TC0-435CSWR-5/2/9f3b5782034320e7912b5a9a4928cad0

Butadiene diepoxide (BDE), a reactive metabolite of 1,3-butadiene that is an important industrial chemical used in synthetic rubber production causes a dose-dependent inhibition of deciduoma development in pseudopregnant Sprague-Dawley rats. This study used 4 daily i.p. BDE doses of 0.20, 0.25, 0.30, 0.35, or 0.40 to characterize mechanisms that may be responsible for the antideciduoma effect. Pseudopregnant rats were treated either before (pseudopregnancy [PPG] days 1-4) or after (PPG days 5-9) deciduoma induction by endometrial trauma with a blunt needle. Animals were killed on PPG day 9 and evaluated for serum progesterone and endometrial protein and DNA. RT-PCR was used to measure message for estrogen receptor (ER) [alpha] and pituitary adenylate cyclase-activating polypeptide (PACAP). Substrate zymography and Western blotting were used respectively to measure matrix metalloproteinase (MMP)-9 and inducible nitric oxide synthase. The antideciduoma effects of BDE were associated with decreases in endometrial weight, protein, and DNA, with decreases in serum progesterone, and with decreases in PACAP message and MMP-9. A reduction in NOS was identified at the highest dose of BDE. Message for estrogen receptor (ER) [alpha] was not affected at any dose. We conclude that the reduction in decidual proliferation was direct and appeared to be associated with either 1) a decrease in the effectiveness of the deciduogenic stimulation and/or a weakened endometrial sensitivity to the stimulus; or 2) an effect on deciduoma development. Molecular mechanisms that apparently contributed to BDE inhibition of decidual metabolism included the synthesis of protein and DNA involved in decidual growth, the synthesis and activation of a matrix metalloproteinase for degradation of the extracellular matrix that is essential for tissue remodeling during deciduoma development, and the nitric oxide/nitric oxide synthase and pituitary adenylate cyclase-activating peptide systems that are involved in promoting vasodilation and increased vascular permeability to enhance the availability of substrates for maximal deciduoma growth. The ovotoxicity of BDE, which has previously been established, may indirectly affect decidual proliferation by reducing progesterone, the preeminent endocrine regulator of deciduoma development. The findings also suggest that BDE may possess no estrogenic action since it was associated with endometrial weight loss and unaltered levels of the estrogen receptor [alpha] mRNA expression.

Research in Microbiology (6)

de Lamballerie, X., C. Zandotti, et al. (1992). "A one-step microbial DNA extraction method using "Chelex 100" suitable for gene amplification." Research in Microbiology **143**(8): 785.

http://www.sciencedirect.com/science/article/B6VN3-47DDBMR-1C/2/701a8333b92d149bcfac19d01fdfeaaa

"Chelex 100" chelating resin has been previously proposed for the rapid extraction of human DNA for polymerase chain reaction. Protocols are given for the rapid extraction of bacterial and viral DNA from cultures or clinical samples. The DNA samples obtained were suitable for use in polymerase chain reaction.

Janvier, B., C. Constantinidou, et al. (1998). "Characterization and gene sequencing of a 19-kDa periplasmic protein of Campylobacter jejuni/coli." Research in Microbiology **149**(2): 95.

http://www.sciencedirect.com/science/article/B6VN3-3V7PB37-9/2/959942c811704a88371e01edf07d6abc

In order to study a 19-kDa protein (p19) of Campylobacter jejuni, we purified this protein to homogeneity from C. jejuni strain 81176 by anion exchange chromatography. The molecular weight of the native protein is 19,000 daltons. P19 was found to be acidic with an isoelectric point of 4.8 and was located in the periplasmic space of the bacteria. The 20 N-terminal amino acids were sequenced and no significant similarities with known proteins were shown. A monoclonal antibody showed that p19 is conserved in the 2 species C. jejuni and C. coli. Analysis of sera from 23 patients with a Campylobacter-related infection indicated that p19 is not immunogenic during natural infection in man. The gene encoding p19 was cloned and no strong homologies with known sequences were identified. The preparation of a knockout mutant in p19 will enable the investigation of the function of this cell wall component of Campylobacter.

Lagatolla, C., A. Lavenia, et al. (1998). "Characterization of oligonucleotide probes for the identification of Acinetobacter spp., A. baumannii and Acinetobacter genomic species 3." Research in Microbiology **149**(8): 557.

http://www.sciencedirect.com/science/article/B6VN3-3XXDWSF-3/2/133e5f862e14c8665a31fb87cfaa233d

The 16S-23S intergenic spacer regions of four Acinetobacter genomic species belonging to the A. calcoaceticus-A. baumannii (Acb) complex, i.e. genomic species 1 (A. calcoaceticus), genomic species 2 (A. baumannii), genomic species 3 and Tjernberg and Ursing (TU) genomic species 13, have been cloned and sequenced. Sequence analysis led to the discovery of a single copy of Ile and Ala tRNA genes within each spacer. Sequence comparison allowed the identification of a 192-base-pair long highly conserved sequence between the 3' end of the 16S rRNA and the 5' end of the tRNAAla genes. Moreover, two short regions, which were specific to, respectively, genomic species 2 and 3, could be identified. Oligonucleotides corresponding to these sequences were constructed and tested for the ability to hybridize with chromosomal DNA extracted from Acinetobacter belonging to different genomic species and with chromosomal DNA of other bacterial genera. One of these oligonucleotides was demonstrated to be useful as a sensitive and specific probe for A. baumannii. A less sensitive probe for Acinetobacter genomic species 3 was also developed.

Paffetti, D., C. Barberio, et al. (1995). "DNA fingerprinting by random amplified polymorphic DNA and restriction fragment length polymorphism is useful for yeast typing." Research in Microbiology **146**(7): 587.

http://www.sciencedirect.com/science/article/B6VN3-3Y5FMKC-8/2/bfffca24f68b2b5eb3602432c4911f1c

Random amplified polymorphic DNA (RAPD) analysis was applied to genomic DNA from nineteen yeast strains belonging to the genera Saccharomyces and Zygosaccharomyces. Results obtained with five primers indicated that this technique is a powerful tool for yeast differentiation and identification. The data were consistent with those derived from restriction fragment length polymorphism (RFLP) using two S. cerevisiae DNA probes. We conclude that RAPD fingerprinting, combined with the analysis of RFLP, can provide unambiguous type assignment in yeasts.

Schvoerer, E., F. Bonnet, et al. (2000). "PCR detection of human enteric viruses in bathing areas, waste waters and human stools in southwestern France." Research in Microbiology **151**(8): 693.

http://www.sciencedirect.com/science/article/B6VN3-41GWN7Y-9/2/10b1617605570d31fc47b17aa77edfab

Detection of human pathogenic viruses by molecular techniques might be suitable for identifying viral pollution in environmental waters and for improving diagnosis in patients. Environmental samples were taken from bathing areas and sewage treatment plants in southwestern France. Small volume samples (50 [mu]L) were tested. Five groups of enteric pathogenic viruses were studied: enteroviruses, Norwalk-like viruses (NLVs), hepatitis A virus, rotaviruses and adenoviruses. Moreover, human samples were tested for NLV. After extraction of viral nucleic acids (Boom's procedure), a nested polymerase chain reaction was conducted before hybridization. Five bathing waters out of 26 were positive for one viral group, without systematic association with bacterial contamination. Eight sewage plant samples out of 13 were positive for at least one viral group. Seven patients out of 45 were NLV-positive. Molecular techniques allow efficient screening of viral contamination in environmental waters and the study of NLV molecular epidemiology.

Wang, J., V. Michel, et al. (1998). "Cloning of the J gene of bacteriophage lambda, expression and solubilization of the J protein: first in vitro studies on the interactions between J and LamB, its cell surface receptor." Research in Microbiology **149**(9): 611.

http://www.sciencedirect.com/science/article/B6VN3-3WB7RD8-C/2/01c1e3e7eeab351a96d8cce2c36ec84f

Bacteriophage [lambda] adsorbs to its Escherichia coli K12 host by interacting with a specific cell surface receptor, the outer membrane protein LamB. Previous genetic analyses led us to define a set of residues at the surface of LamB, which belong to the [lambda] receptor site. Further genetic studies indicated that the C-terminal portion of J, the tail fibre protein of [lambda], was directly involved in the recognition of the receptor site.The present work describes first in vitro studies on the interactions between J and LamB. The J gene of [lambda] was cloned into a plasmid vector under ptac promoter control and expressed in E. coli. We showed that J could be expressed at high levels (up to 28% of whole cell proteins), in an insoluble form. Anti-J antibodies, induced in

rabbits immunized with insoluble J extracts, appeared to specifically neutralize [lambda] infection.Under defined conditions of extraction, the J protein was obtained in a soluble form. We showed that solubilized J was able to interact with LamB trimers in vitro. Implications for future studies on the interactions between LamB and J are discussed.

Research in Veterinary Science (7)

Amin, A. S. (2003). "Application of touchdown enzyme time release (TETR)-PCR for diagnosis of Chlamydophila abortus infection." Research in Veterinary Science **74**(3): 213.

http://www.sciencedirect.com/science/article/B6WWR-48BC03B-1/2/0a4dcfd9bb9df501470d11070e2d5bea

Chlamydophila abortus-DNA was detected using a touchdown enzyme time-release (TETR)-polymerase chain reaction (PCR) assay as an improved test for sensitive and rapid diagnosis of abortion in small ruminants. Two hundred and fifty two placentae, liver or spleen tissue samples from aborting ewes and goats or aborted lambs and kids in which C. abortus infection was suspected were examined by TETR-PCR and the results were compared with cell culture. Sixty-five tissue samples were found to be TETR-PCR positive while only 56 samples were cell culture-positive. After resolution of discrepant samples with a confirmatory nested PCR assay, TETR-PCR had a sensitivity of 97% and a specificity of 99.5% while culture had a sensitivity of 84.8% and a specificity of 100%. The analytical sensitivity of the TETR-PCR assay was determined with DNA extracted from 4-fold serial dilution of C. abortus B577 culture and found to be 0.25 inclusion-forming unit per PCR. No reduction in the analytical sensitivity was noted when the assay was tested with mouse liver samples spiked with 4-fold serial dilution of C. abortus B577 culture. No target product was amplified when DNA from Chlamydophila pecorum was tested. TETR-PCR used in this study is a practical, rapid, sensitive and specific assay that could be used for the detection of C. abortus in infected tissue samples. We recommend the use of this assay as a supplemental diagnostic tool for detection of C. abortus in infected tissue samples.

Awan, A. R., M. Baxi, et al. (1995). "EHV 1-induced abortion in mice and its relationship to stage of gestation." Research in Veterinary Science **59**(2): 139.

http://www.sciencedirect.com/science/article/B6WWR-4CWRXMS-1S/2/db52f8ccd300a9478561256682d0142f

The most important consequence of equine herpesvirus-1 (-1) infection is abortion. The object of the present study was to characterise further a murine -1 abortion model and to make comparisons with the natural host with particular reference to the stage of gestation during which the infection occurs. /c mice at different stages of pregnancy were infected intranasally with -1 (strain A134); they suffered respiratory distress, weight loss, and other constitutional signs of infection. When the virus was inoculated in the late second or early third week of gestation dead or dying fetuses were aborted, whereas infection between seven and nine days of pregnancy led to fetal death and resorption. During the process of resorption, complications were observed. Virus was frequently isolated from the placentas and occasionally from the tissues of the aborting fetuses, depending on the severity of the infection of the placentas. In some cases, therefore, the inoculation resulted in abortion although the infection was restricted to the placenta. Virus antigen

was detected in the placentas, lungs and occasionally in other tissues of the aborting fetuses. The potential of this murine model for testing methods for the diagnosis and control of equine abortion is discussed.

Bashiruddin, J. B., P. de Santis, et al. (2005). "Detection of Mycoplasma mycoides subspecies mycoides SC in bovine lung and lymph node tissues by culture, sandwich ELISA and polymerase chain reaction systems." Research in Veterinary Science **78**(3): 199.

http://www.sciencedirect.com/science/article/B6WWR-4DTKYFF-2/2/0f0eea5bfe5de3998c699c64f39b486e

Cattle from Northern Portugal, many with pulmonary lesions typical of contagious bovine pleuropneumonia, were investigated for the presence of Mycoplasma mycoides subspecies mycoides small colony (MmmSC), which is the causative agent of CBPP, with several detection tests. Sandwich ELISA that included a culture enrichment stage, and 2 different PCR diagnostic systems were used to detect MmmSC in lung and mediastinal lymph node tissues from these animals. The comparison of typical CBPP pathology with the results of detection revealed that no single one of these methods provided a perfect match to the pathological data. Best performing tests were the PCR with laser induced fluorescence and PCR with pleuroTRAP kit (Chemicon, Australia), which are diagnostic systems based on amplification of genomic MmmSC DNA followed by sensitive detection of the amplified products. These were followed by the broth-enriched sandwich ELISA, which uses a monoclonal antibody specific to the M. mycoides cluster, to capture the antigen.

Bucher, K., G. Szalai, et al. (1996). "Tumour suppressor gene p53 in the horse: identification, cloning, sequencing and a possible role in the pathogenesis of equine sarcoid." Research in Veterinary Science **61**(2): 114.

http://www.sciencedirect.com/science/article/B6WWR-4D8W9G1-V/2/80646178c1bdb5a3c3dd257872096696

The tumour suppressor protein p53 enhances the genetic stability of the cell and plays a critical role in tumour suppression. Equine p53 was analysed by sequencing exons 5 to 9, a region which includes most known mutations and all the mutational hotspots in the species that have been investigated. The fragment was amplified, cloned and sequenced from genomic and complementary DNA. A comparison of the predicted amino acid sequences between the horse and other species resulted in identities between 66 per cent with the clawed frog and 92 per cent with the cat. Using the single strand conformation polymorphism technique, exons 5 to 8 amplified from sarcoid tissue and peripheral leucocytes of 28 sarcoid-affected and 11 healthy horses were screened for mutations. No mutations were identified, suggesting that the frequency of p53 mutations in equine sarcoid might be low. However, the high incidence of bovine papillomavirus infection in equine sarcoid may indicate the functional inactivation of p53 by -encoded E6 protein.

Davidson, A. J., J. E. Hodgkinson, et al. (2005). "Cytokine responses to Cyathostominae larvae in the equine large intestinal wall." Research in Veterinary Science **78**(2): 169.

http://www.sciencedirect.com/science/article/B6WWR-4D7K29M-1/2/60a6ad3c68e341cdab3a0a6c3ee3d827

To investigate cytokine responses in cyathostomin infection, we quantified mucosal interleukin-4 (IL-4), interleukin-10 (IL-10), tumour necrosis factor (TNF)-[alpha] and interferon (IFN)-[gamma] by reverse transcriptase-competitive polymerase chain reaction. The analysis was performed on large intestinal wall samples obtained from six anatomical sites spanning the caecum and colon of 17 naturally exposed horses. The numbers of developing larvae (DL) and early third stage larvae (EL3) were ascertained using transmural illumination and pepsin digestion techniques, respectively. Levels of each cytokine transcript were correlated with local intestinal wall burdens of Cyathostominae larvae. IL-4 and IL-10 levels showed significant correlations with EL3 and DL burdens at several sites. No significant correlations were observed with IFN[gamma]. A pro-inflammatory response, typified by detection of TNF[alpha] transcript, was observed at a few sites in some horses with inflammatory enteropathy associated with emerging or emerged larvae. However, this cytokine was measured at an insufficient number of sites to enable statistical analysis. Levels of IL-4, IL-10 and IFN[gamma] transcript were compared between two groups: one group consisting of horses with low to high mucosal burdens (Group A) and the other, of horses with negative/negligible mucosal burdens (Group B). Significant differences in IL-4 (P P < 0.001) transcript levels were observed between the groups, with higher levels observed in Group A. No significant differences in IFN[gamma] were observed. Taken together, these results indicate that Th2 responses predominate in mucosal Cyathostominae infection prior to larval reactivation.

Johansson, K. E., B. Pettersson, et al. (1995). "Identification of the causative agent of granulocytic ehrlichiosis in Swedish dogs and horses by direct solid phase sequencing of PCR products from the 16S rRNA gene." Research in Veterinary Science **58**(2): 109.

http://www.sciencedirect.com/science/article/B6WWR-4CWRXPY-27/2/f0b32412d18efb87350857aea17eb9ea

Seven Swedish isolates of Ehrlichia species from the blood of four dogs and three horses with clinical granulocytic ehrlichiosis, were identified by direct solid phase DNA sequencing of polymerase chain reaction (PCR) products from the 16S rRNA gene. The amplified DNA fragments were produced with primers complementary to the universal regions, Ul, U2, U5 and U8 of the 16S rRNA molecule. Identical sequences were obtained from all seven isolates. This nucleotide sequence was similar to the sequences deposited in GenBank for Ehrlichia phagocytophila and E equi. The sequence of the Swedish ehrlichiae differed in two nucleotide positions from the E phagocytophila sequence and in three positions from the E equi sequence, and it is tentatively proposed that it is a subspecies of one of these two. The alignment of the sequence of the Swedish isolates with a recently deposited sequence from human cases of ehrlichiosis in the USA revealed 100 per cent identity in a segment of about 1400 bp.

Laamanen, U. I., E. P. Neuvonen, et al. (1997). "Comparison of assay and virus isolation in cell cultures for the detection of bovine viral diarrhoea virus in field samples." Research in Veterinary Science **63**(3): 199.

http://www.sciencedirect.com/science/article/B6WWR-4CWRYJJ-BC/2/a4d7ef34cfb1f42b3e7e343f110e0244

The virus isolation-immunoperoxidase test on cell cultures and the reverse transcription-polymerase chain reaction assay were compared for the detection of bovine viral diarrhoea virus directly in serum samples. Material for this study consisted of 403 sera originating from cattle in 41 -infected Finnish dairy herds and one suckler cow herd. The presence of virus was demonstrated in 48 samples by both assays. In addition, two more samples were found to be positive by the assay. Both methods proved to be extremely sensitive, detecting pestiviruses

even in high serum dilutions, and thus to be suitable for demonstrating the occurrence of persistently infected cattle. In conclusion, the method used had the advantage of ascertaining nucleic acid sequences in samples in which the virus had been inactivated, eg during transport or due to the presence of neutralising antibodies.

Review of Palaeobotany and Palynology (1)

Wikstrom, N., P. Kenrick, et al. (2002). "Schizaeaceae: a phylogenetic approach." Review of Palaeobotany and Palynology **119**(1-2): 35.

http://www.sciencedirect.com/science/article/B6V6W-45H0B79-4/2/491b7257f4036364923180f52524d8a2

Schizaeaceae fossils have been documented throughout Mesozoic and Cenozoic deposits, but our understanding of this fossil record is hampered by uncertainties with respect to the relationships of living species. To start building a phylogenetic framework for the family, an initial phylogenetic analysis of living species using plastid rbcL nucleotide sequence data is conducted. The analysis supports Schizaea s. lat. and Lygodium monophyly, but Anemia is resolved as paraphyletic to Mohria. In the Anemia/Mohria clade, monophyly of subgenus Anemiorrhiza is supported, but Coptophyllum is resolved as paraphyletic to subgenus Anemia. In Schizaea s. lat., both Schizaea s. str. and Actinostachys are well supported and Microschizaea is grouped with Schizaea s. str., although only one Microschizaea species (Schizaea pusilla) was included. These results are largely congruent with previous morphology-based analyses. In Lygodium however, results presented contrast with recent morphological analyses highlighting the problems of identifying Lygodium subgeneric groups. Using the resulting phylogeny as a framework, putative relationships of fossil species are discussed, tentative minimum age estimates for generic crown group diversifications are made, and possible conclusions with respect to the origins of habit and habitat preferences are discussed. The fossil evidence indicates that subgeneric groups within the Anemia/Mohria clade are comparatively ancient, originating during the Early Cretaceous, and the putative placement of fossil Anemia within the crown group of living subgenus Anemiorrhiza would indicate that their calcareous habitat preference may be a relic feature that has persisted ever since the Early Cretaceous. Lygodium on the other hand appears to have passed through a diversity bottleneck. Modern species diversity probably originated in the Neogene, and the earliest fossil evidence for the origin of their vining and trailing habit comes from the placement of Miocene fossil Lygodium within the crown group of living species.

Reviews in Molecular Biotechnology (1)

Lovatt, A. (2002). "Applications of quantitative PCR in the biosafety and genetic stability assessment of biotechnology products." Reviews in Molecular Biotechnology **82**(3): 279.

http://www.sciencedirect.com/science/article/B6VR0-44VVW8G-8/2/70bb7db05733e41574524ec8fdffa285

High throughput screening, increased accuracy and the coupling of real-time quantitative PCR (Q-PCR) to robotic set-up systems are beginning to revolutionise biotechnology. Applications of Q-PCR within biotechnology are discussed with particular emphasis on the following areas of biosafety and genetic stability testing: (a) determination of the biodistribution of gene therapy vectors in animals; (b) quantification of the residual DNA in final product therapeutics; (c) detection of viral and bacterial nucleic acid in contaminated cell banks and final products; (d) quantification of the level of virus removal in process validation viral clearance studies; (e) specific detection of retroviral RT activity in vaccines with high sensitivity; and (f) transgene copy number determination for monitoring genetic stability during production. Methods employed for Q-PCR assay validation as required in ICH Topic Q2A Validation of Analytical Methods: Definitions and Terminology (1st June 1995) are also reviewed.

Revue Francaise des Laboratoires (1)

Decre, D., B. Gachot, et al. (2000). "Surveillance epidemiologique des souches de Klebsiella pneumoniae productrices de [beta]-lactamase a spectre etendu (KpBLSE) dans un service de reanimation." Revue Francaise des Laboratoires **2000**(320): 31.

http://www.sciencedirect.com/science/article/B6VRF-46SVDDB-9T/2/ed22165554b2e41f0a5b621ff1a7a462

ResumeL'epidemiologie des souches de Klebsiella pneumoniae productrices de [beta]-lactamase a spectre etendu (KpBLSE), isolees durant une periode de 16 mois dans un service de reanimation, a ete etudiee. Un programme associant le renforcement des mesures d'isolement, le depistage systematique des patients a l'admission puis une fois par semaine et une decontamination digestive (DDS) a ete instaure en 1992. Afin d'analyser les transmissions croisees, differents marqueurs phenotypiques et genotypiques (contenu plasmidique, profils d'ADN total restreint par electrophorese en champ pulse, rep-PCR) ont ete utilises pour comparer 138 souches de KpBLSE isolees chez 64 patients. L'incidence des colonisations et/ou des infections etait de 11,9%. Cinquante cinq cas ont ete consideres comme acquis dans l'unite et 9 cas ont ete importes. Parmi les 45 infections observees chez 32 patients, les infections urinaires ont ete les plus frequentes. L'utilisation d'une DDS n'a pas permis de reduire l'acquisition de KpBLSE. L'utilisation combinee de plusieurs marqueurs s'est averee necessaire pour la differenciation des souches. Un clone producteur de [beta]-lactamase de type SHV-4 a ete a l'origine de 85% des cas acquis dans l'unite. Des cas sporadiques lies a des souches de KpBLSE productrices de [beta]-lactamases variees (TEM-3, SHV-2, SHV-3 et SHV-5) ont ete observes.

Science (2)

Barnes, I., P. Matheus, et al. (2002). "Dynamics of Pleistocene Population Extinctions in Beringian Brown Bears." Science **295**(5563): 2267-2270.

http://www.sciencemag.org/cgi/content/abstract/295/5563/2267

Krishnan, M., V. M. Ugaz, et al. (2002). "PCR in a Rayleigh-Benard Convection Cell." Science **298**(5594): 793-.

http://www.sciencemag.org

Scientia Horticulturae (8)

Dubouzet, J. G., N. Murata, et al. (1997). "RAPD analysis of genetic relationships among Alstroemeria L. cultivars." Scientia Horticulturae **68**(1-4): 181.

http://www.sciencedirect.com/science/article/B6TC3-3W3FGSM-K/2/f048dc1effe2481fa8515af15bd7d9f6

A procedure for the rapid extraction of partially purified nucleic acid extracts from Alstroemeria cultivars and the corresponding polymerase chain reaction (PCR) protocol for the generation of random amplified polymorphic DNA (RAPD) markers were established. Nucleic acid extracts from 23 Alstroemeria cultivars were amplified with 8 random decamers by PCR. OPC02, OPC03, OPD02, OPD05, OPD08, OPD11, OPD13 and OPD18 produced 24, 19, 21, 20, 10, 17, 25 and 29 RAPD bands, respectively. The distinctive RAPD patterns generated from these cultivars could be used as genomic 'fingerprints' to establish the identity of a given genotype. The 'Orchid' and 'Butterfly' types were clearly separated in distinct subclusters in a phyllogram obtained by unweighted pair group method analysis (UPGMA) of the genetic distances. The 'Hybrid' types were distributed in two major subclusters, reflecting the diversity of the parental species used to generate the population. This phyllogram conformed to expectations based on the available pedigree data.

Lazaro, A. and I. Aguinagalde (1996). "Phylogenetic relationships between the wild taxa of the Brassica oleracea L. group (2n = 18) using random amplified polymorphic DNA assay." Scientia Horticulturae **65**(4): 219.

http://www.sciencedirect.com/science/article/B6TC3-41B75WY-V/2/21027b4c6e48be7c48df0076289107d9

The aim of this study was to help establish the phylogenetic relationships between the wild taxa of the Brassica oleracea complex using a random amplified polymorphic DNA (RAPD) assay and also to test the potential use of RAPDs in discriminating among closely related species. A total of 22 populations belonging to 15 taxa were analysed; 20 arbitrary primers were studied and six were selected for the detection of 129 reproducible polymorphic fragments, ranging from 200 to 2400 base pairs. These genetic markers, which allowed us to distinguish the different taxa, were used to study the phylogenetic and evolutive relationships of the wild Brassica (n = 9) species. The dendrogram obtained reflects the already accepted genetic relationships among the 15 taxa. Three clearly separated branches are shown: the Western group, the Sicilian group and the Aegean group. The maximum diversity is detected in the Aegean group and the highest similarity coefficient is shown by the Sicilian group, results which are consistent with previous phytochemical analyses.

Modgil, M., K. Mahajan, et al. (2005). "Molecular analysis of genetic stability in micropropagated apple rootstock MM106." Scientia Horticulturae **104**(2): 151.

http://www.sciencedirect.com/science/article/B6TC3-4DXT80H-1/2/929900307236f64381a06663ecfa3fef

Random amplified polymorphic DNA (RAPD) markers were used to assess the genetic stability of 10 micropropagated plants regenerated through axillary buds of clonal apple (Malus pumila Mill.) rootstock MM106. Eleven random decamer primers were successfully used to analyse genomic DNA from mother plants and in vitro plant material. A total of 129 scorable fragments were amplified with an average of 11.73 bands per primer. Among them, 99 were monomorphic and 30 were polymorphic with 23.2% polymorphism. Among these 30, 12 were found monomorphic across seven plants and parent. Three plants could be regarded as off-types. Our results show that RAPD markers could be used to detect the genetic similarities and dissimilarities in micropropagated material.

Mulcahy, D. L., M. Cresti, et al. (1993). "The use of random amplified polymorphic DNAs to fingerprint apple genotypes." Scientia Horticulturae **54**(2): 89.

http://www.sciencedirect.com/science/article/B6TC3-49S87BR-2B6/2/35f9f06091b8ba27ea12b923f350ec55

Twenty-five accessions of apple, representing eight cultivars ('Golden Delicious', 'Delicious', 'Gala', 'Jonagold', 'Jonathan', 'Florina', 'Fior di Cassia', and 'Imperatore Dallago') have been characterized with Random Amplified Polymorphic DNAs (RAPD). The reliability of the method was tested by analyzing separate scions of the same clone and also by comparing different accessions of the same cultivar. Using two separate ten bp primers, it was possible to obtain a distinctive fingerprint for each of the cultivars. The method is simple, rapid and should provide a useful system for documenting the identity of clonal material.

Schnell, R. J., R. Goenaga, et al. (1999). "Genetic similarities among cocoyam cultivars based on randomly amplified polymorphic DNA (RAPD) analysis." Scientia Horticulturae **80**(3-4): 267.

http://www.sciencedirect.com/science/article/B6TC3-3VTB9DK-F/2/0722a93fa73b5f7ed26fa29b9bfac93e

Eighteen cultivars of cocoyam (Xanthosoma spp.) and two cultivars of taro (Colocasia esculenta (L.) Schott) from the USDA/ARS germplasm collection were evaluated for genetic relatedness using RAPD data. Seven random primers generated 40 RAPD loci. Of the 18 cultivars screened, 11 (61%) were identical at all RAPD loci evaluated. A similarity matrix was constructed on the basis of the presence or absence of bands. Among cocoyam cultivars the genetic similarity ranged from 0.86 to 0.97 with a mean of 0.91. Cluster analysis identified two main clusters with some unexpected groupings. These data indicate that very little genetic variation exists within the accessions used in this study and that this Xanthosoma spp. collection is of limited value as a genetic resource.

Takatsu, Y., M. Miyamoto, et al. (2001). "Interspecific hybridization among wild Gladiolus species of

southern Africa based on randomly amplified polymorphic DNA markers." Scientia Horticulturae **91**(3-4): 339.

http://www.sciencedirect.com/science/article/B6TC3-44CVJ4V-D/2/1b4c37a7184a9c9cf9e1e4a3f445d00b

We obtained information on the genetic relationship in wild Gladiolus species through randomly amplified polymorphic DNA (RAPD) analysis. Out of the 140 tested primers, 32 amplified a total of 133 RAPD bands in 33 Gladiolus species. The genetic distance was calculated from the data of these RAPDs, and a dendrogram was generated. Interspecific crosses were carried out in seven combinations within or between clusters, and F1 seedlings were obtained from most combinations. The RAPD analysis showed that these F1 seedlings were real hybrids. The results suggest that RAPD markers are useful for detecting genetic relationships in Gladiolus species, and for interspecific crosses in breeding programs.

Takatsu, Y., Y. Nishizawa, et al. (1999). "Transgenic chrysanthemum (Dendranthema grandiflorum (Ramat.) Kitamura) expressing a rice chitinase gene shows enhanced resistance to gray mold (Botrytis cinerea)." Scientia Horticulturae **82**(1-2): 113.

http://www.sciencedirect.com/science/article/B6TC3-3Y9H77Y-B/2/8c07f876a743e9951ae40dfa423a3968

Transformation of spray-type chrysanthemum was performed using Agrobacterium tumefaciens strain C58 and MP90 harboring a rice chitinase gene (cDNA clone named: RCC2). Eleven transgenic lines expressing the RCC2 gene were obtained. These lines showed enhanced resistance to gray mold (Botrytis cinerea), although the levels of resistance varied among the transgenic lines. Three higher resistance lines, Y12, Y61 and Y97, showed very slight symptoms against B. cinerea infection and which did not spread even if the incubation period was extended. In these three lines, a higher production of RCC2 protein was detected by enzyme-linked immunosorbent assay (ELISA) compared with non-transgenic plants. These results suggest that the RCC2 gene can be a useful tool to improve resistance to gray mold in chrysanthemum.

Ulanovsky, S., Y. Gogorcena, et al. (2002). "Use of molecular markers in detection of synonymies and homonymies in grapevines (Vitis vinifera L.)." Scientia Horticulturae **92**(3-4): 241.

http://www.sciencedirect.com/science/article/B6TC3-44XM219-5/2/8771b2735fec8dd3fbb652c93bfbe86f

RAPD and microsatellites are used in the present study as molecular markers for characterisation of grapevine germplasm material. The studied varieties were located in two germplasm banks in Spain. Thirty nine accessions were included in the study, including some presumed synonymies and homonymies. Sixty six primers were used for the RAPD study. Twenty were selected for the multivariate analysis and grouping of the varieties. Seven varieties had a monotypic pattern. Twenty three accessions were also analysed with microsatellites with the VVMD7, VVS2, VVS5 and VVS29 loci, obtaining eight different patterns. The high discriminating ability of the RAPD analysis allowed all the studied varieties to be distinguished. A good concordance was obtained for both RAPD and microsatellites when the two analysis were carried out. The following synonymies were confirmed: Moristell and one of the accessions of Monastel; Maturana and Ribadavia; Concejon and one of the accessions of Monastrell; and most of the studied muscat varieties. Homonymies were detected for Miguel de Arco, Monastel, Monastrell and Turruntes. Both RAPD and microsatellites are considered as adequate molecular markers for

characterisation of germplasm banks with the use of one or the other depending on the objectives of the study. When results are intended to compare with other laboratories or germplasm banks, microsatellites give simple and more comparable results.

Sensors and Actuators A: Physical (1)

El-Ali, J., I. R. Perch-Nielsen, et al. (2004). "Simulation and experimental validation of a SU-8 based PCR thermocycler chip with integrated heaters and temperature sensor." Sensors and Actuators A: Physical **110**(1-3): 3.

http://www.sciencedirect.com/science/article/B6THG-4B3JT2Y-1/2/d3b62fa144d94b26897d69b0e27305da

We present a SU-8 based polymerase chain reaction (PCR) chip with integrated platinum thin film heaters and temperature sensor. The device is fabricated in SU-8 on a glass substrate. The use of SU-8 provides a simple microfabrication process for the PCR chamber, controllable surface properties and can allow on chip integration to other SU-8 based functional elements. Finite element modeling (FEM) and experiments show that the temperature distribution in the PCR chamber is homogeneous and that the chip is capable of fast thermal cycling. With heating and cooling rates of up to 50 and 30 [deg]C/s, respectively, the performance of the chip is comparable with the best silicon micromachined PCR chips presented in the literature. The SU-8 chamber surface was found to be PCR compatible by amplification of yeast gene ribosomal protein S3 and Campylobacter gene cadF. The PCR compatibility of the chamber surfaces was enhanced by silanization.

Sensors and Actuators B: Chemical (3)

Liu, R. H., J. Bonanno, et al. (2004). "Single-use, thermally actuated paraffin valves for microfluidic applications." Sensors and Actuators B: Chemical **98**(2-3): 328.

http://www.sciencedirect.com/science/article/B6THH-4BJ77MJ-7/2/32fa825241fe10a15aec5dee54473307

A new thermally actuated valving concept using paraffin as single-use valving material was developed. The paraffin undergoes a phase transition in response to changes in temperature. A variety of single-use paraffin-based microvalves, including "close-open," "open-close-open," "T," and toggle designs, were demonstrated. Fluidic experiments showed that these microvalves had zero leakage and a maximum hold-up pressure of 40 psi in a "closed" position. A DNA polymerase chain reaction microdevice containing paraffin-based microvalves to enclose the sample solution in the reaction chamber during the thermal cycling was demonstrated. The paraffin-based microvalving technique has advantages over many existing active microvalve approaches, including a simple design, ease of fabrication, low cost, and ease of integration into complex microfluidic systems. Moreover, this technique is particularly attractive for single-use and

disposable microfluidic devices.

Sethu, P. and C. H. Mastrangelo (2004). "Cast epoxy-based microfluidic systems and their application in biotechnology." Sensors and Actuators B: Chemical **98**(2-3): 337.

http://www.sciencedirect.com/science/article/B6THH-4BHCMJ5-8/2/d8cafa127afa7cf1e607cc519ccd8039

This paper reports the development of epoxy microcasting technologies for the fabrication of plastic microfluidic platforms. Two new techniques one involving embedding active silicon devices in plastic microsystems using a polymer flip chip process and another involving surface micromachining to build active components like actuators for use in applications like pumping are discussed and explained. Application devices were fabricated for polymerase chain reaction (PCR) and capillary electrophoresis (CE).

Sun, K., A. Yamaguchi, et al. (2002). "A heater-integrated transparent microchannel chip for continuous-flow PCR." Sensors and Actuators B: Chemical **84**(2-3): 283.

http://www.sciencedirect.com/science/article/B6THH-4534CH9-1/2/a11eed4240d2fb663113e9e986293188

A microchannel chip for continuous-flow polymerase chain reaction (PCR) was developed using transparent materials. The microchannel was fabricated on a quartz glass substrate using standard photolithography and wet-etching techniques and was sealed by another quartz glass substrate. Two indium-tin-oxide (ITO) films were deposited on the etched substrate as a thermal source. To confirm the temperature distribution in the microchannel, we measured the fluorescence spectra of an aqueous solution of 1-pyrenesulfonic acid sodium salt (PS-Na), which is a temperature-indicator dye, in the microchannel under a continuous solution flow. The results confirm that the temperature distribution on the microchannel's ITO films was almost uniform (within +/-2 [deg]C) under two flow rates (56 and 152 nl/min). The slightness of this deviation indicates that the ITO films integrated into the microchannel chip can be very useful as a thermal source for PCR. An amplification of a 450 bp segment of Escherichia coli HB101 was successfully performed by two-stage (94 and 67 [deg]C) thermal cycling on the chip device.

Soil Biology and Biochemistry (13)

Bala, A., P. Murphy, et al. (2002). "Occurrence and genetic diversity of rhizobia nodulating Sesbania sesban in African soils." Soil Biology and Biochemistry **34**(11): 1759.

http://www.sciencedirect.com/science/article/B6TC7-46SW58F-6/2/c545c4562f9ec411e532c7211c93452f

The distribution, diversity and relative abundance of Sesbania sesban rhizobia in African soils were investigated by host-trapping and counting of rhizobia and characterization using restriction fragment length polymorphism analysis of PCR-amplified 16S rRNA and the internally transcribed

spacer (ITS) between the 16S and 23S rRNA genes. Isolates representative of the diverse 16S rRNA groups from the various soils were selected for sequence analysis of the first 750 bp of the 16S rRNA. Compatible rhizobia were detected in only 15 out of 55 soils, and were present generally in soils with more than 10% clay, and those from low-lying areas. Populations were small, generally much less than 50 cells g-1 soil. The rhizobia nodulating S. sesban were genetically diverse, with isolates bearing 16S rRNA sequences similar to those of rhizobia belonging to the genera Rhizobium, Mesorhizobium, Sinorhizobium and Allorhizobium. About 1% of the isolates recovered had sequences with close homology withAgrobacterium tumefaciens. Despite the wide phylogenetic distribution of the rhizobial isolates, the Mesorhizobium group was dominant in all soils examined, accounting for 90% of the isolates on average, with individual soil populations usually being comprised of two genera. There was a marked variability in the sequence and size of the ITS region among rhizobia nodulating Sesbania which indicates a broad diversity of 'strain' types both within and between soil populations, and within and between rhizobial genera.

Clegg, C. D., J. M. Anderson, et al. (1996). "Biophysical processes affecting the transit of a genetically-modified Pseudomonas fluorescens through the gut of the woodlouse Porcellio scaber." Soil Biology and Biochemistry **28**(8): 997.

http://www.sciencedirect.com/science/article/B6TC7-3W3NCHS-3/2/75f12c92f91eab9db03e982a3c1853d5

Our objective was to determine the effect of gut transit retention time of genetically-modified bacteria ingested by the woodlouse Porcellio scaber. The experimental animals were supplied ash leaf litter inoculated with the genetically-modified bacterium Pseudomonas fluorescens KTG and bacteria in food and faeces were counted using selective plating and immunofluorescent techniques. The bacteria were also detected using the polymerase chain reaction (PCR). It was found that plate counts of P. fluorescens KTG in fresh faeces were lower than those found in the litter when the GEMMO was supplied to animals at five different population densities, suggesting that a proportion of the GEMMO population was lost during gut transit. There was no significant difference in the survival of freshly cultured and starved cultures of P. fluorescens KTG on gut transit through P. scaber as determined by plate counts in fresh faeces. Retention time of P. fluorescens KTG in the woodlouse gut was found to be longer than that of the food bolus. The passage of bacteria through the gut was modelled and tracked using microbeads of a size similar to bacteria. Fluorescent microbeads added to food litter were detected within the anterior chamber, papillate region and rectum of the woodlouse for at least 17 days after ingestion. Scanning electron microscopy revealed that beads were retained within the cuticular structure of the digestive tract and also within mucopolysaccharide produced within the gut. Immunofluorescent observations of washed hindgut samples provided little evidence to suggest P. fluorescens KTG had become attached to the hindgut wall during transit. Very few colonies of the GEMMO and indigenous bacteria were detected from homogenised hepatopancreas samples. P. fluorescens KTG was however detected in the hepatopancreas of P. scaber using PCR. It is suggested that the retention of bacteria within the guts of woodlice is by physical rather than biological mechanisms such as growth or attachment.

Crecchio, C. and G. Stotzky (2001). "Biodegradation and insecticidal activity of the toxin from Bacillus thuringiensis subsp. kurstaki bound on complexes of montmorillonite-humic acids-Al hydroxypolymers." Soil Biology and Biochemistry **33**(4-5): 573.

http://www.sciencedirect.com/science/article/B6TC7-42991JB-J/2/7aeb75426aeb6838f1c260d6d3ec56a9

The equilibrium adsorption and binding of the active toxin from Bacillus thuringiensis subsp. kurstaki on complexes of montmorillonite-humic acids-Al hydroxypolymers, as well as the biodegradation and the insecticidal activity of the bound toxin, were studied. Seventy percent of the total adsorption occurred within the first hour, and maximal adsorption occurred in 2O and 1 M NaCl. The bound toxin was resistant to utilization by mixed microbial cultures from soil and to enzymatic degradation by Pronase E. Free and bound toxin were active against the larvae of Manduca sexta; the bound toxin retained the same activity after exposure to microbes or Pronase, whereas the toxicity of the free toxin decreased significantly. The results of these studies indicate that the release of transgenic plants and microorganisms expressing truncated genes that encode active insecticidal toxins from B. thuringiensis could result in the accumulation of these toxins in soil as a consequence of binding on surface-active soil particles. This persistence could pose a hazard to nontarget organisms, enhance the selection of toxin-resistant target species, and increase the control of target insect pests.

de Lipthay, J. R., C. Enzinger, et al. (2004). "Impact of DNA extraction method on bacterial community composition measured by denaturing gradient gel electrophoresis." Soil Biology and Biochemistry **36**(10): 1607.

http://www.sciencedirect.com/science/article/B6TC7-4CY5JPN-3/2/d9ebcd3fb705454d19afbf1c4d207e98

The impact of DNA extraction protocol on soil DNA yield and bacterial community composition was evaluated. Three different procedures to physically disrupt cells were compared: sonication, grinding-freezing-thawing, and bead beating. The three protocols were applied to three different topsoils. For all soils, we found that each DNA extraction method resulted in unique community patterns as measured by denaturing gradient gel electrophoresis. This indicates the importance of the DNA extraction protocol on data for evaluating soil bacterial diversity. Consistently, the bead-beating procedure gave rise to the highest number of DNA bands, indicating the highest number of bacterial species. Supplementing the bead-beating procedure with additional cell-rupture steps generally did not change the bacterial community profile. The same consistency was not observed when evaluating the efficiency of the different methods on soil DNA yield. This parameter depended on soil type. The DNA size was of highest molecular weight with the sonication and grinding-freezing-thawing procedures (approx. 20 kb). In contrast, the inclusion of bead beating resulted in more sheared DNA (approx. 6-20 kb), and the longer the bead-beating time, the higher the fraction of low-molecular weight DNA. Clearly, the choice of DNA extraction protocol depends on soil type. We found, however, that for the analysis of indigenous soil bacterial communities the bead-beating procedure was appropriate because it is fast, reproducible, and gives very pure DNA of relatively high molecular weight. And very importantly, with this protocol the highest soil bacterial diversity was obtained. We believe that the choice of DNA extraction protocol will influence not only the determined phylogenetic diversity of indigenous microbial communities, but also the obtained functional diversity. This means that the detected presence of a functional gene--and thus the indication of enzyme activity--may depend on the nature of the applied DNA extraction procedure.

Ellingsoe, P. and K. Johnsen (2002). "Influence of soil sample sizes on the assessment of bacterial community structure." Soil Biology and Biochemistry **34**(11): 1701.

http://www.sciencedirect.com/science/article/B6TC7-473M734-5/2/49412a0c40c9950d801d5af2fbbe1187

When assessing bacterial community structure in soil it is important to establish a satisfactory procedure for sampling. The influence of sample sizes of a forest soil on the assessment of

bacterial community structure was investigated. Four sample sizes (0.01, 0.1, 1.0, and 10.0 g) were evaluated. Time of colony appearance on a nutrient-limited soil extract agar was used to characterise the culturable heterotrophic and Pseudomonas communities. Genetic community structure was assessed with denaturing gradient gel electrophoresis (DGGE) of 16S rDNA using a bacterial primer set. The largest variation in the heterotrophic community structure for bacteria was seen when comparing the 0.01 g replicates. Variation was also seen for the 0.1 and 1.0 g replicates. However, there was no significant difference between the 10.0 g replicates. The 0.01 and 0.1 g replicates showed variation in genetic community structure within the sample sizes, whereas variation between the replicates within the larger sample sizes (1.0, 10.0 g) was negligible. Variation in the heterotrophic community structure for Pseudomonas was seen between replicates of all sample sizes. Hence, the size of soil samples influenced the bacterial community structure observed for bacteria, whereas chance seemed to play an important role when looking at a more narrow community structure.

Klamer, M. and K. Hedlund (2004). "Fungal diversity in set-aide agricultural soil investigated using terminal-restriction fragment length polymorphism." Soil Biology and Biochemistry **36**(6): 983.

http://www.sciencedirect.com/science/article/B6TC7-4C1CBRK-6/2/359b1ed9173ce84cb5c1433b40362f6a

As part of the restoration of biodiversity on former agricultural land there has been focused on methods to enhance the rate of transition from agricultural land towards natural grasslands or forest ecosystems. Management practices such as sowing seed mixtures and inoculating soil of later successional stages have been used. The aim of this study was to determine the effects of a managed plant community on the diversity of soil fungi in a newly abandoned agricultural land. A field site was set up consisting of 20 plots where the plant diversity was managed by either sowing 15 plant species, or natural colonization was allowed to occur. The plant mixture contained five species each of grasses, legumes and forbs that all were expected to occur at the site. A subset of the plots (five from each treatment) was inoculated with soil cores from a late successional stage. The plant community composition was subject to a principal component analysis based on the coverage of each species. Five years after abandonment, soil samples were taken from the plots, DNA was extracted and the ITS region of the rDNA gene was amplified using fluorescently labelled fungal specific primers (ITS 1F/ITS 4). The PCR products were digested using HinfI and TaqI and sequenced. Results from both restriction enzymes were combined and a principal component analysis performed on the presence/absence of fragments. Also the fungal diversity expressed as number of restriction fragments were analysed. There was significantly higher fungal species richness in the experimental plots compared to the forest and field soils, but no differences between sown and naturally colonized plots. The different plant treatments did not influence the below ground fungal community composition. Soil water content on the other hand had an impact on the fungal community composition.

Kozdroj, J. and J. D. van Elsas (2000). "Response of the bacterial community to root exudates in soil polluted with heavy metals assessed by molecular and cultural approaches." Soil Biology and Biochemistry **32**(10): 1405.

http://www.sciencedirect.com/science/article/B6TC7-40PGT9H-B/2/6cdc1b8f118cc2d1e3fc0f1d65923946

We have used PCR based on 16S rDNA sequences followed by denaturing gradient gel electrophoresis (PCR-DGGE) in conjunction with cultivation-based methods to describe the effect of artificial root exudates (ARE), of which the composition simulated maize root exudates, on the structural diversity of bacterial communities in various soils differing in the level of contamination

with heavy metals. The aim of this study was to evaluate the effects of organic compounds of a root exudates as a potential mechanism for selectively enhancing specific bacterial populations in contaminated soils, leading to the development of shifted communities differing in qualitative and quantitative composition. Soil microcosms were either just enriched with ARE or enriched and, additionally, flooded. To characterise the response of the soil microflora to the enrichment, PCR-DGGE was applied for assessment of the total bacterial community structure. Cultivation techniques were used to determine the numbers of total heterotrophic bacteria as well as of pseudomonads (which are considered to be stimulated by components of root exudates). The community structure of culturable bacteria was studied using the concept of r- and K-strategists, and isolates from dominant colonies growing on King's B agar were identified by MIDI-FAME profiling. The results obtained showed a significant effect of root exudates on the development of bacterial populations in soil contaminated with heavy metals. Depending on their availability and conditions prevailing in the habitat (e.g. stronger enrichment by flooding) different bacterial populations were stimulated, resulting in generation of different community patterns by DGGE. The most significant response to root exudates occurred among the culturable fraction of the soil bacteria. Distribution of bacterial classes (i.e. majority of colonies appeared after 24 h), values of EP (from 0.220 to 0.533) and CD (from 43 to 88) indices directly showed that the culturable fraction of bacteria was highly affected by the organic mixture simulating root exudates. These exudates reduced the bacterial diversity towards domination of r-strategists and the reduction of diversity was greater in soil with a higher contamination level. Furthermore, flooding of the soils enhanced the dominance of fast growing bacteria (over 70% formed visible colonies after 24 h even on day 6) and reduced the community diversity (EP and CD indices were from about 0.291 to 0.425 and from 66 to 87, respectively).

Mutch, L. A., S. M. Tamimi, et al. (2003). "Genotypic characterisation of rhizobia nodulating Vicia faba from the soils of Jordan: a comparison with UK isolates." Soil Biology and Biochemistry **35**(5): 709.

http://www.sciencedirect.com/science/article/B6TC7-485X6R1-1/2/182e687dcc2b629321e1ff5d1c05776c

Seven isolates of Rhizobium leguminosarum bv. viciae (Rlv) that nodulate faba beans (Vicia faba) from six sites in Jordan were characterised for chromosomal (glnII) and symbiotic (nodD-F) genotypes using polymerase chain reaction-restriction fragment length polymorphism and sequencing methods. The results were compared to those obtained in a previous UK study, to determine whether or not the UK field population are indigenous or if they were dispersed during the radiation of V. faba domestication. All seven Jordanian isolates displayed novel chromosomal and symbiotic genotypes not identified in the UK population.

Pillai, S. D., K. L. Josephson, et al. (1992). "Specific detection of rhizobia in root nodules and soil using the polymerase chain reaction." Soil Biology and Biochemistry **24**(9): 885.

http://www.sciencedirect.com/science/article/B6TC7-47DKGJP-36/2/9f3ff8cfe4d14388b10a158d10b6d56a

The polymerase chain reaction (PCR) amplification of specific DNA sequences, allows specific and sensitive detection of bacteria at the genus, species or strain level depending on the design of the oligonucleotide primers. In this study we utilized 20 mer primers that flanked a 300 bp region of the npt II gene of the transposon Tn5 thus allowing for the amplification of this region. Insertion of the Tn 5 element into rhizobia allowed for detection of these cells using PCR amplification. Using the npt II-specific primers and Tn5 insertion mutants of Rhizobium leguminosarum bv. phaseoli we were able to detect these specific rhizobia strains in root nodules

of bean plants and in inoculated soils. Utilization of genus-specific gene sequences would allow for estimates of cells of that genus in environmental samples. Conversely, use of gene sequences common to rhizobia, e.g. nif and nod sequences, would give estimates of the population of rhizobia. This paper serves to illustrate the use of PCR, for detecting gene sequences in an environmental sample such as a root nodule.

Renella, G., M. Mench, et al. "Functional activity and microbial community structure in soils amended with bimetallic sludges." Soil Biology and Biochemistry **In Press, Corrected Proof**
http://www.sciencedirect.com/science/article/B6TC7-4FJGSR7-7/2/c27b0d7ae6e097c2198596c06556aebe

Heavy metal availability, microbial biomass and respiration, bacterial diversity and enzyme activity were studied in soils from long-term field experiments contaminated with Mn-Zn- or Cd-Ni-rich sludge, incorporated into soils at two different rates. Soils that never received sludge were used as controls. Microbial biomass C content (BC) and soil respiration (CO2-C) were slightly reduced in soils amended with Mn-Zn at the higher incorporation rate whereas in soils receiving Cd-Ni-rich sludge BC and respiration were unaffected. Metabolic quotient values (qCO2) calculated by the BC-to-CO2-C ratio were not significantly different, regardless of the sludge type whereas the microbial biomass C-to-total organic C (BC-to-TOC) ratios were significantly reduced in the soils receiving the higher rates of both sludge types. Phosphomonoesterase, [beta]-glucosidase and arylsulfatase activities and hydrolase-to-BC ratios, were significantly reduced in soils amended with Ni-Cd-sludge at both rates, whereas the Mn-Zn-sludge only reduced the arylsulfatase activity at the higher rate. Protease activity was generally higher in all the sludge-amended soils as compared to control soils whereas urease activity was unaffected by sludge amendments. The structure of the bacterial community, as determined by denaturing gradient gel electrophoresis (DGGE), was different in the sludge-amended soils as compared to the respective controls. The most important changes were observed in the soils amended with high-level Ni-Cd sludge. Because some of the adverse effects were observed at moderate contamination levels, our results indicate that the presence of certain heavy metal combinations can be a serious limitation for sludge disposal.

Spaccini, R., A. Piccolo, et al. (2002). "Increased soil organic carbon sequestration through hydrophobic protection by humic substances." Soil Biology and Biochemistry **34**(12): 1839.

http://www.sciencedirect.com/science/article/B6TC7-46YXK51-1/2/4fcb5073124632fe5acf1e76b3c6ca26

We studied the effect of humified organic matter (OM) on the mineralization of a representative labile organic compound in soil. In an incubation experiment, a 13C-labeled 2-decanol was added to soil either alone (2-dec*) or in mixture with two humic acids from compost (HAC*) and lignite (HAL*) which had different hydrophobic properties. Isotopic dilution ([delta]13C) showed that after 3 months of incubation about 58, 40 and 28% of the added 13C was retained in the whole soil treated with HAL*, HAC* and 2-dec*, respectively. The higher the hydrophobicity of the employed humic material, the larger was the sequestration of organic carbon in soil. Fractionation of incubated samples revealed that the labeled carbon progressively accumulated in the finest particle-size fractions. However, the high hydrophobicity of the lignite HA favored accumulation of 13C also in the sand-sized fraction. The NMR spectra of humic extracts showed that the 13C-methyl group in the original 2-decanol had been oxidized to a 13C-carboxyl group during incubation for all treatments. This indicated that despite its hydrophilicity, the resulting carboxyl carbon was sequestered into the hydrophobic domains of the humic pool in soil. In fact, the residual 13C was larger in humic than in fulvic extracts for the control sample (2-dec*) and even more so in extracts from soil treated with both exogenous humic acids. Our results suggest that

labile organic compounds may be effectively protected in soil by humified OM and their microbial mineralization substantially reduced. Innovative soil management practices employing hydrophobic humic substances may increase the biological stability of soil OM and thus contribute to significantly mitigate CO2 emissions from agricultural soils.

Tsushima, S., A. Hasebe, et al. (1995). "Detection of genetically engineered microorganisms in paddy soil using a simple and rapid "nested" polymerase chain reaction method." Soil Biology and Biochemistry **27**(2): 219.

http://www.sciencedirect.com/science/article/B6TC7-3YF4MX8-77/2/982db2f637c0a2a7db9b27ededf905ae

A simple method for the detection of small populations of Pseudomonas fluorescens P.B8-1, containing the nptII gene of Tn5 as a unique marker, was applied to a Nyuzen paddy soil using cell extraction (indirect DNA extraction) and a "nested" polymerase chain reaction (PCR). This involved processing samples through a combination of a sucrose gradient centrifugation procedure to isolate bacterial cells, followed by cell lysis with proteinase K and CTAB (hexadecyltrimethyl ammonium bromide)-NaCl. This method allowed the extraction of DNA within about 6 h followed by amplification of DNA. The optimized "nested" PCR comprised a "2-step" PCR (45 cycles) using two 20-mer primers, followed by a "3-step" PCR (30 cycles) using two 26-mer primers which were internal to the first set. After the first PCR step was performed, the amplified DNA was detectable from the inoculated soil containing a minimum of 105 cfu g-1. However, the "nested" PCR procedure permitted the detection of amplified DNA fragments from inoculated non-sterile soils containing 1.3 x 101 cfu g-1. The application of this detection strategy was tested by monitoring the survival of P. fluorescens P.B8-1 in a non-sterile paddy soil during a 53-day period. The P.B8-1 population decreased in soils maintained at either 25 or 10[deg]C after inoculation. After 53 days, samples of soil maintained at 10[deg]C contained 102 cfu g-1 of P.B8-1 (as determined by selective plate count) and permitted amplification of DNA by the "nested" PCR. At the same time, P.B8-1 was not detected in soil maintained at 25[deg]C by either method. The results obtained using this detection strategy suggest that it is highly applicable to monitoring the fate of genetically engineered microorganisms in natural paddy soils.

Vettori, C., L. Calamai, et al. (1999). "Adsorption and binding of AmpliTaq(R) DNA polymerase on the clay minerals, montmorillonite and kaolinite." Soil Biology and Biochemistry **31**(4): 587.

http://www.sciencedirect.com/science/article/B6TC7-3VXYG8T-D/2/06f31ea4f181016838c1a71dd6f43f95

Adsorption at equilibrium and binding of AmpliTaq(R) DNA polymerase (TDP) on clay minerals, two montmorillonites (W-M and Ap-M) and a kaolinite (K), were studied. Equilibrium adsorption isotherms were of the L type, and a plateau was reached with Ap-M and K, whereas no plateau was obtained with W-M. Adsorption of TDP was most rapid on W-M, maximal at pH 6 on all clays, and appeared to involve hydrophobic interactions between the clays and TDP. The amounts of TDP adsorbed and bound on the clays were higher on W-M than on Ap-M and K and occurred only on the external surfaces of the clays. Fourier-transform infrared spectra, SDS-PAGE and scanning electron microscopy of both pure TDP and the W-M-TDP complex showed the presence in the commercial TDP of microspheres (about 100 nm in diameter) of unidentified material, which could influence the adsorption-binding of TDP on clays.

Systematic and Applied Microbiology (1)

Ivanova, E. P., O. M. Onyshchenko, et al. (2005). "Oceanimonas smirnovii sp. nov., a novel organism isolated from the Black Sea." Systematic and Applied Microbiology **28**(2): 131.

http://www.sciencedirect.com/science/article/B7GVX-4F01192-1/2/3ce5fbe22622d5013bdff3d94eeaa5b6

A slightly creamy, melanogenic, Gram-negative, aerobic bacterium was isolated from seawater sample collected in the Karadag Natural Reserve of the Eastern Crimea, the Black Sea. The novel organism was chemoorganotrophic, had no obligate requirement in NaCl, tolerated to 12% NaCl, grew between 10 and 45 [deg]C, was slightly alkaliphilic, and was not able to degrade starch, gelatin, agar, and Tween 80. 16S rRNA gene sequence-based analyses of the new organism revealed that Oceanimonas doudoroffii ATCC 27123T, Oceanimonas baumanii ATCC 700832T, and Oceanisphaera litoralis DSM 15406T were the closest relatives (similarity around 97%-96%). The G+C content of the DNA of the strain 31-13T was 55.5 mol%. Phosphatidylethanolamine (49.0%), phosphatidylglycerol (41.8%), and diphosphatidylglycerol (9.2%) were the predominant phospholipids. The major fatty acids were 16:0 (24.1%), 16:1[omega]7 (40.3%), and 18:1[omega]7 (29.2%). On the basis of the significant differences demonstrated in the phenotypic and chemotaxonomic characteristics, it is suggested that the bacterium be classified as a novel species; the name Oceanimonas smirnovii sp. nov. is proposed. The type strain is 31-13T (UCM B-11076T=LMG 22147T=ATCC BAA-899T).

The International Journal of Biochemistry & Cell Biology (6)

Edgar, A. J. and J. M. Polak (2001). "Flotillin-1: gene structure: cDNA cloning from human lung and the identification of alternative polyadenylation signals." The International Journal of Biochemistry & Cell Biology **33**(1): 53.

http://www.sciencedirect.com/science/article/B6TCH-4292G8K-6/2/b5ecf3c65d6d1abbb50f65e9b300196f

To identify changes in gene expression associated with emphysema, differential display was used to compare RNA extracted from emphysematous lung with that of unused donor tissue taken at the time of transplant. Two expressed clones with sequence homology to the 3' UTR of the murine flotillin-1 cDNA were identified. Flotillin-1 is a plasma membrane protein, which has been associated with detergent-insoluble glycolipid-rich domains and the formation of caveolae. One clone was 95 bp longer than the other. It arose from the use of a second polyadenylation signal and its existence was not due to differential expression nor to polymorphisms in the human flotillin-1 sequence. The 1839 bp human flotillin-1 sequence was completed by 5' RACE from a lung cDNA library. The human mRNA has a 1.9 kbase transcript being highly expressed in brain, heart and lung. The single copy flotillin-1 gene is located at 6p21.3 in the MHC class I region and consists of 13 exons over 15 kb. The ORF encodes a 427 residue protein with a molecular mass 47355 Da, and an isoelectric point 7.08. Human flotillin-1 has a 98% identity with the murine protein and a 47% identity with human flotillin-2. Flotillin-1 belongs to the Band 7.2/stomatin

protein family, possessing a hydrophobic N-terminal region, predicted to form a single, outside to inside, transmembrane domain. The long central [alpha]-helical domain may form a coiled-coil. We have isolated and characterised a cDNA encoding the human flotillin-1 gene, which may play an important role in raft formation.

Kou, S.-Y. and N. S. Cohen (1998). "Ethanol feeding produces deficiencies in left ventricle total RNA, total DNA and mitochondrial ribosomal RNA." The International Journal of Biochemistry & Cell Biology **30**(4): 475.

http://www.sciencedirect.com/science/article/B6TCH-3SY2FGJ-8/2/adcb58a73ac71b02143a990d991384e1

Chronic alcoholism causes a variety of ultrastructural, biochemical and functional alterations in the myocardium, but the underlying mechanisms are not well understood. Molecular changes that developed in the left ventricles of rats fed for 1 to 24 weeks on liquid diets containing ethanol as 36% of total calories were analyzed. Total tissue RNA and DNA were chemically extracted and measured by spectroscopic methods; mitochondrial DNA and mitochondrially-coded ribosomal RNA were measured at the 12s rRNA region by a quantitative polymerase chain reaction method; mitochondrial protein and enzyme activities were assayed. Ethanol-fed rats had 83.9+/-2.9% (mean+/-S.E.M.) as much DNA/g tissue and 74.7+/-3.9% as much total left ventricle DNA as pair-fed controls (PPP<0.001). Total left ventricle 12s rRNA was <40% of normal. There was little or no change in mitochondrial DNA levels measured at the 12s location. Mitochondrial cytochrome contents were reduced 26-38% in the ethanol-fed rats, but only after 24 weeks. This study shows that experimental alcoholism produces rapid and sustained decreases in left ventricle total RNA and DNA and mitochondrial ribosomal RNA. The observed effects would be expected to have a major impact on left ventricle structural integrity and functional capacity.

Puddu, P., P. Borghi, et al. (1998). "Antiviral effect of bovine lactoferrin saturated with metal ions on early steps of human immunodeficiency virus type 1 infection." The International Journal of Biochemistry & Cell Biology **30**(9): 1055.

http://www.sciencedirect.com/science/article/B6TCH-3TYNMH7-F/2/6ba6f6f79b1359590d28038652d3f4a4

Lactoferrin is a mammalian iron-binding glycoprotein present in many biological secretions, such as milk, tears, semen and plasma and a major component of the specific granules of polymorphonuclear leucocytes. The effect of bovine lactoferrin (BLf) in apo-form or saturated with ferric, manganese or zinc ions, on human immunodeficiency virus type 1 (HIV-1) infection in the C8166 T-cell line was studied. Both HIV-1 replication and syncytium formation were efficiently inhibited, in a dose-dependent manner, by lactoferrins. BLf in apo and saturated forms markedly inhibited HIV-1 replication when added prior to HIV infection or during the virus adsorption step, thus suggesting a mechanism of action on the HIV binding to or entry into C8166 cells. Likewise, the addition of Fe3+BLf prior to HIV infection and during the attachment step resulted in a marked reduction of the HIV-1 DNA in C8166 cells 20 h after infection. The potent antiviral effect and the high selectivity index exhibited by BLf suggest for this protein, in apo or saturated forms, an important role in inhibiting the early HIV-cell interaction, even though a post adsorption effect cannot be ruled out.

Suchanek, K. M., F. J. May, et al. (2002). "Peroxisome proliferator-activated receptor [beta] expression in

human breast epithelial cell lines of tumorigenic and non-tumorigenic origin." The International Journal of Biochemistry & Cell Biology **34**(9): 1051.

http://www.sciencedirect.com/science/article/B6TCH-45BHDN9-2/2/48e5c3994b13259ea5162950f8ce2736

Peroxisome proliferator-activated receptor [beta] (PPAR[beta]) is a member of the nuclear hormone receptor superfamily and is a ligand activated transcription factor, although the precise genes that it regulates and its physiological and pathophysiological role remain unclear. In view of the association of PPAR[beta] with colon cancer and increased mRNA levels of PPAR[beta] in colon tumours we sought in this study to examine the expression of PPAR[beta] in human breast epithelial cells of tumorigenic (MCF-7 and MDA-MB-231) and non-tumorigenic origin (MCF-10A). Using quantitative RT-PCR we measured PPAR[beta] mRNA levels in MCF-7, MDA-MB-231 and MCF-10A cells at various stages in culture. After serum-deprivation, MDA-MB-231 and MCF-10A cells had a 4.2- and 3.8-fold statistically greater expression of PPAR[beta] compared with MCF-7 cells. The tumorigenic cell lines also exhibited a significantly greater level of PPAR[beta] mRNA after serum deprivation compared with subconfluence whereas such an effect was not observed in non-tumorigenic MCF-10A cells. The expression of PPAR[beta] was inducible upon exposure to the PPAR[beta] ligand bezafibrate. Our results suggest that unlike colon cancer, PPAR[beta] overexpression is not an inherent property of breast cancer cell lines. However, the dynamic changes in PPAR[beta] mRNA expression and the ability of PPAR[beta] in the MCF-7 cells to respond to ligand indicates that PPAR[beta] may play a role in mammary gland carcinogenesis through activation of downstream genes via endogenous fatty acid ligands or exogenous agonists.

Torres, J. M. and E. Ortega (2004). "Quantitation of mRNA levels of steroid 5[alpha]-reductase isozymes: a novel method that combines quantitative RT-PCR and capillary electrophoresis." The International Journal of Biochemistry & Cell Biology **36**(1): 78.

http://www.sciencedirect.com/science/article/B6TCH-48NJ0G6-2/2/a7a9b75d95fd3414f4d1c2f59645e6d9

A novel, accurate, rapid and modestly labor-intensive method has been developed to quantitate specific mRNA species by reverse transcription-polymerase chain reaction (RT-PCR). This strategy combines the high degree of specificity of competitive PCR with the sensitivity of laser-induced fluorescence capillary electrophoresis (LIF-CE). The specific target mRNA and a mimic DNA fragment, used as an internal standard (IS), were co-amplified in a single reaction in which the same primers are used. The amount of mRNA was then quantitated by extrapolation from the standard curve generated with the internal standard. PCR primers were designed to amplify both a 185 bp fragment of the target cDNA for steroid 5[alpha]-reductase 1 (5[alpha]-R1) and a 192 bp fragment of the target cDNA for steroid 5[alpha]-reductase type 2 (5[alpha]-R2). The 5' forward primers were end-labeled with 6-carboxy-fluorescein (6-FAM). Two synthetic internal standard DNAs of 300 bp were synthesized from the sequence of plasmid pEGFP-C1. The ratio of fluorescence intensity between amplified products of the target cDNA (185 or 192 bp fragments) and the competitive DNA (300 bp fragment) was determined quantitatively after separation by capillary electrophoresis and fluorescence analysis. The accurate quantitation of low-abundance mRNAs by the present method allows low-level gene expression to be characterized.

Yang, Z., A. Paterson, et al. (1999). "Overexpression of sense or antisense human gastrin mRNA does not affect proliferation of normal rat kidney fibroblasts." The International Journal of Biochemistry & Cell Biology **31**(3-4): 509.

http://www.sciencedirect.com/science/article/B6TCH-3VYXT6G-K/2/d8028985b1a3e515d543818207d133fb

Progastrin-derived peptides have been reported to stimulate mitogenesis in Swiss 3T3 fibroblasts [P. Singh, A. Owlia, R. Espeijo, B. Dai, Novel gastrin receptors mediate mitogenic effects of gastrin and processing intermediates of gastrin on Swiss 3T3 fibroblasts: Absence of detectable cholecystokinin (CCK)-A and CCK-B receptors. J. Biol. Chem. 270 (1995) 8429-8438]. The aim of the present study was to determine the generality of these findings, by investigating the effect of endogenous and exogenous progastrin-derived peptides on the proliferation of the normal rat kidney fibroblast cell line NRK. Levels of endogenous progastrin-derived peptides were modified by stable transfection of NRK cells with tetracycline-repressible plasmids containing sequences encoding human gastrin in either the sense or antisense orientation. Expression of sense and antisense gastrin mRNA was demonstrated by reverse transcriptase PCR and by radioimmunoassay, and cell proliferation rates were determined by the colorimetric MTT assay. Sense clones produced full length human progastrin, but significant quantities of glycine-extended or amidated gastrin17 were not detected. Concentrations of endogenous rat progastrin in antisense clones were significantly lower than concentrations in clones transfected with vector only. However no difference in proliferation rate was observed between sense, antisense and vector-transfected clones. No stimulation of proliferation was observed in synchronised untransfected NRK cells after supplementation of media with gastrin17 or gastrin17gly in the concentration range 0.3 to 100 nM. Our results do not provide evidence in support of the hypothesis that endogenous or exogenous progastrin-derived peptides act as growth factors in NRK fibroblasts.

The Journal of Nutritional Biochemistry (2)

Kong, S.-E., J. C. Hall, et al. (2000). "Starvation alters the activity and mRNA level of glutaminase and glutamine synthetase in the rat intestine." The Journal of Nutritional Biochemistry **11**(7-8): 393.

http://www.sciencedirect.com/science/article/B6T8P-41FTT2P-5/2/110e9197ad6c6a08e73b979b0245dc5d

The metabolism of glutamine, the main respiratory fuel of enterocytes, is governed by the activity of glutaminase and glutamine synthetase. Because starvation induces intestinal atrophy, it might alter the rate of intestinal glutamine utilization. This study examined the effect of starvation on the activity, level of mRNA, and distribution of mRNA of glutaminase and glutamine synthetase in the rat intestine. Rats were randomized into groups and were either: (1) fed for 2 days with rat food ad libitum or (2) starved for 2 days. Standardized segments of jejunum and ileum were removed for the estimation of enzyme activity, level of mRNA, and in situ hybridization analysis. The jejunum of the fed rats had a greater activity of both enzymes per centimeter of intestine (P P P < 0.01), a lower level of glutaminase mRNA, and a greater level of glutamine synthetase mRNA. In situ hybridization analysis showed that starvation does not alter the distribution of glutaminase and glutamine synthetase mRNA in the intestinal mucosa. This study confirms that starvation decreases the total intestinal activity per centimeter of both glutaminase and glutamine synthetase. More importantly, the results indicate that the intestine adapts to starvation by accumulating glutaminase mRNA. This process prepares the intestine for a restoration of intake.

Pogribny, I. P. and S. J. James (1997). "A method to estimate the percent loss of cytosine methyl groups at defined CpG sites in liver DNA from methyl-deficient rats." The Journal of Nutritional Biochemistry **8**(6): 355.

http://www.sciencedirect.com/science/article/B6T8P-3S06DYB-B/2/79d610204eb2462ab5eedff6f4cf2575

Dietary methyl deficiency provides an ideal in vivo model system in which to study progressive alterations in DNA methylation patterns as they occur during multistage hepatocarcinogenesis. Weanling male F344 rats were given a semipurified diet deficient in the methyl-donors choline, methionine, and folic acid for a 36-week period with sampling intervals at 3, 9, 24, and 36 weeks. Using a genomic sequencing procedure based on the PCR amplification of bisulfite-modified DNA, the methylation status of individual CpG sites within exons 6 and 7 of the p53 gene in liver samples from control and deficient rats was assessed. Treatment of denatured nuclear DNA with sodium bisulfite converts unmethylated cytosine residues to uracil, which are then amplified as thymine in the PCR reaction. In contrast, methylated cytosines are resistant to bisulfite deamination under these reaction conditions and are amplified as cytosine. In this report, we describe a novel application of automated sequencing technology to estimate the proportion of methylated cytosines present at defined CpG sites within the total population of DNA molecules extracted. Using the bisulfite conversion-PCR genomic sequencing method, we demonstrate the validity of peak height analysis of co-eluting peaks in the autosequencer electrophoregram to estimate the percent methylation at a defined CpG site. The sensitivity of this method is demonstrated by the progressive loss of methyl groups at a defined CpG site in the methyl-deficient rats after 9, 24, and 36 weeks. The application of this sequence-specific technology will allow site-specific definition of the methylation status of each CpG site within a coding sequence or promoter region and should provide new insights into mechanisms and consequences of methylation dysregulation as a result of dietary deprivation of methyl donors.

The Lancet (36)

Ashshi, A. M., R. J. Cooper, et al. (2000). "Detection of human herpes virus 6 DNA in fetal hydrops." The Lancet **355**(9214): 1519.

http://www.sciencedirect.com/science/article/B6T1B-407R3CT-K/2/9a52a09a517b53f2a0d9f1abe5238053

Human herpes virus 6 (HHV6) DNA was detected in two of eight fetuses with hydrops and none of ten non-hydropic dead fetuses. Both cases with HHV6 DNA had chromosomal abnormalities. Positive results were confirmed with a second PCR specific for an alternate region of the HHV6 genome. Restriction endonuclease analysis confirmed that the viral DNA was representative of HHV6 type A.

Bahn, S., M. Mimmack, et al. (2002). "Neuronal target genes of the neuron-restrictive silencer factor in neurospheres derived from fetuses with Down's syndrome: a gene expression study." The Lancet **359**(9303): 310.

http://www.sciencedirect.com/science/article/B6T1B-451NBBT-

D/2/9a3279afd73cc0d16e0edc53550f77db

SummaryBackgroundIdentification of genes and characterisation of their function is an essential step towards understanding complex pathophysiological abnormalities in Down's syndrome. We did a study to investigate abnormalities in gene expression in human neuronal stem cells and progenitor cells from Down's syndrome and control post-mortem human fetal tissue.MethodsIndexing-based differential display PCR was done on neuronal precursor cells derived from the cortex of a fetus with Down's syndrome, and findings were compared with those of two control samples. Findings were validated against neurosphere preparations from three independent Down's syndrome fetuses and five independent controls by real-time quantitative PCR.FindingsResults of differential display PCR analysis showed that SCG10--a neuron-specific growth-associated protein regulated by the neuron-restrictive silencer factor REST--was almost undetectable in the Down's syndrome sample. This finding was validated by real-time PCR. We also found that other genes regulated by the REST transcription factor were selectively repressed, whereas non-REST-regulated genes with similar functions were unaffected. Changes in expression of several key developmental genes in the Down's syndrome stem-cell and progenitor-cell pool correlated with striking changes in neuron morphology after differentiation.InterpretationOur findings suggest a link between dysregulation of the REST transcription factor and some of the neurological deficits seen in Down's syndrome. Experimental REST downregulation has been shown to trigger apoptosis, which could account for the striking and selective loss of neurons in the differentiated Down's syndrome cell preparations.

Baker, E. H., Y. B. Dong, et al. (1998). "Association of hypertension with T594M mutation in [beta] subunit of epithelial sodium channels in black people resident in London." The Lancet **351**(9113): 1388.

http://www.sciencedirect.com/science/article/B6T1B-3Y9H2ND-4Y/2/ff3cc49a315132abaab11e5a750e9df0

Background Liddle's syndrome is a rare inherited form of hypertension in which mutations of the epithelial sodium channel result in increased renal sodium reabsorption. Essential hypertension in black patients also shows clinical features of sodium retention so we screened black people for the T594M mutation, the most commonly identified sodium-channel mutation.Methods In a case-control study, 206 hypertensive (mean age 48.0 [SD 11.8] years, men:women 80:126) and 142 normotensive (48.7 [7.4] years; 61:81) black people who lived in London, UK, were screened for T594M. Part of the last exon of the epithelial sodium-channel [beta] subunit from genomic DNA was amplified by PCR. The T594M variant was detected by single-strand conformational polymorphism analysis of PCR products and confirmed by DNA sequencing.Findings 17 (8.3%) of 206 hypertensive participants compared with three (2.1%) of 142 normotensive participants possessed the T594M variant (odds ratio [OR]=4.17 [95% CI 1.12-18.25], p=0.029). A high proportion of participants with the T594M variant were women (15 of 17 hypertensive participants and all three normotensive participants), whereas women comprised a lower proportion of the individuals screened (61.2% hypertensive, 57.7% normotensive). However, the association between the T594M variant and hypertension persisted after adjustment for sex and body-mass index (Mantel-Haenszel OR=5.52 [1.40-30.61], p=0.012). Plasma renin activity was significantly lower in 13 hypertensive participants with the T594M variant (median=0.19 ng mL-1 h-1 than in 39 untreated hypertensive individuals without the variant (median=0.45 ng mL-1 h-1 p=0.009).Interpretation Among black London people the T594M sodium-channel [beta] subunit mutation occurs more frequently in people with hypertension than those without. The T594M variant may increase sodium-channel activity and could raise blood pressure in affected people by increasing renal tubular sodium reabsorption. These findings suggest that the T594M mutation could be the most common secondary cause of essential hypertension in black people identified to date.

Clay, T. M., J. L. Bidwell, et al. (1991). "PCR-fingerprinting for selection of HLA matched unrelated marrow donors." The Lancet **337**(8749): 1049.

http://www.sciencedirect.com/science/article/B6T1B-49K5B3P-32T/2/d1160bfeb2f462146440a72c0a252723

HLA typing contributes to the delays that occur in the search for HLA-matched unrelated marrow donors, and that result in poor patient survival. A new DNA technique for testing DR match between patient and unrelated marrow donors has been assessed. The technique is based on the formation of heteroduplexes between heterologous amplified coding and non-coding DNA sequences during the final annealing stage of the polymerase chain reaction (PCR), and different HLA-DR/Dw types give unique banding patterns (PCR fingerprints) on non-denaturing polyacrylamide gel electrophoresis. HLA-DR matching is by visual comparison of patients' with donors' fingerprints. Identity can be confirmed by mixing donor and recipient DNA before the final stage of the PCR (DNA crossmatching). In an assessment of the technique in 53 unrelated HLA-A and HLA-B matched patient-donor pairs, 42 pairs gave the same results with PCR fingerprinting as with DNA-RFLP analysis. In the 11 other pairs DR/Dw mismatches were detected by PCR fingerprinting but not by the standard DNA-RFLP method; PCR-SSO typing with selected sequence-specific oligonucleotides (SSO) confirmed that mismatches were due to different subtypes of DR4. PCR fingerprinting might thus accelerate the selection of unrelated marrow donors by simplifying the logistics of the donor search.

Fischereder, M., B. Luckow, et al. (2001). "CC chemokine receptor 5 and renal-transplant survival." The Lancet **357**(9270): 1758.

http://www.sciencedirect.com/science/article/B6T1B-436W05H-H/2/9c30d00629369dcc58f86f59057c5f1c

SummaryBackgroundAbout 1% of white populations are homozygous carriers of an allele of the gene for the CC chemokine receptor 5 (CCR5) with a 32 bp deletion (CCR5[Delta]32), which leads to an inactive receptor. During acute and chronic transplant rejection, ligands for CCR5 are upregulated, and the graft is infiltrated by CCR5-positive mononuclear cells. We therefore investigated the influence of CCR5[Delta]32 on renal-transplant survival.MethodsGenomic DNA from peripheral-blood leucocytes of 1227 renal-transplant recipients was screened by PCR for the presence of CCR5[Delta]32. Demographic and clinical data were extracted from hospital records. Complete follow-up data were available for 576 recipients of first renal transplants. Graft survival was analysed by Fisher's exact test and Kaplan-Meier plots compared with a log-rank test.FindingsPCR identified 21 patients homozygous for CCR5[Delta]32 (frequency 1[middle dot]7%). One patient died with a functioning graft. Only one of the remaining patients lost transplant function during follow-up (median 7[middle dot]2 years) compared with 78 of the 555 patients with a homozygous wild-type or heterozygous CCR5[delta]32 genotype. Graft survival was significantly longer in the homozygous CCR5[Delta]32 group than in the control group (log-rank p=0[middle dot]033; hazard ratio 0[middle dot]367 [95% CI 0[middle dot]157-0[middle dot]859]).InterpretationPatients homozygous for CCR5[Delta]32 show longer survival of renal transplants than those with other genotypes, suggesting a pathophysiological role for CCR5 in transplant loss. This receptor may be a useful target for the prevention of transplant loss.

Gilks, W. P., P. M. Abou-Sleiman, et al. (2005). "A common LRRK2 mutation in idiopathic Parkinson's disease." The Lancet **365**(9457): 415.

http://www.sciencedirect.com/science/article/B6T1B-4FBVXJV-

14/2/2f2237832e72339635c19cb0c53ebff7

Mutations in the leucine-rich repeat kinase 2 (LRRK2) gene have been shown to cause autosomal dominant Parkinson's disease. Few mutations in this gene have been identified. We investigated the frequency of a common heterozygous mutation, 2877510 g->A, which produces a glycine to serine aminoacid substitution at codon 2019 (Gly2019 ser), in idiopathic Parkinson's disease. We assessed 482 patients with the disorder, of whom 263 had pathologically confirmed disease, by direct sequencing for mutations in exon 41 of LRRK2. The mutation was present in eight (1.6%) patients. We have shown that a common single Mendelian mutation is implicated in sporadic Parkinson's disease. We suggest that testing for this mutation will be important in the management and genetic counselling of patients with Parkinson's disease.

Gu, M., J. M. Cooper, et al. (2000). "Oxidative-phosphorylation defects in liver of patients with Wilson's disease." The Lancet **356**(9228): 469.

http://www.sciencedirect.com/science/article/B6T1B-411G2C5-F/2/dc49b8cf18626bde7effa50ad3143051

Background Wilson's disease (WD) is caused by mutations in a P-type ATPase and is associated with copper deposition in liver and brain. The WD protein is present in the trans-Golgi network and may also be imported into mitochondria. The WD protein functions as a P-type copper transporting ATPase in the Golgi but any action in mitochondria is at present unknown.Methods We studied mitochondrial function and aconitase activity in WD liver tissue and compared the results with those in a series of healthy controls and patients without WD.Findings There was evidence of severe mitochondrial dysfunction in the livers of patients with WD. Enzyme activities were decreased as follows: complex I by 62%, complex II+III by 52%, complex IV by 33%, and aconitase by 71%. These defects did not seem to be secondary to penicillamine use, cholestasis, or poor hepatocellular synthetic function.Interpretation The results show that there is a defect of energy metabolism in WD. The pattern of enzyme defects suggests that free-radical formation and oxidative damage, probably mediated via mitochondrial copper accumulation, are important in WD pathogenesis. These results provide a rationale for a study of the use of antioxidants in WD.

Guy, E. and R. Farquhar (1991). "Borrelia burgdorferi in urban parks." The Lancet **338**(8761): 253.

http://www.sciencedirect.com/science/article/B6T1B-49K0C6T-3SB/2/d484f26c8dc3e03cbb0c88e3ad058ec9

Hatzakis, A., G. Touloumi, et al. (2000). "Effect of recent thymic emigrants on progression of HIV-1 disease." The Lancet **355**(9204): 599.

http://www.sciencedirect.com/science/article/B6T1B-3YTB3WH-B/2/ff8ab7a1c64ccd0f063daa07e51e5b62

Background The concentration of T-cell receptor-rearrangement excision DNA circles (TREC) in peripheral-blood T cells is a marker of recent thymic emigrant [alpha]b T cells. We studied the predictive ability of measurements of TREC for clinical outcome in HIV-1-infected individuals.Methods We measured TREC in peripheral-blood mononuclear cells with a real-time PCR assay. We studied 131 Greek participants in the Multicenter Hemophilia Cohort Study who

had known HIV-1 seroconversion dates. The prognostic value of baseline TREC, CD4 T-cell count, and HIV-1 RNA concentration was assessed by Kaplan-Meier and Cox's regression analysis.Findings Four participants had progressed to AIDS by first blood sampling. Among the remaining 127 individuals, the median value of TREC per 106 cells was 6900 (IQR 2370-15 604). Baseline TREC values were lower in the 53 who progressed to AIDS than in those who did not (geometric mean 2843 [95% CI 1468-5504] vs 6560 [4723-9113] TREC per 106 cells; p=0.017). The relative hazard of AIDS, adjusted for plasma viral load, CD4 T-cell count, and age at seroconversion was 1.44 (95% CI 1.04-2.01; p=0.031) per ten-fold increase in TREC; that for death was 1.52 (1.12-2.06; p=0.007). The adjusted relative hazards of death were 2.91 (1.91-4.44; pInterpretation The concentration of TREC in the peripheral T-cell pool complements HIV-1 RNA load and CD4 T-cell count in predicting the rate of HIV-1 disease progression. Recent thymic emigrants have a role in the pathogenesis of HIV-1 disease.

Helweg-Larsen, J., T. L. Benfield, et al. (1999). "Effects of mutations in Pneumocystis carinii dihydropteroate synthase gene on outcome of AIDS-associated P carinii pneumonia." The Lancet **354**(9187): 1347.

http://www.sciencedirect.com/science/article/B6T1B-3XYFJJ5-F/2/2a6659976699f596831e59e09a3773e9

Background Sulpha drugs are widely used for the treatment and long-term prophylaxis of Pneumocystis carinii pneumonia (PCP) in HIV-1-infected individuals. Sulpha resistance in many microorganisms is caused by point mutations in dihydropteroate synthase (DHPS), an enzyme that is essential for folate biosynthesis. We assessed whether mutations in the DHPS gene of P carinii were associated with exposure to sulpha drugs and influenced outcome from PCP.Methods We studied bronchoalveolar samples collected in 1989-99 from a prospective cohort of HIV-1-infected patients who had PCP. In 144 patients with 152 episodes of PCP, we analysed portions of DHPS using PCR and direct sequencing. The relation between survival, P carinii DHPS mutations, and other predictors of treatment failure was assessed by Kaplan-Meier and multivariate Cox regression analysis.Findings P carinii DHPS mutations were found in 31 (20.4%) of 152 PCP episodes. 3-month survival was significantly lower in patients infected with mutant P carinii DHPS strains than in those with wild-type strains (p=0.002). After adjustment for other prognostic variables, presence of DHPS mutations remained the most important predictor of mortality (hazard ratio 3.1 [95% CI 1.2-8.1]). DHPS mutations were significantly more common in patients who had previous exposure to sulpha drugs (18 of 29 [62%]) than in those who had no exposure (13 of 123 [10.5%]; pDHPS mutations (p=0.01 for trend) was closely correlated with the rate of previous or current use of sulpha drugs as chemoprophylaxis.Interpretation Mutations in DHPS are associated with impaired prognosis in PCP, and may develop as a result of exposure to sulpha drugs.

Hill, A. V. S., C. E. M. Allsopp, et al. (1991). "HLA class I typing by PCR: HLA-B27 and an African B27 subtype." The Lancet **337**(8742): 640.

http://www.sciencedirect.com/science/article/B6T1B-49K2KBM-1H1/2/53214cf820778f8cbb3f5b51735ef164

We describe a rapid method of HLA class I typing using the polymerase chain reaction and oligonucleotide hybridisation that eliminates the requirements for viable lymphocytes and allows subtypes to be defined. We have used this to demonstrate that the predominant subtype of H LA-B27 in the Gambia, West Africa, is HLA-B*2703, which is very rare or absent in other racial groups. This subtype differs from the common Caucasian HLA-B27 subtypes in its recognition by cytotoxic T cells. We propose that HLA*B-2703, unlike other HLA-B27 subtypes, may not be

associated with ankylosing spondylitis, thus accounting in part for the rarity of this condition in black populations.

Holding, C. and M. Monk (1989). "DIAGNOSIS OF BETA-THALASSAEMIA BY DNA AMPLIFICATION IN SINGLE BLASTOMERES FROM MOUSE PREIMPLANTATION EMBRYOS." The Lancet **334**(8662): 532.

http://www.sciencedirect.com/science/article/B6T1B-49HFW1P-4CR/2/35da56adf530a8c756d89df6d86d4690

Mouse preimplantation embryos were accurately diagnosed as normal or mutant at the beta-major haemoglobin locus by amplification of specific DNA sequences in a single cell. A DNA sequence containing the whole of exon 3 and some 3' untranslated sequences within the beta-major haemoglobin gene was amplified in single blastomeres by means of the polymerase chain reaction (PCR). Blastomeres were removed from embryos of four to eight cells from normal BALB/c mice and from mutant (thalassaemic) BALB/c mice homozygous for a deletion of the whole beta-major haemoglobin gene. The sensitivity of the amplification procedure was enhanced by the sequential use of two sets of oligonucleotide primers for 30 cycles of amplification each, the second pair being located within the segment amplified by the first pair. The product (204 base-pairs) could be easily visualised in ethidium bromide-stained agarose gels. Stringent precautions to prevent contamination were taken, and with these precautions the PCR amplification procedure could be carried out under normal laboratory conditions. These procedures for diagnosis of genetic disease before

Ingrosso, D., A. Cimmino, et al. (2003). "Folate treatment and unbalanced methylation and changes of allelic expression induced by hyperhomocysteinaemia in patients with uraemia." The Lancet **361**(9370): 1693.

http://www.sciencedirect.com/science/article/B6T1B-48KVF5R-B/2/3d77a37ffeeae4d8f7f353993dfe2401

SummaryBackgroundHyperhomocysteinaemia occurs in several genetically determined and acquired disorders and is highly prevalent in patients with uraemia. In these disorders, homocysteine precursor S-adenosylhomocysteine, a powerful competitive inhibitor of S-adenosylmethionine-dependent methyltransferases, is increased, suggesting unbalanced methylation. We aimed to investigate whether DNA hypomethylation is present in patients with uraemia who also have hyperhomocysteinaemia and whether regulation of specific classes of genes, dependent on DNA methylation, is compromised.MethodsWe selected men with hyperhomocysteinaemia and uraemia who were having standard haemodialysis treatment, and compared them with healthy male controls. We measured the homocysteine concentration from plasma samples and obtained DNA and RNA samples from peripheral mononuclear cells. DNA methylation was assessed by cytosine extension assay and by Southern blotting. Allelic expression of pseudoautosomal and imprinted genes was investigated by analysis of suitable restriction fragment length polymorphisms.FindingsTotal DNA hypomethylation was higher in patients than in controls (z score -4[middle dot]593, p=0[middle dot]0006) and allelic expression was changed in both sex-linked and imprinted genes. The shift from monoallelic to biallelic expression was dependent on homocysteine concentrations. Folate therapy, a common method to reduce hyperhomocysteinaemia, restored DNA methylation to normal levels and corrected the patterns of gene expression.InterpretationOur results suggest that hyperhomocysteinaemia affects epigenetic control of gene expression, which can be reverted by folate treatment. Our data support the hypothesis that the toxic action of homocysteine can be mediated by macromolecule hypomethylation.

Joynson, D. H. M., C. Verhofstede, et al. (1990). "Congenital toxoplasmosis and TORCH." The Lancet **336**(8715): 622.

http://www.sciencedirect.com/science/article/B6T1B-49M0JHS-20R/2/a055fc745e2c578f5f4c912ed171d1b9

Kahn, R., H. Herwald, et al. (2002). "Contact-system activation in children with vasculitis." The Lancet **360**(9332): 535.

http://www.sciencedirect.com/science/article/B6T1B-46HNW1W-D/2/3593cd67943d813e92da0a08f435bedd

SummaryBackgroundThe contact system triggers the kallikrein-kinin cascade, liberating bradykinin from high-molecular-weight kininogen. Effectors of the contact system have proinflammatory and vasoactive properties. Vasculitis is a condition characterised by inflammation around vessel walls, leading to secondary tissue damage for which the underlying molecular mechanisms are poorly understood. Our aim was to investigate contact-system activation in children with vasculitis.MethodsWe compared 17 children, aged 4-19 years, with vasculitis, engaging the skin, joints, intestines, or kidneys, with 21 controls, aged 2-18 years. We analysed proteolysis of high-molecular-weight kininogen by immunoblotting. Plasma bradykinin concentrations were quantified by ELISA. Kidney and skin biopsies were stained in situ for kinins. Concentrations of heparin binding protein (HBP) were quantified by ELISA.FindingsWe noted extensive proteolysis of high-molecular-weight kininogen in the plasma of 13 of 17 patients, but in only one of 21 controls (pInterpretationActivation of the contact system could play a part in the pathogenesis of vasculitis, and explain the inflammation, pain, vasodilatation, and oedema seen in patients.

Kawakami, T., K. Okamoto, et al. (2004). "XISTunmethylated DNA fragments in male-derived plasma as a tumour marker for testicular cancer." The Lancet **363**(9402): 40.

http://www.sciencedirect.com/science/article/B6T1B-4BC1S83-J/2/12d29dfac4096e6364ae164ccf25cba3

SummaryTesticular germ-cell tumours (TGCTs) are the most common malignant diseases among young men, with peak incidence at age 20-40 years. We developed a DNA tumour marker for TGCTs based on the unmethylated DNA profile of a neoplasm. The 5' end of the XIST gene is mainly hypomethylated in TGCTs irrespective of XIST expression. Male somatic cells, however, show complete methylation through the CpG sites, including the minimum promoter and XIST-conserved repeats. Identification of a XIST unmethylated fragment in male plasma might be diagnostic for TGCTs.

Laule, M., I. Cascorbi, et al. (1999). "A1/A2 polymorphism of glycoprotein IIIa and association with excess procedural risk for coronary catheter interventions: a case-controlled study." The Lancet **353**(9154): 708.

http://www.sciencedirect.com/science/article/B6T1B-3W9KVPB-C/2/3ccb53a874d0f2ca0139bb43f8b86ef5

Background A five-fold increase in risk of stent thrombosis in carriers of A1/A2 (Leu33Pro) polymorphism of glycoprotein IIIa has been described. Whether this increased procedural risk applies to other coronary interventions is unknown. We investigated the role of A1/A2 polymorphism as a putative risk factor.Methods We genotyped 1000 consecutive patients with angiographically confirmed coronary-artery disease and 1000 controls matched for age and sex. 653 of the 1000 patients received interventions (271 coronary angioplasty, 102 directional coronary atherectomy, and 280 stenting) and were assessed for a 30-day composite endpoint of need for target-vessel revascularisation, myocardial infarction, and death.Findings The composite endpoint occurred in 41 (6.3%) patients. There was no evidence that the A2 allele was associated with excess procedural risk (relative risk 1.36 [95% CI 0.70-2.70], p=0.37). Nor, in subgroup analyses, did A2 predict events that complicated coronary angioplasty (1.17 [0.40-2.70]), directional coronary atherectomy (1.50 [0.30-8.70]), or stenting (1.45 [0.60-3.50]). Neither heterozygotes (A1/A2) nor homozygotes (A2/A2) were over-represented in any subgroup, including those with acute coronary syndromes, early disease manifestation (age Interpretation A1/A2 polymorphism is not a major risk factor for 30-day adverse events that complicate coronary angioplasty, directional coronary atherectomy, or stenting. Furthermore, A1/A2 polymorphism has no apparent impact on more chronic processes such as atherogenesis of the coronary arteries.

Lewis, D. L., M. Arens, et al. (1992). "Cross-contamination potential with dental equipment." The Lancet **340**(8830): 1252.

http://www.sciencedirect.com/science/article/B6T1B-49K57JH-18X/2/42d0cf88fde351239acb240791367319

Some types of reused dental equipment, especially handpieces and their attachments for drilling and cleaning teeth, might be responsible for cross-contamination if patient material were to lodge temporarily in difficult-to-disinfect internal mechanisms. This possibility is worrisome with respect to transmission of hepatitis B and human immunodeficiency viruses (HBV, HIV). Previous cross-contamination studies have relied on laboratory experiments with bacteria or dye tracers. To assess possible risks more thoroughly, we tested 30 new prophylaxis angles and 12 new high-speed handpieces to see whether they would take up and expel contaminants in laboratory and clinical trials.In treatments of three patients, including two infected with HIV, human-specific DNA ([beta]-globin, HLA DQ[alpha]) and HIV proviral DNA were detected inside or coming back from the devices. Similarly, when handpieces were operated in contact with blood pooled from HBV-infected patients, HBV DNA was detected in samples taken from inside the equipment and from their attached air/water hoses. When we used bacteriophage [phi]X174 as a model virus in laboratory tests, many infective viral particles were recovered from internal mechanisms of handpieces, their connecting air/water hoses, and from water spray expelled when the equipment was reused.We recommend that reused high-speed, air-driven handpieces and prophylaxis angles should be cleaned and heat-treated between patients. Further studies are needed to determine ways of eliminating the risks associated with exhaust hoses and air/water input lines.

Limaye, A. P., L. Corey, et al. (2000). "Emergence of ganciclovir-resistant cytomegalovirus disease among recipients of solid-organ transplants." The Lancet **356**(9230): 645.

http://www.sciencedirect.com/science/article/B6T1B-41357RN-F/2/ea698fd2a271021da108258eaf25c646

SummaryBackgroundConcerns have been raised about emergence of ganciclovir resistance as a result of the advent of both routine oral ganciclovir prophylaxis and highly potent immuno-suppression. We retrospectively assessed the occurrence of ganciclovir-resistant cytomegalovirus disease among transplant recipients who had received oral ganciclovir

prophylaxis and highly potent immunosuppression.MethodsWe studied 240 recipients of liver, kidney, or pancreas transplants. Antiviral susceptibility testing of blood cytomegaloviral isolates was done when patients failed to respond to intravenous ganciclovir treatment for symptomatic cytomegalovirus infection. Portions of the UL97 gene associated with ganciclovir resistance were sequenced in cytomegalovirus isolates with phenotypic resistance to ganciclovir.FindingsGanciclovir-resistant cytomegalovirus disease developed in five (7%) of 67 seronegative recipients of cytomegalovirus-seropositive organs (D+/R-) compared with none of 173 seropositive recipients (p=0[middle dot]002). Among the 25 (10[middle dot]4%) patients who developed cytomegalovirus disease within 1 year after transplantation, five had ganciclovir-resistant cytomegalovirus disease. Among D+/R- transplant recipients, ganciclovir-resistant cytomegalovirus disease was more common among the group receiving the most potent immunosuppression--ie, recipients of kidney and pancreas or pancreas alone (four of 19) compared with all other transplant recipients (one of 48, p=0[middle dot]02). Ganciclovir-resistant cytomegalovirus disease was diagnosed at a median of 10 months after transplantation (range 7-12) after lengthened exposure to ganciclovir, was associated with previously described mutations of the UL97 gene, and led to serious clinical complications.InterpretationGanciclovir-resistant cytomegalovirus is an important cause of late morbidity among D+/R- transplant recipients who have had lengthened exposure to ganciclovir and have received highly potent immunosuppression. Strategies to reduce this complication, especially among D+/R- patients, are warranted.

Lipschik, G. Y., V. A. Andrawis, et al. (1992). "Improved diagnosis of Pneumocystis carinii infection by polymerase chain reaction on induced sputum and blood." The Lancet **340**(8813): 203.

http://www.sciencedirect.com/science/article/B6T1B-49K5G3B-7N/2/99bf161b0209ccf0359f3db8a841141b

Detection of Pneumocystis carinii by the polymerase chain reaction (PCR) may facilitate non-invasive diagnosis of P carinii pneumonia and study of its epidemiology. We have compared the sensitivity and specificity of two PCR methods with those of conventional staining for detection of P carinii in induced sputum, bronchoalveolar lavage fluid (BAL), and blood. Of 71 sputum samples, 17 were from patients with microbiologically confirmed P carinii pneumonia. A nested PCR method correctly identified the presence of P carinii in all 17 (100% sensitive, 95% confidence interval [Cl] 81-100%) and found no organisms in 50 of 54 microbiologically negative samples (93% specific, 95% Cl 82-98%). PCR with a single primer pair was 71% sensitive (44-90%) and 94% specific (85-99%). The sensitivity of conventional staining methods (direct and indirect fluorescence antibody and toluidine-blue-O tests) was significantly less (38-53%) than that of nested PCR (pP carinii was detected in BAL or sputum from 10 immunocompromised patients without microbiological evidence of P carinii pneumonia, which suggests that symptom-free carriers or subclinical infection can exist. P carinii was detected by nested PCR in blood from 2 of 3 patients with disseminated pneumocystosis but in only 1 of 11 patients with P carinii infection restricted to the lungs. Nested PCR on induced sputum is more sensitive than conventional staining methods for the diagnosis of P carinii pneumonia and provides a non-invasive method of detecting disseminated disease.

Luoma, P., A. Melberg, et al. "Parkinsonism, premature menopause, and mitochondrial DNA polymerase [gamma] mutations: clinical and molecular genetic study." The Lancet **364**(9437): 875.

http://www.sciencedirect.com/science/article/B6T1B-4D8V8SH-19/2/97c2d16510ef0a7f6f0d4e0da9896ab5

SummaryBackgroundMutations in the gene encoding mitochondrial DNA polymerase [gamma]

(POLG), the enzyme that synthesises mitochondrial DNA (mtDNA), have been associated with a mitochondrial disease--autosomal dominant or recessive progressive external ophthalmoplegia--and multiple deletions of mtDNA. Mitochondrial dysfunction is also suspected to participate in the pathogenesis of Parkinson's disease. However, no primary gene defects affecting mitochondrial proteins causing mendelian transmission of parkinsonism have been characterised. We aimed to analyse the gene sequence of POLGin patients with progressive external ophthalmoplegia and their healthy relatives.MethodsIn seven families of various ethnic origins we assessed patients with progressive external ophthalmoplegia and unaffected individuals by clinical, biochemical, morphological, and molecular genetic characterisation and positron emission tomography (PET).FindingsWe recorded mutations in POLG in members of all seven families. Clinical assessment showed significant cosegregation of parkinsonism with POLGmutations (pPOLG gene defect resulted in secondary accumulation of mtDNA deletions in patients' tissues.InterpretationDysfunction of mitochondrial POLG causes a severe progressive multisystem disorder including parkinsonism and premature menopause, which are not typical of mitochondrial disease. Cosegregation of parkinsonism and POLG mutations in our families suggests that when defective, this gene can underlie mendelian transmission of parkinsonism.Relevance to practiceAwareness that mitochondrial POLG mutations can underlie parkinsonism is important for clinicians working in diagnosis of movement disorders, as well as for studies of the genetics of Parkinson's disease. Further, progressive external ophthalmoplegia with muscle weakness and neuropathy can mask symptoms of parkinsonism, and clinicians should pay special attention to detect and treat parkinsonism in those individuals.

Mitchell, T. J., A. J. Walley, et al. (2000). "Delta 32 deletion of CCR5 gene and association with asthma or atopy." The Lancet **356**(9240): 1491.

http://www.sciencedirect.com/science/article/B6T1B-41PVXTP-M/2/287ce1e5dd5a24f5f33b7a7eef5ddc8b

SummaryThe CCR5-[Delta]32 deletion polymorphism (CCR5-[Delta]32) was investigated for linkage and association to asthma and atopy using two panels of nuclear families containing 1284 individuals. No statistically significant linkage to asthma/ wheeze or atopy was observed in either of the two panels of families. Multiallelic transmission disequilibrium tests (TDT) of the combined data found no significant association for atopy (52 independent alleles transmitted, 51 non-transmitted) or asthma/wheeze (39 transmitted, 44 non-transmitted). Although functional evidence might suggest that CCR5 is a good candidate gene for atopic asthma, this study provides no genetic evidence from CCR5-[Delta]32 polymorphism to support this hypothesis.

Monk, M. and C. Holding (1990). "Amplification of a [beta]-haemoglobin sequence in individual human oocytes and polar bodies." The Lancet **335**(8696): 985.

http://www.sciencedirect.com/science/article/B6T1B-49M0N8R-373/2/1e7485d6a509b2e1d656648127268f79

A 680 base-pair sequence of the human [beta]-haemoglobin gene was reproducibly amplified in individual unfertilised human oocytes and in first polar bodies isolated from them. Specificity and sensitivity of amplification were achieved by two sequential reactions with two sets of primers, amplifying first a 725 base-pair sequence and secondly a 680 base-pair sequence from within the first amplified fragment. A restriction enzyme digest of the DNA amplified from a single oocyte with the endonuclease Dde I confirmed the identity of the amplified [beta]-haemoglobin fragment; this technique provides a diagnostic test for the genetic defect responsible for sickle cell anaemia. Analysis of the DNA from the first polar body may enable detection of such defects in unfertilised eggs from carrier women. Selection of eggs without the defect for fertilisation may therefore

obviate the need for diagnostic procedures on embryos.

Munroe, P. B., H. M. Mitchison, et al. (1996). "Prenatal diagnosis of Batten's disease." The Lancet **347**(9007): 1014.

http://www.sciencedirect.com/science/article/B6T1B-4B8JK7N-169/2/02abac5f95591fd0c1d66dda15a92f07

SummaryBackground Batten's disease is the most common progressive encephalopathy of childhood in Western countries. The major mutation is a 1 kb deletion, which is carried by 81% of Batten's disease patients. We report on the use of direct gene analysis in the prenatal diagnosis of this disease.Methods and findings A Finnish woman with a son with Batten's disease came for genetic counselling for her current pregnancy. Electron microscopy of a chorionic villus sample gave suggestive findings. We used PCR to look for the intragenic microsatellite marker D16S298; 96% of Finnish Batten's disease patients carry allele 6 at this marker. The fetus and the affected son both carried the same high-risk genotype, 6/6. Both were homozygous for the 1 kb deletion. The pregnancy was terminated. Electron microscopy of the fetus showed typical Batten's disease changes.Interpretation We have successfully used direct gene analysis in the prenatal diagnosis of Batten's disease.

Naylor, J. A., P. M. Green, et al. (1991). "Detection of three novel mutations in two haemophilia A patients by rapid screening of whole essential region of factor VIII gene." The Lancet **337**(8742): 635.

http://www.sciencedirect.com/science/article/B6T1B-49K2KBM-1GY/2/54e17c75b8fae4185bf0bbc088fa13a1

In an attempt to replace the existing, DNA-based, 50% effective, carrier and prenatal diagnoses of haemophilia A with the 100% successful direct detection of defective genes, a new procedure was developed to screen and identify mutations in all the essential regions of the factor VIII gene (putative promoter, coding sequence, and the cleavage and polyadenylation region). Genomic DNA and cDNA obtained by reverse transcription of the leaky mRNA found in peripheral lymphocytes were amplified by means of the polymerase chain reaction to yield a set of eight segments comprising the essential gene sequences. The segments were then screened individually for mutations by the amplification mismatch detection method, which detects and locates any type of sequence discrepancy between the test DNA and the control probe by cleavage of the probe at the site of mismatches. Two haemophilia A patients were studied. The first showed two single-base changes: one (substitution of tryptophan 2229 by cysteine in the C2 domain) is the probable cause of the disease, since it affects a conserved residue of factor VIIIa, whereas the other (the conservative substitution of aspartic acid at position 1241 by glutamic acid) occurs in a domain (B) irrelevant to factor VIII activity. The second patient showed a complete failure of pre-mRNA splicing due to a single-base substitution that changes the obligatory AG acceptor splice site of intron 5 to GG. The method characterises the gene defect in 10 days or less and should lead to the rapid accumulation of information on the molecular biology of haemophilia A.

Old, J. M., N. Y. Varawalla, et al. (1990). "Rapid detection and prenatal diagnosis of [beta]-thalassaemia: studies in Indian and Cypriot populations in the UK." The Lancet **336**(8719): 834.

http://www.sciencedirect.com/science/article/B6T1B-49M0MHX-

2WH/2/ee54c2e8ae7ce7266c4d57e02db9e134

The application of the amplification refractory mutation system (ARMS) to the detection of individual [beta]-thalassaemia mutations in heterozygous parents and at risk fetuses has been assessed in Indian and Cypriot immigrant populations in the UK. 100 first trimester prenatal diagnoses have been done, entailing the detection of 17 different mutations. The method, which allows the determination of the mutations in both parental and fetal DNA on the same day, should have wide application to the carrier detection and prenatal diagnosis of monogenic diseases with heterogeneous molecular defects.

Price, C. G. A., F. E. Cotter, et al. (1989). "POLYMERASE CHAIN REACTION TO CONFIRM EXTRANODAL PROGRESSION OF FOLLICULAR LYMPHOMA." The Lancet **333**(8647): 1132.

http://www.sciencedirect.com/science/article/B6T1B-49KJXD5-147/2/a76c783b8db34652886a5a633022622e

Quillent, C., E. Oberlin, et al. (1998). "HIV-1-resistance phenotype conferred by combination of two separate inherited mutations of CCR5 gene." The Lancet **351**(9095): 14.

http://www.sciencedirect.com/science/article/B6T1B-3TXSRB9-4/2/7ca76ab386c17ac1ef25bff5bbb5f0db

Background Despite multiple exposures to HIV-1, some individuals remain uninfected, and their peripheral-blood mononuclear cells (PBMC) are resistant to in-vitro infection by primary HIV-1 isolates. Such resistance has been associated with a homozygous 32-base-pair deletion ([Delta]32) in the C-C chemokine receptor gene CCR5. We examined other mutations of the CCR5 gene that could be associated with resistance to HIV-1 infection.Methods We assessed the susceptibility of PBMC to in-vitro infection by HIV-1 isolates that use the CCR5 as the major coreceptor for viral entry in 18 men who had frequent unprotected sexual intercourse with a seropositive partner. We also did genotypic analysis of CCR5 alleles. One of the 18 exposed but uninfected men (who we refer to as ExU2) showed total resistance to in-vitro infection by CCR5-dependent viruses, and was found to carry a CCR5 [Delta]32 allele and a single point mutation (T->A) at position 303 on the other allele. To find out whether the CCR5 mutation was restricted to ExU2's family or existed in the general population, we did genetic analyses of the CCR5 genotype in ExU2's father and sister and also in 209 healthy blood donors who were not exposed to HIV-1.Findings The m303 mutation found in ExU2 introduced a premature stop codon and prevented the expression of a functional coreceptor. The family studies revealed that the m303 mutant allele was inherited as a single mendelian trait. Genotype analysis showed that three of the 209 healthy blood donors were heterozygous for the mutant allele.Interpretation We characterise a new CCR5 gene mutation, present in the general population, that prevents expression of functional coreceptors from the abnormal allele and confers resistance to HIV-1 infection when associated to the [Delta]32 CCR5 mutant gene.

Roberts, R. G., D. R. Bentley, et al. (1990). "Direct diagnosis of carriers of Duchenne and Becker muscular dystrophy by amplification of lymphocyte RNA." The Lancet **336**(8730-8731): 1523.

http://www.sciencedirect.com/science/article/B6T1B-4B023PS-RY/2/8fd3065f966705ba91ee5744cdf4ed3a

Rapid detection of deletion and duplication mutations that cause Duchenne and Becker muscular dystrophy was achieved in patients and carriers after amplification of small amounts of mRNA from peripheral blood lymphocytes. The entire coding region of the dystrophin mRNA was amplified in 10 sections by reverse transcription and nested polymerase chain reaction, and the products were directly visualised on acrylamide minigels with ethidium staining. Major structural gene mutations were identified by the appearance of a band of different size to that of the wild type. The altered band was readily detected in all patients and heterozygous relatives. This nonradioactive test of venous blood samples can be used for unambiguous and rapid identification of virtually all carriers of deletions or insertions within the dystrophin gene.

Roth, W. K., M. Weber, et al. (1999). "Feasibility and efficacy of routine PCR screening of blood donations for hepatitis C virus, hepatitis B virus, and HIV-1 in a blood-bank setting." The Lancet **353**(9150): 359.

http://www.sciencedirect.com/science/article/B6T1B-3W49589-6/2/662c95d38f4c48bc31c59b324a6df469

Background Despite sensitive antibody-based blood-donor screening, a residual risk of transfusion-transmitted viral infections exists. Only direct monitoring by sensitive nucleic-acid tests would provide data accurately to measure the risk and to assess risk-reduction procedures. We investigated the feasibility and efficacy of routine screening of donors for hepatitis C virus (HCV), hepatitis B virus (HBV), and HIV-1 by PCR.Methods For PCR testing, individual donor plasma samples were pooled (96 x 100 [mu]L) overnight by two automatic pipetting machines. Viruses were concentrated by centrifugation and nucleic acids were extracted. HCV PCR was done on the Cobas Amplicor system (Hoffmann-La Roche, Mannheim, Germany). HBV and HIV-1 sequences were amplified by single (non-nested) in-house PCRs and detected by agarose-gel electrophoresis. Detection limits were 1000-5000 genome equivalents/mL in the donor blood.Findings PCR testing was done in parallel to antibody screening with a maximum throughput of 3000 samples in 7-8 h. Positive samples were identified 1-2 days later. 111 of 373423 donations (107 of 4500 pools) were PCR and antibody/antigen-confirmed positive. We found one HCV PCR-positive antibody-negative donation with normal alanine aminotransferase and one HCV PCR-positive donation with an elevated alanine aminotransferase (100 IU), which was negative in the AxSYM 2.0 and Matrix 1.0, but positive after control in the Abbott Prism test (Abbott GmbH, Wiesbaden, Germany).Interpretation PCR is a suitable and fast blood-donor screening procedure and contributes to a reduction in viral transmission by transfusion of blood components. In our selected donor population, the yield of detected contaminated donations from donors in the time window in which they are highly infectious but do not have any symptoms or detectable antigen and antibody concentrations (diagnostic window), confirms theoretical estimates.

Wakefield, A. E., F. J. Pixley, et al. (1990). "Detection of Pneumocystis carinii with DNA amplification." The Lancet **336**(8713): 451.

http://www.sciencedirect.com/science/article/B6T1B-49M0HT4-1K1/2/782aac00830cfaa0c559ab64dcc5ce73

Oligonucleotide primers and probes were used in the polymerase chain reaction to amplify Pneumocystis carinii specific DNA sequences from alveolar lavage samples from 47 diagnostic bronchoscopies. No P carinii DNA was found in lavage from 10 immunocompetent patients; only low levels were found in 3 of 13 samples from immunosuppressed individuals without P cariniipneumonia (PCP), and the highest levels, readily demonstrated by simple ethidium bromide staining, were found in all of 16 samples from immunosuppressed patients with PCP confirmed

by means of standard silver staining and in 4 from patients with clinical PCP but negative silver staining. DNA amplification provides a highly sensitive and specific technique for the identification of Pcarinii that should be valuable in epidemiological studies on this parasitic infection and in diagnosis.

Wenham, P., W. Price, et al. (1991). "Apolipoprotein E genotyping by one-stage PCR." The Lancet **337**(8750): 1158.

http://www.sciencedirect.com/science/article/B6T1B-49K5BJG-3DT/2/ee69fa32cbc4832c8c462800ecb94228

Wong, Z. Y. H., M. Stebbing, et al. (1999). "Genetic linkage of [beta] and [gamma] subunits of epithelial sodium channel to systolic blood pressure." The Lancet **353**(9160): 1222.

http://www.sciencedirect.com/science/article/B6T1B-3WK3BSG-B/2/f186398e4702485929c6f6857d84871f

Background Mutations in the genes on chromosome 16p12 that encode the [beta] and [gamma] subunits of the epithelial sodium channel (SCNNIB and SCNNIG, respectively) have been linked with rare sodium-dependent forms of low and high blood pressure. Other DNA variants in or around these genes may contribute to variation in blood pressure and the risk of coronary heart disease and stroke.Methods We studied 286 white families from the general population in Victoria, Australia. Each family comprised both parents and two natural children. All participants were genotyped at chromosome 16p12 by use of four highly polymorphic microsatellite markers. Quantitative phenotype measurements were correlated with genotype in identity-by-descent sibling-pair linkage analyses.Findings We found significant linkage between systolic blood pressure and chromosome 16p12 after parametric analyses (p=0.0003) and non-parametric analyses (p=0.001). The mean difference in systolic blood pressure between siblings identical-by-descent at these loci was half as large (7.1 mm Hg) as the difference between siblings non-identical at these loci (14.0 mm Hg, p=0.001). No linkage between chromosome 16p12 and diastolic blood pressure or body-mass index was observed.Interpretation Chromosome 16p12 and the SCNNIB and SCNNIG genes are implicated in the physiological variation of systolic blood pressure. Our findings are important in explaining individual cardiovascular risk within the general population.

Wright, T. L., D. Mamish, et al. (1992). "Hepatitis B virus and apparent fulminant non-A, non-B hepatitis." The Lancet **339**(8799): 952.

http://www.sciencedirect.com/science/article/B6T1B-49K572T-XT/2/817f081654d906ee8a2d097d4205efe1

While there is evidence that hepatitis C virus (HCV) does not cause fulminant non-A, non-B hepatitis, the causal agent remains unknown. To evaluate the role of hepatitis B virus (HBV) in this disease, we used a two-step polymerase chain reaction (PCR) to amplify the surface and core regions of HBV DNA in serum and liver samples taken prospectively from twenty-six patients (mean age 36 years, range 1 to 64) with acute hepatic failure undergoing liver transplantation. HBV DNA was absent from the serum of all patients before transplantation. Seventeen patients were diagnosed as having non-A, non-B hepatitis because they lacked serological evidence of hepatitis A virus or HBV infection. Liver samples were taken from twelve of these patients, and six

samples were positive for HBV DNA. By contrast HBV DNA was not detected in liver from three patients with acute liver failure caused by hepatitis A or toxins. HCV RNA was not found in pretransplant samples by PCR. Four of the six patients with detectable HBV DNA in liver and presumptive non-A, non-B hepatitis had detectable HBV DNA in serum after transplantation. One additional patient who did not donate pretransplant liver had HBV DNA in a post-transplant serum sample. Thus, HBV DNA was present before or after transplantation in seven of seventeen patients with apparent non-A, non-B hepatitis. Three of five patients with detectable post-transplant serum HBV DNA were serologically positive for HBV surface antigen. These findings indicate that HBV may be a common cause of fulminant hepatic failure in patients lacking serological evidence of HBV infection.

Yagi, H., Y. Furutani, et al. (2003). "Role of TBX1 in human del22q11.2 syndrome." The Lancet **362**(9393): 1366.

http://www.sciencedirect.com/science/article/B6T1B-49V58KN-B/2/a1dfc425a7e308d963895e91bba51c7f

SummaryBackgroundDel22q11.2 syndrome is the most frequent known chromosomal microdeletion syndrome, with an incidence of 1 in 4000-5000 livebirths. It is characterised by a 3-Mb deletion on chromosome 22q11.2, cardiac abnormalities, T-cell deficits, cleft palate facial anomalies, and hypocalcaemia. At least 30 genes have been mapped to the deleted region. However, the association of these genes with the cause of this syndrome is not clearly understood.MethodsTo test for the chromosomal deletion at 22q11.2, we did fluorescence in-situ hybridisation analysis with ten probes on 22q11.2 in 235 unrelated patients with clinically diagnosed del22q11.2 syndrome. To investigate mutations in the coding sequence of TBX1, we also did genetic analysis in 13 patients from ten families who have the 22q11.2 syndrome phenotype but no detectable deletion of 22q11.2.Findings96% (225 of 235) of patients had a defined 1[middle dot]5-3-Mb deletion at 22q11.2. We identified three mutations of TBX1 in two unrelated patients without the 22q11.2 deletion--one with sporadic conotruncal anomaly face syndrome/velocardiofacial syndrome and one with sporadic DiGeorge's syndrome--and in three patients from a family with conotruncal anomaly face syndrome/velocardiofacial syndrome. We did not record these three mutations in 555 healthy controls (1110 chromosomes; pInterpretationOur results suggest that the TBX1 mutation is responsible for five major phenotypes in del22q112 syndrome. Therefore, we conclude that TBX1 is a major genetic determinant of the del22q11.2 syndrome.

Yerly, S., L. Kaiser, et al. (1999). "Transmission of antiretroviral-drug-resistant HIV-1 variants." The Lancet **354**(9180): 729.

http://www.sciencedirect.com/science/article/B6T1B-3XFTGMD-D/2/8bd731cae2532a0c2241dab7b74cf73e

Background Resistance of HIV-1 to antiretroviral drugs is the main cause of antiretroviral-treatment failure. We assessed the transmission of drug-resistant variants among individuals with primary HIV-1 infection.Methods Population-based sequencing of the viral reverse-transcriptase and protease genes derived from plasma viral RNA was done in 82 consecutive individuals with documented primary HIV-1 infection from January, 1996, to July, 1998. Phenotypic resistance to protease inhibitors was assessed by recombinant virus assay in individuals with two or more mutations associated with resistance to protease inhibitors.Findings Zidovudine-resistance mutations were detected in seven (9%) of 82 individuals. Mutations associated with resistance to other reverse-transcriptase inhibitors (RTIs) were detected in two individuals. Primary-resistance mutations associated with protease inhibitors (V82A, L90M) were detected in three (4%) of 70

individuals; two of these had also RTI-resistance mutations. Decreased sensitivity to three or four protease inhibitors was seen in three individuals, one of whom was infected with HIV-1 variants that harboured 12 mutations associated with resistance to multiple RTI and protease inhibitors.Interpretation To introduce the best antiretroviral treatment, resistance testing should be done in recently HIV-1-infected individuals.

The Science of The Total Environment (2)

Biscardi, D., A. Castaldo, et al. (2002). "The occurrence of cytotoxic Aeromonas hydrophila strains in Italian mineral and thermal waters." The Science of The Total Environment **292**(3): 255.

http://www.sciencedirect.com/science/article/B6V78-44X036P-3/2/f9f16bfa7eec7af633dd8eb1f70771ca

Bacteria of the genus Aeromonas are ubiquitous in aquatic environments, including mineral drinking and thermal waters. Motile species are related to different diseases, mostly gastrointestinal disorders. Criteria for Aeromonas pathogenicity in humans and animals are still unclear and neither is the relationship between production virulence and pathogenicity factors. In the present study, strains of Aeromonas hydrophila, from 61 samples of bottled mineral waters and 23 thermal Italian sources have been isolated and identified by biochemical tests, for toxicity and detection of the aerolysin gene by the Polymerase Chain Reaction (PCR). Six strains were isolated from the mineral waters and were found to be cytotoxic and in possession of the aerolysin gene. For the twelve strains isolated from thermal waters, seven were cytotoxic and eleven contained the aerolysin gene.

Cousinou, M., B. Nilsen, et al. (2000). "New methods to use fish cytochrome P4501A to assess marine organic pollutants." The Science of The Total Environment **247**(2-3): 213.

http://www.sciencedirect.com/science/article/B6V78-3YVM1DK-F/2/42fb9b90f24f6ff003ec8adb32b72ebb

A new methodology has been developed to assess cytochrome P4501A expression in two South Atlantic Spanish fish, guilthead seabream (Sparus aurata) and grey mullet (Liza aurata), used as pollution bioindicators. Degenerate oligos were used to amplify by reverse transcription and PCR (RT-PCR) specific cyp1A cDNA sequences, used subsequently to design specific primers to get the full cDNA by rapid amplification of cDNA ends. A new assay has been developed to quantitate cyp1A expression by RT-PCR in an automated DNA sequencer. The effect of [beta]-naphthoflavone inducing biotransformation has been used to compare three distinct pollution biomarkers: EROD activity, ELISA determination of CYP1A, and 2-aminoanthracene (2-AA) activation. Immunodetection by ELISA or Western blot was inconsistent in S. aurata and L. aurata. EROD activity yielded satisfactory results; the higher induction was observed by bioactivation of 2-AA to mutagens detected with strain BA149 of Salmonella typhimurium, in agreement with the high sensitivity previously described for this biomarker. The present paper summarizes the current status of our research.

The Veterinary Journal (2)

Greco, G., A. Madio, et al. "Clostridium perfringens toxin-types in lambs and kids affected with gastroenteric pathologies in Italy." The Veterinary Journal **In Press, Corrected Proof** http://www.sciencedirect.com/science/article/B6WXN-4DJBRB8-1/2/e2757eb0a9cada2c9e3ffd62676907d0

A study was carried out in the South of Italy to assess the role of clostridia in neonatal diseases of lambs and kids. Eighty-seven lambs and 15 kids belonging to 25 flocks were examined and Clostridium perfringens was the microorganism most commonly identified. C. perfringens isolates were analysed by polymerase chain reaction (PCR), in order to determine the prevalence of the genes cpa, cpb, cpb2, etx, iap and cpe. The most prevalent toxin-type of C. perfringens was found to be type A found in 84% of the cases with clostridial enterotoxaemia. No C. perfringens type B, C or E were found.C. perfringens type D was isolated in 16% of the cases. About 24% of the isolates were cpb2 positive. The prevalence of cpb2 across the different C. perfringens types varied. The [beta]2-toxin gene cpb2 was detected in 4/21 (19%) type A isolates, in 1/2 type D isolates, and in 1/2 type DE (cpe-carrying type D) isolates.The high rate of positivity to cpb2 among the isolates suggests that a vaccine based on the [beta]2-toxin, should be included in the vaccination schedule of the animals to confer adequate protection and to prevent the disease.

Grom, J., P. Hostnik, et al. "Molecular detection of BHV-1 in artificially inoculated semen and in the semen of a latently infected bull treated with dexamethasone." The Veterinary Journal **In Press, Corrected Proof** http://www.sciencedirect.com/science/article/B6WXN-4F6SSHH-1/2/2c259616d4733bcc5480fbe7da5b0060

Two polymerase chain reaction (PCR) assays specific for glycoprotein B (gB) and glycoprotein E (gE) gene detection, respectively, were adopted for the detection of bovine herpesvirus-1 (BHV-1) in naturally infected bulls. The methods were tested on bovine semen artificially inoculated with BHV-1 and were compared with an optimised virus isolation method. Raw and extended semen samples were diluted in minimal essential medium (MEM) and spiked with equal dose of BHV-1. The extended semen was found to be more toxic for the cells than the raw semen, while the viral DNA could be detected by the PCR method in all tested dilutions of raw and extended semen samples. The sensitivity of both methods was compared also for BHV-1 detection in semen, nasal swabs and leucocytes of a seropositive bull in a different time period after virus reactivation with dexamethasone treatment. The sensitivity of virus detection by the PCR method was equivalent to that of virus isolation in cell culture. However, PCR was shown to be faster and easier to perform and may be a good alternative to virus isolation especially when bovine semen has to be screened for BHV-1 prior to artificial insemination.

Theriogenology (11)

Blocher, S., R. Behr, et al. (2003). "Different CREM-isoform gene expression between equine and human normal and impaired spermatogenesis." Theriogenology **60**(7): 1357.

http://www.sciencedirect.com/science/article/B6TCM-48PDMBD-3/2/e4dcd43d4d514e2e0699077fa7f34bdc

Histone-to-protamine exchange causes chromatin condensation ceasing gene expression in elongating spermatids. Gene expression of protamines is regulated by the transcription factor cAMP-responsive element modulator (CREM). Altered CREM expression results in male infertility, as shown by CREM-knock-out mice being sterile due to round spermatid maturation arrest and patients exhibiting round spermatid maturation arrest revealing a lack or substantial reduction of both CREM-mRNA and CREM-protein. Similar defects in histone-to-protamine exchange have been suggested in infertile stallions exhibiting enlarged sperm heads. The CREM-gene consists of 14 exons. Alternative exon splicing results in the production of both activator and repressor proteins. To further clarify the role of different CREM-isoforms for male infertility, the expression pattern of various CREM-isoforms during equine and human normal and impaired spermatogenesis was investigated by RT-PCR. Stallions with normal spermatogenesis expressed six activators and three repressors. In men three activators and seven different repressors were detected. In one stallion and patients with impaired spermatogenesis, only repressors were found. It is concluded that (i) stallion and man reveal a different CREM expression pattern, (ii) the expression of CREM activators is a prerequisite for normal spermatogenesis, and (iii) the lack of CREM activator expression results in male infertility.

Bureau, M., S. Dea, et al. "Evaluation of virus decontamination techniques for porcine embryos produced in vitro." Theriogenology **In Press, Corrected Proof**
http://www.sciencedirect.com/science/article/B6TCM-4F05GD0-4/2/e911158ef64bb0539b4ab4a2b97a9233

The objective of this study was to explore approaches to decontaminate embryos either contaminated naturally or under experimental conditions with different viruses. Embryos were obtained from in vitro maturation and fertilisation of porcine oocytes. After 7 days of development, morula and blastocyst stages were exposed for 1 h to the following viruses: encephalomyocarditis virus (EMCV), porcine circovirus type 2 (PCV2), porcine parvovirus (PPV), porcine reproductive and respiratory syndrome virus (PRRSV), and bovine viral diarrhea virus (BVDV) at an infectivity of 100 TCID50/mL. Embryos samples were treated with different washing procedures, which all included the following standard washing solutions: PBS+0.4% BSA (five times for 10 s), Hank's + 0.25% trypsin (two times for 60-90 or 120-150 s, or one time of 5 min), Hank's + 0.1 mg/mL DNase 1 + 20 U/mL RNase One (one time of 30 min) and PBS + 0.4% BSA again (five times for 10 s). Two new approaches were used to improve trypsin treatment, 0.1% hyaluronidase (one time for 5 min) instead of trypsin and a pre-incubation with oviductal cells. Therefore, in the first experiment, oocytes received standard maturation treatments and in the second, they were also co-incubated with oviductal cells for the last 3 h of maturation. The effectiveness of the different washing techniques in removing viruses was evaluated by polymerase chain reaction (PCR) analysis. In the first experiment, trypsin treatment did not eliminate PRRSV, PPV, PCV, and EMCV from contaminated embryos. Surprisingly, treatment with hyaluronidase eliminated all tested viruses. In the second experiment, all viruses tested were removed from the oocytes following the different enzymatic treatments. In conclusion, in vitro embryo decontamination was more effective following exposure to oviductal secretions and hyaluronidase eliminated more virions than trypsin in washing techniques.

Fieni, F., J. Rowe, et al. (2002). "Presence of caprine arthritis-encephalitis virus (CAEV) infected cells in flushing media following oviductal-stage embryo collection." Theriogenology **57**(2): 931.

http://www.sciencedirect.com/science/article/B6TCM-44XCDXK-3/2/7d81006d231ddc375bcd93c6a0dc32e7

To improve the knowledge on the risk of transmission of the caprine arthritis-encephalitis virus (CAEV) during embryo manipulations, we conducted a double-nested polymerase chain reaction (PCR) for CAEV proviral-DNA on flushing media recovered from the oviducts 48 h after the beginning of estrus and on blood from 89 donor does. Sixty-four does had negative blood and flushing media by PCR. Among the 25 CAEV infected goats (blood PCR positive), 11 were PCR flushing media positive (PThe mean number of embryos recovered was not significantly different between goats with flushing media PCR positive and goats with flushing media PCR negative (6.0+/-5.4 versus 7.8+/-4.4, respectively: mean+/-S.D.) nor between goats with blood PCR positive and goats with blood PCR negative (7.0+/-5.0 versus 5.9+/-5.3: mean+/-S.D.).The presence of CAEV infected cells in oviductal flushing media from infected donor does was indicated for the first time during this study. The absence of flushing media PCR positive for goat blood PCR negative seemed to allow the use of the blood PCR test to confidently predict the absence of CAEV provirus in the oviductal fluid.

Fieni, F., J. Rowe, et al. (2003). "Presence of caprine arthritis-encephalitis virus (CAEV) proviral DNA in genital tract tissues of superovulated dairy goat does." Theriogenology **59**(7): 1515.

http://www.sciencedirect.com/science/article/B6TCM-4728DTG-2/2/fa35c7d8604f893259e13efaca1db8fc

Transmission of caprine arthritis-encephalitis virus (CAEV) is not completely understood and the vertical route of infection from the goat to the embryo or to the fetus needs to be investigated. This route of infection involves the presence of CAEV in the genital tract tissues. Prior studies have detected CAEV-infected cells in genital secretions and in flushing media recovered during embryo collection from infected goats. To specify the origin of these cells, we conducted a double-nested polymerase chain reaction (PCR) test on embryo flushing media and on mammary gland, mammary lymph node, synovial membrane, pelvic lymph node, uterus and oviduct tissues from 25 CAEV-infected (blood PCR positive) embryo donor goats for the presence of CAEV proviral DNA.The presence of proviral DNA was found in 22 of 25 mammary gland samples, 14 of 25 uterus samples, and in 16 of 25 oviduct samples. Nineteen of 25 goats had at least one positive genital tract sample. Flushing media from 11 goats were PCR positive. All goats with positive-flushing media were oviduct positive. Of this group of does, except for 1 of the 11, infection of flushing media correlated with infection of almost all the other tissues examined. The frequency of positive tissues for flushing media-positive goats (61/66; 92%) was significantly higher than that for flushing media-negative goats (50/84; 60%) (PThis study demonstrated the presence of CAEV-infected cells in the goat genital tract. The presence of CAEV-infected cells in the uterus and oviducts suggests potential for vertical transmission of CAEV from doe to embryo or fetus.

Fujishiro, A., K. Kawakura, et al. (1995). "A fast, convenient diagnosis of the bovine freemartin syndrome using polymerase chain reaction." Theriogenology **43**(5): 883.

http://www.sciencedirect.com/science/article/B6TCM-3YS90XS-14/2/45ae21f799b4939dff1acfa79a8bd05b

To establish the polymerase chain reaction (PCR) method for detecting the XY cells in cases suspected to have the bovine freemartin syndrome, a PCR reaction test was conducted on blood from a normal bull diluted in blood from a normal cow. From the results obtained, it was shown that the Y-specific sequence was detectable down to a concentration of 0.1%. Various types of the bovine freemartin syndrome, which occurs in heterosexual twins, single-born sterile heifers, and heifers born with Acardius amorphus, were examined by the chromosome analysis and the PCR method. The Y-specific sequence was detected in all 26 cases that showed chromosome

chimerism but which was absent in the 5 cases without a chimerism. The PCR method was found to be effective and convenient for quickly diagnosing the various types of bovine freemartin syndrome.

Hilgers, L. J. and C. Herr (1993). "DNA contamination of reagents used in embryo transfer and culture." Theriogenology **40**(5): 923.

http://www.sciencedirect.com/science/article/B6TCM-49PSB4V-BX/2/ac8d75b6f6b522251357ea6aefbde64a

Commonly used reagents in the culture and transfer of embryos are isolated from blood and tissue samples and thus have the potential for chromosomal and or mitochondrial DNA contamination. In this study, we evaluated the results obtained from PCR analysis of bovine trypsin, bovine sera, and bovine albumin precipitates. Bovine sera samples that were tested yielded minor to heavy DNA contamination signals depending on the manufacturer and specific type of sera. Bovine albumin precipitates showed very little DNA contamination or none at all. Bovine trypsin samples yielded moderate DNA contamination signals depending on the ability of the trypsin to be inactivated prior to PCR analysis.

O'Brien, J. K., T. Stojanov, et al. (2005). "Flow cytometric sorting of non-human primate sperm nuclei." Theriogenology **63**(1): 246.

http://www.sciencedirect.com/science/article/B6TCM-4CVVC2S-1/2/3dece4b0253af601c2ee985ae6ce38b5

Pre-determination of the sex of offspring has implications for management and conservation of captive wildlife species, particularly those with single sex-dominated social structures. Our goal is to adapt flow cytometry technology to sort spermatozoa of non-human primate species for use with assisted reproductive technologies. The objectives of this study were to: (i) determine the difference in DNA content between X- and Y-bearing spermatozoa (ii) sort sperm nuclei into X- and Y-enriched samples; and (iii) assess the accuracy of sorting. Spermatozoa were collected from two common marmosets (Callithrix jacchus), seven hamadryas baboons (Papio hamadryas) and two common chimpanzees (Pan troglodytes). Human spermatozoa from one male were used as a control. Sperm nuclei were stained (Hoechst 33342), incubated and analyzed using a high-speed cell sorter. Flow cytometric reanalysis of sorted samples (sort reanalysis, 10,000 events/sample) and fluorescence in situ hybridization (FISH; 500 sperm nuclei/sample) were used to evaluate accuracy of sorting. Based on fluorescence intensity of X- and Y-bearing sperm nuclei, the difference in DNA content between X and Y populations was 4.09 +/- 0.03, 4.20 +/- 0.03, 3.30 +/- 0.01, and 2.97 +/- 0.05%, for marmoset, baboon, chimpanzee and human, respectively. Sort reanalysis and FISH results were similar; combined data revealed high levels of purity for X- and Y-enriched samples (94 +/- 0.9 and 93 +/- 0.8%, 94 +/- 0.7 and 94 +/- 0.5%, 91 +/- 0.9 and 97 +/- 0.6%, 94 +/- 0.6 and 94 +/- 0.9%, for marmoset, baboon, chimpanzee and human, respectively). These data indicate the potential for high-purity sorting of spermatozoa from non-human primates.

Oshima, K., H. Watanabe, et al. (2003). "Gene expression of leukemia inhibitory factor (LIF) and macrophage colony stimulating factor (M-CSF) in bovine endometrium during early pregnancy." Theriogenology **60**(7): 1217.

http://www.sciencedirect.com/science/article/B6TCM-48R7F4N-1/2/aa6bafb79651ab7680e0244288aa5eae

Leukemia inhibitory factor (LIF) and macrophage colony stimulating factor (M-CSF), members of the group of hemopoietic cytokines, play a primary role in the control of embryo development and implantation and in the growth of the placenta in humans and mice. Gene expressions of LIF and M-CSF were investigated using quantitative RT-PCR in bovine endometrial tissues during early and mid-pregnancy (Days 16-17, 20-21, 30-36, 48-49 and 74-140) and during the estrous cycle (Days 13-14). Leukemia inhibitory factor and M-CSF genes were expressed in all samples examined. Significant differences were found between the gene expression patterns of LIF and M-CSF. Leukemia inhibitory factor expression level at Days 48-49 was the highest in caruncular endometrium, however, the large variability negated any significant differences. Leukemia inhibitory factor expression levels in intercaruncular endometrium at Days 48-49 and 74-140 of pregnancy were greater than at Days 13-14 of the estrous cycle and at other days of pregnancy. No significant change was recognized in M-CSF expression levels in caruncular endometrium. Macrophage colony stimulating factor expression level in intercaruncular endometrium at Days 74-140 was greater than those of the other samples. These results suggest that LIF and M-CSF are produced in the endometrium and may play different roles in early and mid-pregnancy.

Rui, R., H. Shim, et al. (2004). "Attempts to enhance production of porcine chimeras from embryonic germ cells and preimplantation embryos." Theriogenology **61**(7-8): 1225.

http://www.sciencedirect.com/science/article/B6TCM-4B4S5RN-7/2/2eeae34ac1b63f30a5423c563878854c

Porcine embryonic germ (EG) cells share common features with porcine embryonic stem (ES) cells, including morphology, alkaline phosphatase activity and capacity for in vitro differentiation. Porcine EG cells are also capable of in vivo development by producing chimeras after blastocyst injection; however, the proportion of injected embryos that yield a chimera and the proportion of cells contributed by the cultured cells in each chimera are too low for practical use in genetic manipulation. Moreover, somatic, but not germ-line chimerism, has been reported from blastocyst injection using porcine ES or EG cells. To test whether efficiency of chimera production from blastocyst injection can be improved upon by changing the host embryo, we used as host embryos four groups according to developmental stage or length in culture: fresh 4-cell and 8-cell stage embryos subsequently cultured into blastocysts, fresh morulae, fresh blastocysts, and cultured blastocysts. Injection and embryo transfer of fresh and cultured blastocysts produced similar percentages of live piglets (17% versus 19%). Four piglets were judged to have a small degree of pigmentation chimerism, but microsatellite analysis failed to confirm chimerism in these or other piglets. Polymerase chain reaction analysis for detection of the porcine SRY gene in female piglets born from embryos injected with male EG cells identified six chimeras, at least one, but not more than two, from each treatment. Chimerism was confirmed in two putative pigmentation chimeras and in four piglets without overt signs of chimerism. The low percentage of injected embryos that yielded a chimera and the small contribution by EG cells to development of each confirmed chimera indicated that procedural changes in how EG cells were combined with host embryos were unsuccessful in increasing the likelihood that porcine EG cells will participate in embryonic development. Alternatively, our results suggested that improvements are needed in EG cell isolation and culture procedures to ensure in vitro maintenance of EG cell developmental capacity.

van Rens, B. T. T. M., G. J. Evans, et al. (2003). "Components of litter size in gilts with different prolactin receptor genotypes." Theriogenology **59**(3-4): 915.

http://www.sciencedirect.com/science/article/B6TCM-46SG670-5/2/d82c19ecd4b0991fdf4e42175ba07ff6

Behavioral estrus and components of litter size at Day 35/36 of pregnancy were studied in gilts with prolactin receptor (PRLR) genotype AA (n=9), AB (n=25), and BB (n=22). This PRLR polymorphism (two alleles, A and B) has been associated with litter size, although it is not known whether the polymorphism itself causes differences in litter size or whether it is a marker for a closely linked causative gene. Estrus length in three successive estrous cycles was not affected by genotype, but estrous cycle length tended (PPPPP2) than embryos of BB (42.0+/-2.3 g, P2, PP2, P<0.05) gilts. Results of this experiment show that the PRLR gene or a very closely linked gene affects porcine ovaries, uterus, and placenta in a way that might lead to differences in litter size. Since other genes and also environmental factors, however, might change the effect within the 112 days to parturition, it is preferable to state that the PRLR gene is a candidate gene for ovulation rate rather than for litter size.

van Rens, B. T. T. M. and T. van der Lende (2002). "Litter size and piglet traits of gilts with different prolactin receptor genotypes." Theriogenology **57**(2): 883.

http://www.sciencedirect.com/science/article/B6TCM-44X0BGC-9/2/2cc8807b0c87967270316dae96cf4a9a

Seventy-seven Large White x Meishan F2 crossbred gilts with prolactin receptor (PRLR) genotype AA (n=26), AB (n=36) and BB (n=15) were compared for teat number (FTm), age at first estrus, gestation length (GL), litter size, and litter means of functional teat number (FTp), birthweight (BW), and pre-weaning growth rate (GR). Own placental information was available for 88% of 620 live-born piglets (62 gilts), since placentae were labeled during farrowing. The effect of PRLR genotype of the mother on average placenta weight (PLW) and placenta efficiency (EFF=BW/PLW), was therefore, also analyzed. PRLR genotype significantly (PP=0.056) and number of piglets born alive (NBA, P=0.072), but it did not affect (P>0.3) GL, BW or GR, neither before nor after correction for litter size. BB gilts were significantly younger at first estrus and younger and lighter at insemination than AA gilts (PP=0.047) and tended to have a larger NBA (P=0.062) than BB gilts. TNB was 11.4+/-0.7, 10.8+/-0.6, and 8.8+/-0.9; NBA was 11.1+/-0.6, 10.5+/-0.6, and 8.7+/-0.9; BW was 1309+/-40, 1277+/-34, and 1290+/-53 g; and GL was 113.6+/-0.3, 113.8+/-0.3, and 113.5+/-0.4 days for AA, AB and BB gilts, respectively. The effects on litter size and age at first estrus are independent effects. PRLR affected PLW (P=0.050) and EFF (P=0.066), resulting in a difference between AA and BB gilts. PLW was 160+/-9, 181+/-7 and 196+/-11 g and EFF was 7.6+/-0.2, 7.3+/-0.2 and 6.7+/-0.3 for AA (n=19), AB (n=29) and BB (n=14) gilts, respectively. After correction for TNB, the differences disappeared. Functional teat number of the AA, AB and BB gilts was 15.35+/-0.22, 15.53+/-0.18, and 15.60+/-0.29, respectively, and was not affected by PRLR genotype (P=0.7). Functional teat number of piglets from AA, AB and BB mothers was 14.20+/-0.10, 14.37+/-0.08, and 14.63+/-0.13, respectively. Piglets from BB mothers had on average larger numbers of functional teats compared to piglets from AA mothers (P=0.028). In conclusion, PRLR gene is a major gene or marker for age at first estrus, litter size, and litter average of number of functional teats in the Large White x Meishan F2 crossbred gilts studied. The favorable allele for litter size (A allele) is the unfavorable allele for age at first estrus and for litter mean of functional teat number.

Abel, E. L., T. K. Bammler, et al. (2004). "Biotransformation of Methyl Parathion by Glutathione S-Transferases." Toxicol. Sci. **79**(2): 224-232.

http://toxsci.oupjournals.org/cgi/content/abstract/79/2/224

The organo(thio)phosphate esters are one of the most widely used classes of insecticides. Worldwide, organophosphate insecticides (OPs) result in numerous poisonings each year. In insects, glutathione S-transferases (GSTs) play an important role in OP resistance; limited data suggest that GST-mediated O-dealkylation occurs in humans as well. To characterize the capacity of mammalian GSTs to detoxify OPs, we investigated mammalian GST biotransformation of the widely used OP, methyl parathion (MeP). Cytosolic fractions isolated from rat, mouse, and ten individual adult human livers biotransformed 300 {micro}M MeP at rates of 2.36, 1.76, and 0.70 (mean rate) nmol desmethyl parathion/min/mg, respectively. Our study focused on human GSTs; in particular, we investigated hGSTs M1-1 and T1-1, since deletion polymorphisms occur commonly in these genes. However, we found no correlation between hGSTM1/T1 genotypes and MeP O-dealkylation activities of the ten human liver cytosolic samples. We also measured MeP O-dealkylation activities of several purified recombinant GSTs belonging to the alpha (human GSTs A1-1 and A2-2, mouse GSTA3-3, rat GSTA5-5), mu (human GSTs M1a-1a, M2-2, M3-3, M4-4), pi (human GSTP1-1, mouse GSTs P1-1, P2-2), and theta (human GSTT1-1) classes. At 1 mM glutathione and 300 {micro}M MeP concentrations, hGSTT1-1 and hGSTA1-1 exhibited the highest O-dealkylation activities: 545.8 and 65.0 nmol/min/mg, respectively. When expression level and enzymatic activity are considered, we estimate that hGSTA1-1 is responsible for the majority of MeP O-dealkylation in human hepatic cytosol. In target organs such as brain and skeletal muscle, where hGSTT1-1 is expressed, hGSTT1-1-mediated biotransformation of MeP may be important.

Faiola, B., A. K. Bauer, et al. (2003). "Variations in Prkdc and Susceptibility to Benzene-Induced Toxicity in Mice." Toxicol. Sci. **75**(2): 321-332.

http://toxsci.oupjournals.org/cgi/content/abstract/75/2/321

Benzene, a carcinogen that induces chromosomal breaks, is strongly associated with leukemias in humans. Possible genetic determinants of benzene susceptibility include proteins involved in repair of benzene-induced DNA damage. The catalytic subunit of DNA-dependent protein kinase (DNA-PKcs), encoded by Prkdc, is one such protein. DNA-PKcs is involved in the nonhomologous end-joining (NHEJ) pathway of DNA double-strand break (DSB) repair. Here we compared the toxic effects of benzene on mice (C57BL/6 and 129/Sv) homozygous for the wild-type Prkdc allele and mice (129/SvJ) homozygous for a Prkdc functional polymorphism that leads to diminished DNA-PK activity and enhanced apoptosis in response to radiation-induced damage. Male and female mice were exposed to 0, 10, 50, or 100 ppm benzene for 6 h/d, 5 d/week for 2 weeks. Male mice were more susceptible to benzene toxicity compared with females. Hematotoxicity was evident in all male mice but was not seen in female mice. We observed similar, large increases in both micronucleated erythrocyte populations in all male mice. Female mice had smaller but significant increases in micronucleated cells. The p53-dependent response was induced in all strains and genders of mice following benzene exposure, as indicated by an increase in p21 mRNA levels in bone marrow that frequently corresponded with cell cycle arrest in G2/M. Prkdc does not appear to be a significant genetic susceptibility factor for acute benzene toxicity. Moreover, the role of NHEJ, mediated by DNA-PK, in restoring genomic integrity following benzene-induced DSB remains equivocal.

Farraj, A. K., J. R. Harkema, et al. (2004). "Allergic Rhinitis Induced by Intranasal Sensitization and Challenge with Trimellitic Anhydride but Not with Dinitrochlorobenzene or Oxazolone in A/J Mice." Toxicol. Sci. **79**(2): 315-325.

http://toxsci.oupjournals.org/cgi/content/abstract/79/2/315

Allergic airway diseases induced by low molecular weight (LMW) chemicals, including trimellitic anhydride (TMA), are characterized by airway mucus hypersecretion and an infiltration of eosinophils and lymphocytes. Many experimental models have linked LMW chemical-induced allergic airway disease to Th2 cytokines. Most murine models, however, use dermal exposure to sensitize mice. The present study was designed to test the hypothesis that intranasal sensitization and challenge with the known chemical respiratory allergen TMA, but not the nonrespiratory sensitizers dinitrochlorobenzene (DNCB) and oxazolone (OXA), will induce characteristic features of LMW chemical-induced allergic airway disease in the nasal and pulmonary airways. A/J mice were intranasally sensitized and challenged with TMA, DNCB, or OXA. Only mice that were intranasally sensitized and challenged with TMA had a marked allergic rhinitis with an influx of eosinophils, lymphocytes, and plasma cells, increased intraepithelial mucusubstances, and a regenerative hyperplasia. Cytokine mRNA levels in the nasal airway of TMA treated mice also revealed an increase in the mRNA levels of the Th2 cytokines IL-4, IL-5, and IL-13, but no change in the level of the Th1 cytokine IFN-{gamma}. No lesions were found in the nasal airways of mice exposed to DNCB or OXA. TMA increased lung-derived IL-5 mRNA while DNCB and OXA caused no change in lung-derived cytokine mRNA levels. Both TMA and DNCB caused increases in total serum IgE, unlike OXA-exposed mice. However, no adverse alterations were found microscopically in the lungs of mice treated with TMA, DNCB, or OXA. This study is the first to demonstrate that intranasal administration of a known chemical respiratory allergen is an effective method of sensitization resulting in the hallmark features of allergic rhinitis after challenge with a concomitant increase in nasal airway-derived Th2 cytokine mRNA, lung-derived IL-5 mRNA, and total serum IgE. In contrast, DNCB and OXA failed to elicit the pathologic changes in the nasal airways and cytokine changes in the lung. This model may be useful for identifying other chemical respiratory allergens.

Gurel, V., D. A. Sens, et al. (2005). "Post-Transcriptional Regulation of Metallothionein Isoform 1 and 2 Expression in the Human Breast and the MCF-10A Cell Line." Toxicol. Sci.: kfi155.

http://toxsci.oupjournals.org/cgi/content/abstract/kfi155v1

Studies have shown using immunohistochemical staining that the MT-1 and MT-2 proteins (MT-1/2) are overexpressed in a substantial sub-set of ductal breast cancers, that overexpression occurs early in the disease process, and is indicative of a poor prognosis. Normal ductal breast epithelium fails to immunostain for the MT-1/2 protein where as the myoepithelial cells of the ducts stain intensely. There is no information regarding the expression of the mRNAs for the 8 active MT-1 and MT-2 genes in normal breast duct epithelium. Microdissection of normal breast samples was used to obtain total RNA from enriched populations of ductal epithelium and myoepithelium. Analysis by RT-PCR demonstrated that the identity of the MT isoform-specific genes expressed (MT-2A and MT-1X) and their relative levels of expression were similar between the myoepithelial and ductal components. These findings indicate that the ductal and myoepithelial components express similar amounts of MT-2A and MT-1X mRNAs, but have distinctly different expression of the MT-1/2 protein. Confluent cultures of MCF-10A breast epithelial cells were exposed to Cd+2 to test for evidence of post-transcriptional regulation of MT-1/2 protein accumulation in ductal epithelium. It was demonstrated that Cd+2 elicited only a marginal induction of MT-1E, MT-1X or MT-2A mRNAs; where as, there was a marked increase in MT-1/2 protein, reaching levels of 6% of total cell protein under conditions of extended exposure. This study suggests that the mechanism underlying the finding of increased MT-1/2 protein expression in ductal breast cancer may involve in part the post-transcriptional regulation

of MT-1/2 protein expression.

Hamadeh, H. K., P. R. Bushel, et al. (2002). "Gene Expression Analysis Reveals Chemical-Specific Profiles." Toxicol. Sci. **67**(2): 219-231.

http://toxsci.oupjournals.org/cgi/content/abstract/67/2/219

The application of gene expression profiling technology to examine multiple genes and signaling pathways simultaneously promises a significant advance in understanding toxic mechanisms to ultimately aid in protection of public health. Public and private efforts in the new field of toxicogenomics are focused on populating databases with gene expression profiles of compounds where toxicological and pathological endpoints are well characterized. The validity and utility of a toxicogenomics is dependent on whether gene expression profiles that correspond to different chemicals can be distinguished. The principal hypothesis underlying a toxicogenomic or pharmacogenomic strategy is that chemical-specific patterns of altered gene expression will be revealed using high-density microarray analysis of tissues from exposed organisms. Analyses of these patterns should allow classification of toxicants and provide important mechanistic insights. This report provides a verification of this hypothesis. Patterns of gene expression corresponding to liver tissue derived from chemically exposed rats revealed similarity in gene expression profiles between animals treated with different agents from a common class of compounds, peroxisome proliferators [clofibrate (ethyl-p-chlorophenoxyisobutyrate), Wyeth 14,643 ([4-chloro-6(2,3-xylidino)-2-pyrimidinylthio]acetic acid), and gemfibrozil (5-2[2,5-dimethylphenoxy]2-2-dimethylpentanoic acid)], but a very distinct gene expression profile was produced using a compound from another class, enzyme inducers (phenobarbital).

Hilscherova, K., P. D. Jones, et al. (2004). "Assessment of the Effects of Chemicals on the Expression of Ten Steroidogenic Genes in the H295R Cell Line Using Real-Time PCR." Toxicol. Sci. **81**(1): 78-89.

http://toxsci.oupjournals.org/cgi/content/abstract/81/1/78

The potential for a variety of environmental contaminants to disturb endocrine function in wildlife and humans has been of recent concern. While much effort is being focused on the assessment of effects mediated through steroid hormone receptor-based mechanisms, there are potentially several other mechanisms that could lead to endocrine disruption. Recent studies have demonstrated that a variety of xenobiotics can alter the gene expression or activity of enzymes involved in steroidogenesis. By altering the production or catalytic activity of steroidogenic or steroid-catabolizing enzymes, these chemicals have the potential to alter the steroid balance in organisms. To assess the potential of chemicals to alter steroidogenesis, an assay system was developed using a human adrenocortical carcinoma cell line, the H295R cell line, which retains the ability to synthesize most of the important steroidogenic enzymes. Methods were developed, optimized, and validated to measure the expression of 10 genes involved in steroidogenesis by the use of real-time quantitative reverse transcriptase PCR. The effects of several model chemicals known to alter steroid metabolism, both inducers and inhibitors, were assessed. Similar expression patterns were observed for chemicals acting through common mechanisms of action. Time-course studies demonstrated distinct time-dependent expression profiles for chemicals able to modulate steroid metabolism. The assay, which allows simultaneous analysis of the expression of numerous steroidogenic enzymes, would be useful as a sensitive and integrative screen for the many effects of chemicals on steroidogenesis.

Johnson, V. J., S.-H. Kim, et al. (2005). "Aluminum-Maltolate Induces Apoptosis and Necrosis in Neuro-2a Cells: Potential Role for p53 Signaling." Toxicol. Sci. **83**(2): 329-339.

http://toxsci.oupjournals.org/cgi/content/abstract/83/2/329

Aluminum maltolate (Al-malt) causes neurodegeneration following in vivo exposure, and apoptosis plays a prominent role. The objective of this study was to define the form of cell death induced by Al-malt and to establish an in vitro model system amenable to mechanistic investigations of Al-malt-induced cell death. Neuro-2a cells, a murine neuroblastoma cell line, were treated with Al-malt for 24 h, following which mode of cell death and alterations in apoptosis-related gene expression were studied. Al-malt concentration-dependently increased cell death. The mode of cell death was a combination of apoptosis and necrosis. Treatment with Al-malt resulted in caspase 3 activation and the externalization of phosphatidyl serine, both indicative of apoptosis. In addition, nuclear condensation and fragmentation were evident. Interestingly, pretreatment with cycloheximide (CHX), a potent protein synthesis inhibitor markedly reduced Al-malt-induced apoptosis, indicating that altered gene expression was critical for this form of cell death. Pretreatment with CHX had no effect on necrosis induced by Al-malt. Analysis of gene expression showed that p53 mRNA was increased following treatment with Al-malt. This increase was accompanied by a marked inhibition of Bcl2 expression and an increase in BAX expression, a pattern of gene expression suggestive of a pro-apoptotic shift. Results show for the first time that p53 is induced by Al in neuron-like cells and suggest that the p53-dependent intrinsic pathway may be responsible for Al-induced apoptosis. Future studies investigating the role of p53 in Al neurotoxicity both in vivo and in vitro are warranted.

Kato, Y., K. Haraguchi, et al. (2003). "Effects of Polychlorinated Biphenyls, Kanechlor-500, on Serum Thyroid Hormone Levels in Rats and Mice." Toxicol. Sci. **72**(2): 235-241.

http://toxsci.oupjournals.org/cgi/content/abstract/72/2/235

Effects of a commercial polychlorinated biphenyls mixture, Kanechlor-500 (KC500), on the levels of serum thyroid hormones such as total thyroxine (T4) and triiodothyronine (T3) were examined comparatively in male Wistar rats and ddy mice. Serum T4 levels were significantly decreased in both rats and mice 4 days after a single ip injection of KC500 (100 mg/kg body weight), whereas decreased levels of T3 were observed in mice but not in rats. In addition, no significant change in the level of serum thyroid stimulating hormone was observed in either rats or mice. Hepatic UDP-glucuronosyltransferases (UDP-GTs) UGT1A1 and UGT1A6, which efficiently mediate glucuronidation of T4 and promote the excretion of the hormones, were induced by KC500 in rats but not in mice. Hepatic microsomal cytochrome P450 (P450) content and the microsomal activity for 7-ethoxy-, 7-pentoxy-, and 7-benzoyloxy-resorufin dealkylations were significantly increased by KC500 in both rats and mice, although the magnitude of increase in the enzyme activities was higher in rats than in mice. The difference in the increase in the activity of microsomal enzymes, including UDP-GT and P450, between KC500-treated rats and mice was not correlated with that in the level of hepatic methylsulfonyl-PCB metabolites. In the present study, we found for the first time that the decrease in serum T4 levels by KC-500 in mice occurred without increase in hepatic UDP-GTs, UGT1A1 and UGT1A6, responsible for T4 glucuronidation. The present findings further suggested that although the decrease in serum T4 levels in KC500-treated rats would occur at least in part through the induction of the UDP-GTs, it might not be dependent on only the increase in the enzymes.

Kuruvilla, S., C. W. Qualls, Jr., et al. (2003). "Effects of Minimally Toxic Levels of Carbonyl Cyanide P-(Trifluoromethoxy) Phenylhydrazone (FCCP), Elucidated through Differential Gene Expression with Biochemical and Morphological Correlations." Toxicol. Sci. **73**(2): 348-361.

http://toxsci.oupjournals.org/cgi/content/abstract/73/2/348

Uncouplers of oxidative phosphorylation have relevance to bioenergetics and obesity. The mechanisms of action of chemical uncouplers of oxidative phosphorylation on biological systems were evaluated using differential gene expression. The transcriptional response in human rhabdomyosarcoma cell line (RD), was elucidated following treatment with carbonyl cyanide p-(trifluoromethoxy) phenylhydrazone (FCCP), a classical uncoupling agent. Changes in mitochondrial membrane potential were used as the biological dosimeter. There was an increase in membrane depolarization with increasing concentrations of FCCP. The concentration at 75% uncoupling (20 {micro}M) was chosen to study gene expression changes, using cDNA-based large-scale differential gene expression (LSDGE) platforms. At the above concentration, subtle light microscopic and clear gene expression changes were observed at 1, 2, and 10 h. Statistically significant transcriptional changes were largely associated with protein synthesis, cell cycle regulation, cytoskeletal proteins, energy metabolism, apoptosis, and inflammatory mediators. Bromodeoxyuridine (BrdU) and propidium iodide (PI) assays revealed cell cycle arrest to occur in the G1 and S phases. There was a significant initial decrease in the intracellular adenosine triphosphate (ATP) concentrations. The following seven genes were selected as potential molecular markers for chemical uncouplers: seryl-tRNA synthetase (Ser-tRS), glutamine-hydrolyzing asparagine synthetase (Glut-HAS), mitochondrial bifunctional methylenetetrahydrofolate dehydrogenase (Mit BMD), mitochondrial heat shock 10-kDa protein (Mit HSP 10), proliferating cyclic nuclear antigen (PCNA), cytoplasmic beta-actin (Act B), and growth arrest and DNA damage-inducible protein 153 (GADD153). Transcriptional changes of all seven genes were later confirmed with reverse transcription-polymerase chain reaction (RT-PCR). These results suggest that gene expression changes may provide a sensitive indicator of uncoupling in response to chemical exposure.

Lambert, A. L., J. B. Mangum, et al. (2003). "Ultrafine Carbon Black Particles Enhance Respiratory Syncytial Virus-Induced Airway Reactivity, Pulmonary Inflammation, and Chemokine Expression." Toxicol. Sci. **72**(2): 339-346.

http://toxsci.oupjournals.org/cgi/content/abstract/72/2/339

Exposure to particulate matter (PM) may exacerbate preexisting respiratory diseases such as asthma, chronic obstructive pulmonary disease (COPD), bronchitis, and pneumonia. However, few experimental studies have addressed the effects of PM on lower respiratory tract (LRT) viral infection. Respiratory syncytial virus (RSV) is a major etiological agent for LRT infections in infants, the elderly, and the immunocompromised and may lead to chronic wheezing and the development of asthma in children. In this study, we examined the effects of carbon black (CB) on RSV-induced pulmonary inflammation, chemokine and cytokine expression, and airway hyperresponsiveness in a mouse model of RSV. Female BALB/c mice were instilled via the trachea (i.t.) with 1 x 106 plaque forming units (pfu) RSV or with uninfected culture media. On day 3 of infection, mice were i.t. instilled with either 40 {micro}g ultrafine CB particles or with saline. End points were examined on days 4, 5, 7, and 14 of RSV infection. Viral titer and clearance in the lung were unaffected by CB exposure. Neutrophil numbers were elevated on days 4 and 7, and lymphocyte numbers were higher on days 4 and 14 of infection in CB-exposed, RSV-infected mice. CB exposure also enhanced RSV-induced airway hyperresponsiveness to methacholine, bronchoalveolar lavage (BAL) total protein, and virus-associated chemokines monocyte chemoattractant protein (MCP-1), macrophage inflammatory protein (MIP-1{alpha}), and regulated upon activation, normal T cell expressed and secreted (RANTES). MIP-1{alpha} mRNA expression was increased in the alveolar epithelium, where ultrafine particles deposit in the lung. These data demonstrate a synergistic effect of ultrafine CB particles on RSV infection, and suggest a potential mechanism for increased respiratory infections in human populations after PM exposure.

Loertscher, J. A., T.-M. Lin, et al. (2002). "In Utero Exposure to 2,3,7,8-Tetrachlorodibenzo-p-dioxin Causes Accelerated Terminal Differentiation in Fetal Mouse Skin." Toxicol. Sci. **68**(2): 465-472.

http://toxsci.oupjournals.org/cgi/content/abstract/68/2/465

2,3,7,8 Tetrachlorodibenzo-p-dioxin (TCDD), a ubiquitous environmental toxin, has been shown to cause a human skin pathology called chloracne. The majority of laboratory mouse strains, with the exception of mice bearing a mutation in thehairless gene, fail to display overt signs of chloracne upon exposure to TCDD. As a result, only minimal data exist on the effects of TCDD in adult haired mice and no data exist on the effects of TCDD in developing mouse skin. Here we report that TCDD affects the temporal expression of protein markers of keratinocyte terminal differentiation during murine skin morphogenesis. Immunohistochemical analysis of E16 mice reveals accelerated expression of the intermediate filament-associated protein filaggrin in response to TCDD. At a later developmental time and after birth, expression of filaggrin and loricrin is indistinguishable between treatment and control groups. At E16 expression of keratins 5, 6, and 10 are unaltered in TCDD-exposed individuals and TUNEL analysis shows no apoptotic cells in the basal and spinous layers of either treatment or control groups. At E16, immunohistochemical analysis of AhR-null mouse skin reveals accelerated filaggrin expression in both vehicle and TCDD exposed animals. We therefore hypothesize that AhR acts as a modulator of late stage keratinocyte terminal differentiation.

Merrill, C. L., H. Ni, et al. (2002). "Etomoxir-Induced Oxidative Stress in HepG2 Cells Detected by Differential Gene Expression Is Confirmed Biochemically." Toxicol. Sci. **68**(1): 93-101.

http://toxsci.oupjournals.org/cgi/content/abstract/68/1/93

Although they are known to be effective antidiabetic agents, little is published about the toxic effects of carnitine palmitoyltransferase-1 (CPT-1) inhibitors, such as etomoxir (ET). These compounds inhibit mitochondrial fatty acid {beta}-oxidation by irreversibly binding to CPT-1 and preventing entry of long chain fatty acids into the mitochondrial matrix. Treatment of HepG2 cells with 1 mM etomoxir for 6 h caused significant modulations in the expression of several redox-related and cell cycle mRNAs as measured by microarray analysis. Upregulated mRNAs included heme oxygenase 1 (HO1), 8-oxoguanine DNA glycosylase 1 (OGG1), glutathione reductase (GSR), cyclin-dependent kinase inhibitor 1A (CDKN1 [p21waf1]) and Mn+ superoxide dismutase precursor (SOD2); while cytochrome P450 1A1 (CYP1A1) and heat shock 70kD protein 1 (HSPA1A) were downregulated. Real time quantitative PCR (RT-PCR) confirmed the significant changes in 4 of 4 mRNAs assayed (CYP1A1, HO1, GSR, CDKN1), and identified 3 additional mRNA changes; 2 redox-related genes, {gamma}-glutamate-cysteine ligase modifier subunit (GCLM) and thioredoxin reductase (TXNRD1) and 1 DNA replication gene, topoisomerase II{alpha} (TOP2A). Temporal changes in selected mRNA levels were examined by RT-PCR over 11 time points from 15 min to 24 h postdosing. CYP1A1 exhibited a 38-fold decrease by 4 h, which rebounded to a 39-fold increase by 20 h. GCLM and TXNRD1 exhibited 13- and 9-fold increases, respectively at 24 h. Etomoxir-induced oxidative stress and impaired mitochondrial energy metabolism were confirmed by a significant decrease in reduced glutathione (GSH), reduced/oxidized glutathione ratio (GSH/GSSG), mitochondrial membrane potential (MMP), and ATP levels, and by concurrent increase in oxidized glutathione (GSSG) and superoxide generation. This is the first report of oxidative stress caused by etomoxir.

Richards, V. E., B. Chau, et al. (2004). "Hepatic Gene Expression and Lipid Homeostasis in C57Bl/6 Mice

Exposed to Hydrazine or Acetylhydrazine." Toxicol. Sci. **82**(1): 318-332.

http://toxsci.oupjournals.org/cgi/content/abstract/82/1/318

Hydrazine (HD) and acetylhydrazine (AcHD) are metabolites of the antituberculosis drug isoniazid (INH) that have been implicated in INH-induced liver damage. The hepatotoxicity of AcHD and HD were compared in adult male C57Bl/6J mice by evaluating hepatic histopathology, plasma biochemistry, and hepatic gene expression. By all measures, HD had significantly greater effects than AcHD. There was no evidence of liver damage following exposure to AcHD (300 mg/kg, po). However, HD at this dose caused marked hepatic necrosis, macrovesicular degeneration, and steatosis. Lipid accumulation was initiated 2 h after HD exposure, with hepatic macrovesicular degeneration evident after 4 h, and severe necrosis by 36 h. Gene expression profiles were compared 24 h following 100 mg/kg po of HD or AcHD. HD changed the hepatic expression of more genes than AcHD, particularly lipid synthesis, transport, and metabolism genes that may be involved in steatosis. Hepatic expression of genes regulated by peroxisome proliferator activated receptors (PPAR) and sterol regulatory element binding protein (SREBP) transcription factors was increased only by HD. The hepatotoxicty and hepatic gene expression profile of HD, but not AcHD, indicate that exposure to HD initiates a process whereby the production and intracellular transport of hepatic lipids is favored over the removal of fatty acids and their metabolites.

Vickers, A. E. M., M. Saulnier, et al. (2004). "Organ Slice Viability Extended for Pathway Characterization: An in Vitro Model to Investigate Fibrosis." Toxicol. Sci. **82**(2): 534-544.

http://toxsci.oupjournals.org/cgi/content/abstract/82/2/534

Liver slice viability is extended to 96 h for rat, expanding the use of this in vitro model for studying mechanisms of injury and repair, including pathways of fibrosis. The contributing factors to increased organ slice survival consist of the use of a preservation solution for liver perfusion and slice preparation, obtaining rats that are within the weight range of 250-325 g, placing a cellulose filter atop the titanium mesh roller-insert to support the slice, and maintaining the slices in an optimized culture medium which is replaced daily. The liver slices remain metabolically active, synthesizing adenosine triphosphate (ATP), glutathione, and glycogen, and exhibit preserved organelle integrity and slice morphology. Slice preparation results in 2-cut surfaces which likely triggers a repair and regenerative response. The fibrogenic pathways are evident by the activation of stellate cells, the proliferation of myofibroblast-like cells, and an increased collagen deposition by 48 h. Markers indicative of activated stellate cells, {alpha}-smooth muscle actin, collagen 1a1, desmin, and HSP47 are substantiated by real time-PCR. Increased staining of {alpha}-smooth muscle actin initially around the vessels and by 72-96 h in the tissue is accompanied by increased collagen staining. Microarray gene expression revealed extracellular matrix changes with the up-regulation of cytoskeleton, filaments, collagens, and actin genes; and the down-regulation of genes linked with lipid metabolism. The improvements in extending liver slice survival, in conjunction with its three-dimensional multi-cellular complexity, increases the application of this in vitro model for investigating pathways of injury and repair, and fibrosis.

Zodrow, J. M. and R. L. Tanguay (2003). "2,3,7,8-Tetrachlorodibenzo-p-dioxin Inhibits Zebrafish Caudal Fin Regeneration." Toxicol. Sci. **76**(1): 151-161.

http://toxsci.oupjournals.org/cgi/content/abstract/76/1/151

Adult zebrafish completely regenerate their caudal fins following partial amputation. Fin regrowth can easily be monitored in vivo and regenerating tissues can be used to study this dynamic

developmental process. In this study we determined that fin regeneration is significantly affected by exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Zebrafish caudal fins were partially amputated, and the fish received intraperitoneal (ip) injection of 2.8, 14, or 70 ng/g weight TCDD or vehicle control. By 7 days postamputation, fish exposed to the highest concentration of TCDD regenerated 15% of their fin compared to 65% regrowth in control fish. To determine if this effect was stage specific, zebrafish were exposed to 70 ng/g TCDD on 1, 2, 3, or 4 days postamputation. Fin regeneration was significantly inhibited at all time points following TCDD exposure. TCDD exposure also induced hyperpigmentation in de novo tissue. Zebrafish were dosed with BrdU, following fin amputation and TCDD exposure, to study changes in cell proliferation. By 4 days postamputation, cell proliferation rates were significantly lower in TCDD-exposed fish. TCDD toxicity is mediated through the aryl hydrocarbon receptor (AHR), and RT-PCR experiments confirmed AHR2, ARNT2b, and TCDD-dependent CYP1A expression in the regenerating tissue. These results demonstrate that zebrafish caudal fin regeneration is a unique model to investigate molecular mechanism(s) of TCDD toxicity.

Toxicology (13)

Dai, J., C. Huang, et al. (2004). "Iron-induced interleukin-6 gene expression: possible mediation through the extracellular signal-regulated kinase and p38 mitogen-activated protein kinase pathways." Toxicology **203**(1-3): 199.

http://www.sciencedirect.com/science/article/B6TCN-4CTTN3G-2/2/34806a6e6d9e0568713ef46ccf50218b

Increased iron store in the body may increase the risk of many diseases such as cancer and inflammation. However, the precise pathogenic mechanism of iron has not yet been elucidated. In the present study, the early biological responses of cells to iron treatment were investigated in AP-1 luciferase reporter stably transfected mouse epidermal JB6 cells and primary rat hepatocytes. It was shown that water-soluble iron compounds, such as FeSO4 and Fe2(SO4)3, were more active in inducing AP-1 in JB6 cells than water-insoluble iron compounds, such as Fe2O3 and FeS. Iron stimulated mitogen-activated protein kinase (MAPK) family members of extracellular signal-regulated kinases (ERKs) and p38 MAPK but not c-jun NH2 terminal kinases (JNKs), both in JB6 cells and in primary rat hepatocytes, as determined by the phosphorylation assay. Interestingly, the increase in AP-1 luciferase activity by iron was inhibited by the pretreatment of the cells with PD98059, a specific MEK1 inhibitor, and SB202190, a p38 kinase inhibitor. Levels of interleukin-6 (IL-6), a pro-inflammatory cytokine, were increased in JB6 cells by iron in a dose-dependent manner. The increase in IL-6 and its mRNA by iron was also eliminated by the pretreatment of the cells with PD98059 and SB202190. Since the IL-6 promoter contains an AP-1 binding site, our studies indicate that the iron-induced IL-6 gene expression may be mediated through ERKs and p38 MAPK pathways, possibly one of the important mechanisms for the pathogenesis of iron overload.

del Carmen, E. M., V. Souza, et al. (2002). "Cadmium induces [alpha]1collagen (I) and metallothionein II gene and alters the antioxidant system in rat hepatic stellate cells." Toxicology **170**(1-2): 63.

http://www.sciencedirect.com/science/article/B6TCN-44N3BXJ-7/2/61cb99fe146dde6b6895c57f0a3efa41

The mechanism of cadmium-mediated hepatotoxicity has been the subject of numerous investigations, principally in hepatocytes. Although, some uncertainties persist, sufficient evidence has emerged to provide a reasonable account of the toxic process in parenchymal cells. However, there is no information about the effect of cadmium in other hepatic cell types, such as stellate cells (fat storing cells, Ito cells, perisinusoidal cells, parasinusoidal cells, lipocytes). Hepatic stellate cells (HSC) express a quiescent phenotype in a healthy liver and acquire an activated phenotype in liver injury. These cells play an important role in the fibrogenic process. The objective of this study was to investigate the effect of a 24 h treatment of low Cd concentrations in glutathione content, lipid peroxidation damage, cytosolic free Ca, antioxidant enzyme activities: glutathione peroxidase, glutathione reductase, superoxide dismutase and catalase along with the capacity of this heavy metal to induce metallothionein II and [alpha]1collagen (I) in an hepatic stellate cell line (CFSC-2G). Cd-treated cells increased lipid peroxidation and the content of cytosolic free calcium, decreased glutathione content and superoxide dismutase, glutathione peroxidase and catalase activity. Cd was able to induce the expression of the metallothionein II and [alpha]1collagen (I) gene, that was not described in this cell type. Cadmium may act as a pro-fibrogenic agent in the liver probably by inducing oxidative damage by enhancing lipid peroxidation and altering the antioxidant system of the cells. Although, the exact role metallothionein induction plays in this process is unknown, it probably, provides a cytosolic pool of potential binding sites to sequester ionic Cd, thereby decreasing its toxicity.

Hester, S. D., G. B. Benavides, et al. (2003). "Formaldehyde-induced gene expression in F344 rat nasal respiratory epithelium." Toxicology **187**(1): 13.

http://www.sciencedirect.com/science/article/B6TCN-47WDB67-2/2/c7fbae1878b4afc5f9b9016b09cf5d57

Formaldehyde (FA), an occupational and environmental toxicant used extensively in the manufacturing of many household and personal use products, is known to induce squamous cell carcinomas in the nasal turbinates of rats and mice and squamous metaplasia in monkey noses. Tissue responses to FA include a dose dependent epithelial degeneration, respiratory cell hypertrophy, and squamous metaplasia. The primary target for FA-induced toxicity in both rodents and monkeys is the respiratory nasal epithelium. FA increases nasal epithelial cell proliferation and DNA-protein crosslinks (DPX) that are associated with subsequent nasal cancer development. To address the acute effects of FA exposure that might contribute to known pathological changes, cDNA gene expression analysis was used. Two groups of male F344 rats received either 40 ul of distilled water or FA (400 mM) instilled into each nostril. Twenty-four hours following treatment, nasal epithelium was recovered from which total RNA was used to generate cDNA probes. Significance analysis of microarrays (SAM) hybridization data using Clontech(TM) Rat Atlas 1.2 arrays revealed that 24 of the 1185 genes queried were significantly up-regulated and 22 genes were significantly downregulated. Results for ten of the differentially expressed genes were confirmed by quantitative real time RT PCR. The identified genes with FA-induced change in expression belong to the functional gene categories xenobiotic metabolism, cell cycle, apoptosis, and DNA repair. These data suggest that multiple pathways are dysregulated by FA exposure, including those involved in DNA synthesis/repair and regulation of cell proliferation. Differential gene expression profiles may provide clues that could be used to define mechanisms involved in FA-induced nasal cancer.

Lundby, C., H. Pilegaard, et al. (2003). "Oxidative DNA damage and repair in skeletal muscle of humans exposed to high-altitude hypoxia." Toxicology **192**(2-3): 229.

http://www.sciencedirect.com/science/article/B6TCN-49MF283-2/2/9e4ca0ae5875f002e0e495199aad8855

Recent research suggests that high-altitude hypoxia may serve as a model for prolonged oxidative stress in healthy humans. In this study, we investigated the consequences of prolonged high-altitude hypoxia on the basal level of oxidative damage to nuclear DNA in muscle cells, a major oxygen-consuming tissue. Muscle biopsies from seven healthy humans were obtained at sea level and after 2 and 8 weeks of hypoxia at 4100 m.a.s.l. We found increased levels of strand breaks and endonuclease III-sensitive sites after 2 weeks of hypoxia, whereas oxidative DNA damage detected by formamidopyrimidine DNA glycosylase (FPG) protein was unaltered. The expression of 8-oxoguanine DNA glycosylase 1 (OGG1), determined by quantitative RT-PCR of mRNA levels did not significantly change during high-altitude hypoxia, although the data could not exclude a minor upregulation. The expression of heme oxygenase-1 (HO-1) was unaltered by prolonged hypoxia, in accordance with the notion that HO-1 is an acute stress response protein. In conclusion, our data indicate high-altitude hypoxia may serve as a good model for oxidative stress and that antioxidant genes are not upregulated in muscle tissue by prolonged hypoxia despite increased generation of oxidative DNA damage.

Nabekura, T., M. Tomohiro, et al. (2004). "Changes in plasma membrane Ca2+-ATPase expression and ATP content in lenses of hereditary cataract UPL rats." Toxicology **197**(2): 176.

http://www.sciencedirect.com/science/article/B6TCN-4BP9KKJ-5/2/4c1a66db48b7e789c61352242aa73269

The UPL rat is a newly developed hereditary cataract model. We previously found that Ca2+ concentrations in UPL rat lenses increase with the development of cataract, and that the administration of disulfiram and aminoguanidine ameliorates the increase in Ca2+ and the development of cataract in UPL rats. In this study, we determined the expression and activity of plasma membrane Ca2+-ATPase (PMCA) in lenses of normal and UPL rats. We also determined the ATP content in UPL rat lenses and the effects of disulfiram and aminoguanidine administration. Expression of PMCA mRNA in UPL rat lenses, determined by a reverse transcription-PCR method, increased during the development of cataract. Ca2+-ATPase activity in UPL rat lenses also increased with the progression of lens opacification. On the other hand, ATP decreased markedly in UPL rat lenses, and the administration of disulfiram and aminoguanidine attenuate the ATP decrease. These results suggest that an ATP decrease cause cataract development and an increased Ca2+ may upregulate PMCA expression in UPL rat lenses. Disulfiram and aminoguanidine attenuate the decrease in ATP, resulting in a delay in cataract development.

Paschke, T., M. Riefler, et al. (2001). "Comparison of cytochrome P450 2A6 polymorphism frequencies in Caucasians and African-Americans using a new one-step PCR-RFLP genotyping method." Toxicology **168**(3): 259.

http://www.sciencedirect.com/science/article/B6TCN-448BG0N-6/2/02862c8f8d95353a92b75ba46a97be4e

CYP2A6 (cytochrome P450 2A6), which was first identified as the human coumarin 7-hydroxylase, is the most important enzyme in nicotine C-oxidation. The enzyme also metabolically activates the tobacco specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in vitro. Polymorphisms in the CYP2A6 gene may thus impact on both smoking behavior and lung cancer susceptibility. Several different genotyping methods have been reported with conflicting results in the frequencies of CYP2A6 polymorphic variants. Thus we decided to perform a sequence analysis of the entire CYP2A6 gene. Sequencing confirmed the published CYP2A6 cDNA sequence. However, intron sequences differed considerably from the reported sequence of the CYP2A6*3 (v2) variant. Our analyses revealed that parts of introns

shared homologies with the published sequence of CYP2A13. Based on our sequence data we developed a one step protocol for specific amplification of exon 3 of CYP2A6. The resulting PCR product can be used directly for restriction endonuclease digestion with XcmI and DdeI to determine the frequencies of the reported variant alleles CYP2A6*2 and CYP2A6*3. In a population of 305 African-Americans and 145 Caucasians, we found allele frequencies of 0.003 (2/610) for CYP2A6*2 and 0 (0/610) for CYP2A6*3 in African-Americans and allele frequencies of 0.014 (4/290) and 0 (0/290) in Caucasians. We conclude that both alleles are considerably less frequent in populations than previously reported.

Pylkkanen, L., H. Gullsten, et al. (2004). "Exposure to Aspergillus fumigatus spores induces chemokine expression in mouse macrophages." Toxicology **200**(2-3): 255.

http://www.sciencedirect.com/science/article/B6TCN-4CHS17S-1/2/3a68eb8e91c766a6ab812c701b353e87

Inhalation of fungal spores may cause inflammation and respiratory diseases, such as bronchitis, allergic alveolitis, and asthma. Alveolar macrophages provide the first line of defense in the respiratory tract. To examine the cellular mechanisms involved in Aspergillus fumigatus-induced airway inflammation, mouse macrophage cell line (RAW 264.7) cells were exposed for 2 h or 6 h to graded doses of A. fumigatus spores that were either alive or heat-killed. Furthermore, the ability of the cells to phagocytize the spores was visualized by electron microscopy. Expression of selected cytokines and chemokines was assessed by a real time quantitative PCR method and by enzyme-linked immunoabsorbent assay (ELISA) after exposure. A significant increase in mRNA expression of TNF-[alpha], MIP-1[alpha], MIP-1[beta], and MCP-1 was observed with a maximal induction at 6 h after exposure to the highest (1 x 107) concentration of live spores. Similar response was not detected with heat-killed spores in the expression of chemokines and cytokines, even though there were no differences between the phagocytosis of live and heat-killed spores. These results suggest that exposure to live spores of A. fumigatus can modulate the expression of proinflammatory cytokines and chemokines in mouse macrophages and thus influence the development of inflammatory processes in the airways.

Takaki, A., S. Jimi, et al. (2004). "Long-term cadmium exposure accelerates age-related mitochondrial changes in renal epithelial cells." Toxicology **203**(1-3): 145.

http://www.sciencedirect.com/science/article/B6TCN-4CTTN3G-5/2/d66cb5d571105fcc3e9bc66f65a6da0b

Long-term cadmium exposure leads to mitochondrial dysfunction in the proximal tubular epithelial cells. Mitochondrial DNA deletion may contribute to the pathogenesis of cadmium-induced nephropathy. The aim of our study is to clarify the accumulation of mitochondrial DNA deletion and mitochondrial dysfunction in the renal cortex of rats injected three times/week with 1 ml of 1 mM CdCl2 or saline for 80 weeks. After 40-week cadmium injection, mitochondrial number diminished, and cadmium in the renal cortex reached a saturation level. At this time interval, nearly 30% of cadmium in the whole cell fraction was found in the mitochondria. Cytochrome c oxidase (COX) activity in the proximal tubular epithelial cells decreased after 40-week exposure of cadmium. Oxidized phosphatidylcholine (oxPC) started to accumulate in the cytochrome c-positive mitochondria in some tubular epithelial cells after 80-week exposure. After 40 weeks, accumulation of the 4834-bp deletion in mitochondrial DNA was evident in both control and cadmium-treated groups. However, the amount of accumulated mitochondrial DNA deletion tended to increase after 40-week exposure, and was significantly greater after 80 weeks of exposure, compared to the control. Our results indicate that long-term cadmium exposure in rats accelerates accumulation of 4834-bp mitochondrial DNA deletions and impairment of

mitochondrial function associated with accumulation of oxidized product.

Teixeira, J. P., J. Gaspar, et al. (2004). "Occupational exposure to styrene: modulation of cytogenetic damage and levels of urinary metabolites of styrene by polymorphisms in genes CYP2E1, EPHX1, GSTM1, GSTT1 and GSTP1." Toxicology **195**(2-3): 231.

http://www.sciencedirect.com/science/article/B6TCN-4B7D8RY-1/2/cef8d9f6ac600aeceaa7096a47ee876c

Styrene is widely used in the production of various plastics, synthetic rubber and resins. The aim of this study was to evaluate if individual polymorphisms in xenobiotic metabolizing enzymes, related with the metabolic fate of styrene, could modify individual susceptibility to the possible genotoxic effects of the styrene exposure. Twenty-eight reinforced plastic workers and 28 control subjects were studied. In the selected population the urinary styrene metabolites mandelic (MA) and phenylglyoxylic (PGA) acids were quantified, sister chromatid exchanges (SCE) and micronuclei (MN) were assessed in peripheral lymphocytes and all the subjects were genotyped for GSTM1, GSTT1 (gene deletions), GSTP1 (codon 105 ile => val), EPHX1 (codons 113 tyr => his and 139 his => arg) and CYP2E1 (DraI polymorphism in intron 6).The results obtained showed a significant difference between the levels of SCE, but not in MN levels, in exposed workers as compared with the control group. The GSTP1 and CYP2E1 individual genotypes modulate the baseline levels of SCE that are lower in non-wild type individuals for both polymorphisms. The GSTM1 null individuals with low levels of exposure have significantly higher urinary levels of MA+PGA. The present data seem to suggest that apart from the methodology usually used for monitoring populations occupationally exposed to styrene (urinary metabolites and biomarkers of early biological effects) the analysis of individual genotypes associated with the metabolic fate of styrene should also be carried out in order to evaluate the individual genetic susceptibility of exposed populations.

Valles, E. G., A. R. Laughter, et al. (2003). "Role of the peroxisome proliferator-activated receptor [alpha] in responses to diisononyl phthalate." Toxicology **191**(2-3): 211.

http://www.sciencedirect.com/science/article/B6TCN-497RD7N-2/2/56e4781b2216419f6fb6498ec77c026b

Diisononyl phthalate (DINP) is a compound widely used as a plasticizer in the production of polyvinyl chloride products. Chronic exposure to DINP leads to liver cancer in rats and mice. Many phthalates are considered to be relatively weak peroxisome proliferators (PP), a group of rodent hepatocarcinogens that cause a variety of adaptive responses in liver through the PP-activated receptor alpha (PPAR[alpha]). The objectives of this study were to determine whether DINP-induced effects in the liver associated with carcinogenesis are mediated by PPAR[alpha] and to identify novel gene targets of DINP. Male and female SV129 wild-type, SV129 PPAR[alpha]-null, and B6C3F1 mice were administered DINP by gavage or in the feed. Transcript profile technology and reverse transcriptase (RT)-polymerase chain reaction (PCR) were used to identify gene targets. Dose-dependent increases in relative liver weights were dependent on PPAR[alpha] in 10- or 12-week-old male and female mice and 30-week-old male mice. Female 30-week-old mice exhibited PPAR[alpha]-independent increases in relative liver weights. Increases in hepatocyte proliferation, palmitoyl-CoA oxidase (PCO) activity, and levels of enzymes involved in [beta]- and [omega]-oxidation of fatty acids were shown to be dependent on PPAR[alpha]. Five novel genes were shown to be altered in the livers of female wild-type mice after a 3-week exposure, but not in PPAR[alpha]-null, mice. These genes included those involved in DNA repair and recombination (ATP-dependent helicase and Endonuclease III homolog), drug metabolism (Cyp2a4) and protein trafficking (FKBP-1, FKBP-13). An additional gene (Cyp2d9)

was shown to be down-regulated in wild-type mice but up-regulated in PPAR[alpha]-null mice indicating more complex regulation by PPAR[alpha] and additional factors. These data support the hypothesis that PPAR[alpha] plays a dominant role in mediating the effects associated with hepatocarcinogenesis after DINP exposure.

Van Och, F. M. M., H. Van Loveren, et al. "Assessment of potency of allergenic activity of low molecular weight compounds based on IL-1[alpha] and IL-18 production by a murine and human keratinocyte cell line." Toxicology **In Press, Corrected Proof**
http://www.sciencedirect.com/science/article/B6TCN-4FGXVB0-2/2/bef972feec3bc22c863ec4a74fde1c2c

Assessment of allergenic potency of low molecular weight compounds is generally performed using animal models, such as the guinea pig maximisation test and the murine local lymph node assay (LLNA). Progress in unravelling the mechanisms of skin sensitisation, including effects on the production of cytokines by the different cell types of the skin, provides us with the opportunity to develop in vitro tests as an alternative to in vivo sensitisation testing. The aim of the present study was to establish an in vitro method to assess the potency of allergens, on the basis of their induction of cytokine production by murine and human keratinocytes. In the present study we used test systems comprised of the murine epidermal keratinocyte cell line HEL-30 and the human keratinocyte cell line HaCaT. We exposed these cell lines to the allergens ethyl-p-aminobenzoate (benzocaine), diethylamine (DEA), 2,4-dinitrochlorobenzene (DNCB), and phthalic anhydride (PA). IL-1[alpha] and IL-18 dose-response data were evaluated by non-linear regression analysis and at a stimulation index of 3 of cytokine production of treatment versus control, the corresponding allergen concentration was calculated. For HEL-30, for both cytokines DNCB showed the strongest potency followed in this order by PA, benzocaine, and DEA. This classification was similar to our previous findings obtained in the LLNA. For HaCaT, unfortunately, such ranking proved to be much less feasible. In conclusion, to assess the potency of allergens the murine keratinocyte cell line HEL-30 may be a useful in vitro test system, alternative to in vivo models, although this requires further testing using a much wider range of compounds.

Vandebriel, R. J., W. H. De Jong, et al. (2003). "Impact of exposure duration by low molecular weight compounds on interferon-[gamma] and interleukin-4 mRNA expression and production in the draining lymph nodes of mice." Toxicology **188**(1): 1.

http://www.sciencedirect.com/science/article/B6TCN-47WDB67-1/2/74e429efa78f09a513609cc9afd22d3c

The local lymph node assay (LLNA) is used to identify allergens by means of dermal exposure. For hazard identification, besides identification also the distinction between contact and respiratory allergens is of importance. We have previously shown that a modified LLNA can be used to identify respiratory allergens, on the basis of Con A induced IL-4 production. Here we show a good qualitative correlation between mRNA expression and production of IFN-[gamma] and IL-4. This suggests that distinction between contact and respiratory allergens may also be studied at the mRNA expression level. Secondly, another assay, similar to the modified LLNA but differing in the duration and the number of allergen applications as well as in the ex vivo culture conditions, here denoted as 'longer' assay, has been reported to be able to identify contact allergens, on the basis of (spontaneous) IFN-[gamma] production. In the present study we have compared these assays. Similar to our previous findings, in the modified LLNA exposure to the respiratory allergen trimellitic anhydride (TMA) resulted in a ~10-fold higher Con A induced IL-4 production compared with the contact allergen dinitrochlorobenzene (DNCB), while exposure to both allergens resulted in a similar Con A induced IFN-[gamma] production. In the 'longer' assay,

TMA exposure resulted in Con A induced IL-4 production whereas DNCB exposure did not. Importantly, only a 2-fold higher spontaneous IFN-[gamma] production was induced by DNCB compared with TMA, the difference being not statistically significant. Thus, although the 'longer' assay indeed showed a somewhat higher IFN-[gamma] induction by DNCB compared with TMA, the magnitude and robustness of this effect question its applicability. These results favor the modified LLNA since it is shorter, and combines identification of allergens (by cell proliferation) with identification of respiratory allergens (by IL-4 production). Compounds that induce cell proliferation with a low concomitant IL-4 production may thus be identified as contact allergens, although the need to positively identity such allergens remain.

Vandebriel, R. J., S. W. Spiekstra, et al. (1999). "In vitro exposure effects of cyclosporin A and bis(tri-n-butyltin)oxide on lymphocyte proliferation, cytokine (receptor) mRNA expression, and cell surface marker expression in rat thymocytes and splenocytes." Toxicology **135**(1): 49.

http://www.sciencedirect.com/science/article/B6TCN-3Y3XPWF-6/2/ffbd5822a8507b5e69c17fd4f8b24422

Rat thymocytes and splenocytes were exposed in vitro to the model compounds Cyclosporin A (CsA), an immunosuppressive drug, and bis(tri-n-butyltin)oxide (TBTO), an immunotoxic environmental contaminant. The lymphocyte transformation test (LTT), cytokine (receptor) mRNA expression (RT-PCR and dot blot hybridisation), and flow cytometry were evaluated as assays for in vitro immunotoxicity, at dose levels that did not show effects on viability, this being the aim of the study. LTT and RT-PCR proved useful assays. Lymphocyte transformation was suppressed by both compounds, while IL-2 mRNA expression was suppressed by CsA but not by TBTO, and both compounds suppressed IL-2R mRNA expression in splenocytes but not in thymocytes. Furthermore, the data obtained suggest that antiproliferative effects may be more relevant than apoptosis induction for TBTO induced thymus atrophy.

Toxicology and Applied Pharmacology (7)

Hwan Kim, S., K. Ok Hong, et al. (2004). "Abrogation of cisplatin-induced hepatotoxicity in mice by xanthorrhizol is related to its effect on the regulation of gene transcription." Toxicology and Applied Pharmacology **196**(3): 346.

http://www.sciencedirect.com/science/article/B6WXH-4C0V5S4-1/2/63ea8338a7b897b93357b23949f74a86

Cisplatin is a widely used anticancer drug, but at high dose, it can produce undesirable side effects such as hepatotoxicity. Because Curcuma xanthorrhiza Roxb. (Zingiberaceae) has been traditionally used to treat liver disorders, the protective effect of xanthorrhizol, which is isolated from C. xanthorrhiza, on cisplatin-induced hepatotoxicity was evaluated in mice. The pretreatment of xanthorrhizol (200 mg/kg/day, po) for 4 days prevented the hepatotoxicity induced by cisplatin (45 mg/kg, ip) with statistical significance. Interestingly, it abrogated cisplatin-induced DNA-binding activity of nuclear factor-kappaB (NF-[kappa]B), which consequently affected mRNA expression levels of NF-[kappa]B-dependent genes, inducible nitric oxide synthase (iNOS), and cyclooxygenase-2 (COX-2), even in part. It also attenuated the cisplatin-suppressed DNA-binding activity of activator protein 1 (AP-1). Using differential display reverse transcription-polymerase

chain reaction (DDRT-PCR), seven upregulated genes including S100 calcium binding protein A9 (S100A9) mRNA and antigenic determinant for rec-A protein mRNA and five downregulated genes including caseinolytic protease X (ClpX) mRNA and ceruloplasmin (CP) mRNA by cisplatin were identified. Although these mRNA expression patterns were not totally consistent with gel shift patterns, altered expression levels by cisplatin were reversed by the pretreatment of xanthorrhizol. In conclusion, the ability of xanthorrhizol to regulate the DNA-binding activities of transcription factors, NF-[kappa]B and AP-1, could be one possible mechanism to elucidate the preventive effect of xanthorrhizol on cisplatin-induced hepatotoxicity. Furthermore, genes identified in this study could be helpful to understand the mechanism of cisplatin-induced hepatotoxicity. Finally, the combination treatment of xanthorrhizol and cisplatin may provide more advantage than single treatment of cisplatin in cancer therapy.

Kenyon, N. J., R. W. Ward, et al. (2003). "Airway fibrosis in a mouse model of airway inflammation." Toxicology and Applied Pharmacology **186**(2): 90.

http://www.sciencedirect.com/science/article/B6WXH-47RYSNF-4/2/e14863329ee59f7df018f4cf3cd54ff1

BALB/c mice were sensitized to ovalbumin by systemic injection and then exposed for up to 8 weeks to ovalbumin aerosols in whole body chambers. A pattern of airway inflammation, mucous cell hypertrophy and hyperplasia, and airway remodeling with submucosal fibrosis was observed as lesions evolved over time. Larger conducting airways were removed from the lungs by microdissection. Airway fibrosis was quantified by direct assay for collagen content, which was significantly increased after 4 and 8 weeks of exposure to ovalbumin aerosol. Based upon PCR analysis of mRNA levels in the airways, most of the newly synthesized collagen was Type I. Relaxin, administered by continuous infusion over the second half of a 4-week exposure to ovalbumin, was able to inhibit the accumulation of collagen in the airways of exposed mice. Thus, stimulation of collagen degradation by an activator of collagen breakdown by matrix metalloproteinases appears to be an effective therapeutic strategy in prevention of airway fibrosis in this animal model. Whole body plethysmography of unrestrained mice indicated functional changes in airway reactivity in the lungs of exposed animals occurring in conjunction with the reported structural changes. This result indicates that the ovalbumin-exposed mouse may be a suitable model for examining structure-function relationships in the lungs of animals with a predictable time course of airway inflammation, remodeling, and fibrosis and for testing potential new drugs for treatment of asthma or chronic bronchitis at a mechanistic level.

Konno, Y., M. Sekimoto, et al. (2004). "Sex difference in induction of hepatic CYP2B and CYP3A subfamily enzymes by nicardipine and nifedipine in rats." Toxicology and Applied Pharmacology **196**(1): 20.

http://www.sciencedirect.com/science/article/B6WXH-4BT1SB3-1/2/d033257ac2462d714c4155e378292b73

Male and female of F344 rats were treated per os with nicardipine (Nic) and nifedipine (Nif), and changes in the levels of mRNA and protein of hepatic cytochrome P450 (P450) enzymes, CYP2B1, CYP2B2, CYP3A1, CYP3A2, CYP3A9, and CYP3A18 were examined. Furthermore, hepatic microsomal activities for pentoxyresorufin O-dealkylation (PROD) and nifedipine oxidation, which are mainly mediated by CYP2B and CYP3A subfamily enzymes, respectively, were measured. Analyses of RT-PCR and Western blotting revealed that Nic and Nif induced predominantly CYP3A and CYP2B enzymes, respectively. As for the gene activation of CYP2B enzymes, especially CYP2B1, Nif showed high capacity in both sexes of rats, whereas Nic did a definite capacity in the males but little in the females. Gene activations of CYP3A1, CYP3A2, and

CYP3A18 by Nic occurred in both sexes of rats, although that of CYP3A9 did only in the male rats. Although gene activations of CYP3A1 and CYP3A2 by Nif were observed in both sexes of rats, a slight activation of the CYP3A9 gene occurred only in female rats, and the CYP3A18 gene activation, in neither male nor female rats. Thus, changes in levels of the mRNA or protein of CYP2B and CYP3A enzymes, especially CYP2B1 and CYP3A2, were closely correlated with those in hepatic PROD and nifedipine oxidation activities, respectively. The present findings demonstrate for the first time the sex difference in the Nic- and Nif-mediated induction of hepatic P450 enzymes in rats and further indicate that Nic and Nif show different specificities and sex dependencies in the induction of hepatic P450 enzymes.

Lai, Y.-L., S. C. Yu, et al. (2003). "RNA interference prevents lipopolysaccharide-induced preprotachykinin gene expression." Toxicology and Applied Pharmacology **193**(1): 47.

http://www.sciencedirect.com/science/article/B6WXH-49NXGG3-4/2/57529a79531b0125153204071dc3125c

We showed previously that lipopolysaccharide (LPS) induces noncholinergic airway hyperreactivity to capsaicin via an upregulation of tachykinin synthesis. This study was designed to test whether double-stranded preprotachykinin (ds PPT) RNA, RNA interference (RNAi), prevents the LPS-induced alterations. First, cultured primary nodose ganglial cells of newborn Brown-Norway rats were divided into four groups: control; LPS; LPS+RNAi; and LPS+RNAi+liposome. Second, young Brown-Norway rats for the in vivo study were divided into three groups (control; LPS; and LPS+RNAi), and ds PPT RNA was microinjected bilaterally into the nodose ganglia in the LPS+RNAi group. Then, ganglial cells were collected from the culture whereas the nodose ganglia and lungs were sampled from the animals, and PPT mRNA and substance P (SP) levels were analyzed. Also, airway reactivity to capsaicin was performed in vivo. LPS induced significant increases in PPT mRNA and SP levels in vitro and in vivo and an increase in airway reactivity to capsaicin in vivo. However, ds PPT RNA, but not scrambled RNA, prevented all LPS-induced alterations. The effect of ds PPT RNA was not enhanced by liposome in vitro. Therefore, we demonstrated that the local application of RNAi prevents effectively the activation of the noncholinergic system modulating the lungs/airways.

Patterson, R. M., R. Stachlewitz, et al. (2003). "Induction of apoptosis by 2,3,7,8-tetrachlorodibenzo-p-dioxin following endotoxin exposure." Toxicology and Applied Pharmacology **190**(2): 120.

http://www.sciencedirect.com/science/article/B6WXH-48XD53J-2/2/8a01d8f42ed8df66bb286a26860e1b70

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is a potent and persistent environmental toxin that induces hepatotoxicity and increases endotoxin-induced liver injury. The objective of this study was to evaluate whether TCDD could modulate apoptosis and cytokine-controlled apoptotic signaling pathways following lipopolysaccharide (LPS) exposure in female B6C3F1 mice. The effects of TCDD treatment were most dramatic late in the time course (10-14 days posttreatment). Serum enzyme activities were elevated at day 10 (100 [mu]g TCDD/40 [mu]g LPS treatment) and day 14 (100 [mu]g TCDD/saline treatment), indicating peak liver damage occurred at those times. Histological examination of perfused livers showed an increase in apoptotic cells at day 14 in animals treated with 10 [mu]g TCDD. Caspase-1 activity was suppressed at 14 days in mice treated with 100 [mu]g TCDD/40 [mu]g LPS and 100 [mu]g TCDD/4 [mu]g LPS compared to the respective corn oil (CO)/LPS-treated controls. Caspase-3 activity was suppressed at 14 days in 100 [mu]g TCDD/saline-100 [mu]g TCDD/40 [mu]g LPS- and 100 [mu]g TCDD/4 [mu]g LPS-treated mice compared to respective CO/saline- or CO/LPS-treated control mice. At 40 [mu]g LPS, caspase activity was stimulated in TCDD (100 [mu]g)-exposed mice at 3 and 7 days

and then suppressed at 10 and 14 days. Western blot analysis, electrophoretic mobility shift assay, and ELISA did not show any effect by TCDD (100 [mu]g) on I[kappa]B-[beta] and I[kappa]B-[alpha] protein expression or on DNA binding activity of the nuclear NF[kappa]B protein. These data indicate that TCDD induces apoptosis 14 days posttreatment; however, we found no evidence of suppression of the antiapoptotic transcription factor NF[kappa]B.

Rojas-Garcia, A. E., M. J. Solis-Heredia, et al. "Genetic polymorphisms and activity of PON1 in a Mexican population." Toxicology and Applied Pharmacology **In Press, Corrected Proof**
http://www.sciencedirect.com/science/article/B6WXH-4F0118T-1/2/342e096c045e31d69f1b45f05721a129

Human paraoxonase (PON1) plays a role in detoxification of organophosphorus (OP) compounds by hydrolyzing the bioactive oxons, and in reducing oxidative low-density lipoproteins, which may protect against atherosclerosis. Some PON1 polymorphisms have been found to be responsible for variations in catalytic activity and expression and have been associated with susceptibility to OP poisoning and vascular diseases. Both situations are of public health relevance in Mexico. Therefore, the aim of this study was to evaluate PON1 phenotype and the frequencies of polymorphisms PON1 -162, -108, 55, and 192 in a Mexican population. The studied population consisted of unrelated individuals (n = 214) of either gender, 18-52 years old. Serum PON1 activity was assayed using phenylacetate and paraoxon as substrates. PON1 variants, -162, 55, and 192, were determined by real-time PCR using the TaqMan System, and PON1 -108 genotype by PCR-RFLP. We found a wide interindividual variability of PON1 activity with a unimodal distribution; the range of enzymatic activity toward phenylacetate was 84.72 to 422.0 U/mL, and 88.37 to 1645.6 U/L toward paraoxon. All four PON1 polymorphisms showed strong linkage disequilibrium (D% >90). PON1 polymorphisms -108, 55, and 192 were independently associated with arylesterase activity; whereas the activity toward paraoxon was related only with PON1 192 polymorphism, suggesting that this polymorphism is determinant to infer PON1 activity. A better understanding of the phenotype and genotypes of PON1 in Mexican populations will facilitate further epidemiological studies involving PON1 variability in OP poisoning and in the development of atherosclerosis.

Zheng, Y. i.-M., K. R. Henne, et al. (2003). "Genotyping and site-directed mutagenesis of a cytochrome P450 meander Pro-X-Arg motif critical to CYP4B1 catalysis." Toxicology and Applied Pharmacology **186**(2): 119.

http://www.sciencedirect.com/science/article/B6WXH-47RYSNF-3/2/0246741551aadbdef0ffe5508494ff4c

CYP4B1 isoforms from rodents and other common laboratory animals are involved in the bioactivation of a range of protoxins, including 2-aminofluorene, 4-ipomeanol, and valproic acid. However, an earlier study provided evidence for a human allele encoding a nonfunctional CYP4B1 enzyme due to a Pro427Ser transversion in the meander region of the protein. In the present study, the CYP4B1 gene from several racial groups, Caucasians, African-Americans, and Hispanics, and from six nonhuman primate species was genotyped using a PCR-Hinf1 restriction enzyme fragment length polymorphism assay or by direct sequencing. All human populations examined were found to possess only the Ser allele at codon 427 (1279TCT) and all of the nonhuman primate species possessed only the Pro (CCT) allele. Therefore, an inactivating 1279C->T mutation in the human CYP4B1 gene likely arose following divergence of the Homo and Pan clades. Amino acid sequence alignments revealed further that this key Pro residue is located two amino acid residues N-terminal to the distal Arg of a Glu-Arg- triad thought to participate in heme binding and/or redox partner interactions. Mutation of the corresponding Arg424 residue in rabbit CYP4B1 to Leu, but not His, resulted in a loss of lauric acid hydroxylase

activity and ability to generate a reduced-CO binding spectrum. These data provide additional evidence for the importance of this meander region Pro-X-Arg motif in CYP4B1 heme binding and catalytic function.

Toxicology in Vitro (6)

Hildebrand, H., U. Schmidt, et al. (1999). "An in vitro model for peroxisome proliferation utilizing primary hepatocytes in sandwich culture." Toxicology in Vitro **13**(2): 265.

http://www.sciencedirect.com/science/article/B6TCP-3WRB2B0-6/2/a26a09192f8c3aa3813cdac0fc601a51

Peroxisome proliferators comprise a group of structurally diverse chemicals which share as a common biologic effect the induction of peroxisomal fatty acid degrading enzymes. Concomitantly, the number and size of peroxisomes within hepatocytes increases. Following chronic administration some peroxisome proliferators act as non-genotoxic hepatocarcinogens in susceptible species such as rodents. To establish an in vitro model for the toxicological investigation of peroxisome proliferation, primary hepatocytes of rats, dogs and humans were cultivated in an organotypic cell culture model (sandwich model). By employing a panel of diverse compounds in this model a graded response was observed in the induction of carnitine acetyl transferase (CAT), the activity of which was determined as an endpoint. The following results were obtained in the order of decreasing inducing potential for rat hepatocytes: FOE 3798>nafenopin>fenofibrate (ciprofibrate>bezafibrate >> DEHP[ap]ETYA>DEHA. Induction of CAT activity was generally higher than reported in earlier cell culture systems, probably reflecting the effect of the extracellular matrix provided by the collagen gel sandwich. In parallel, transcription of the rat CYP4A1 gene was induced by a similar order of magnitude as measured by TaqMan RT-PCR. In accordance with literature data, human and dog hepatocytes did not display such a strong and graded response but rather were not susceptible to this effect. In addition, 3H-thymidine incorporation data demonstrated that nafenopin was able to induce DNA synthesis in rat hepatocytes whereas it did not in human hepatocytes.

Pawlowski, S., M. Islinger, et al. (2000). "Temperature-dependent vitellogenin-mRNA expression in primary cultures of rainbow trout (Oncorhynchus mykiss) hepatocytes at 14 and 18[deg]C." Toxicology in Vitro **14**(6): 531.

http://www.sciencedirect.com/science/article/B6TCP-41CP350-7/2/eb6babd6ef637553d04350ff95f28b85

In order to study the influence of temperature on vitellogenin gene and estrogen receptor gene expression in primary hepatocytes from rainbow trout (Oncorhynchus mykiss), cells were exposed to 17[beta]-estradiol, bisphenol-A and nonylphenol for 48 and 96 hr. Induction of vitellogenin-mRNA expression was detected in a non-radioactive dot blot/RNAse protection assay and by RT-PCR. In the dot blot/RNAse protection assay, the estrogenic potentials of bisphenol-A and nonylphenol were about 104- to 105-fold and 105-fold lower than that of 17[beta]-estradiol, respectively. The relative estrogenic potential did not show any difference between 14 and 18[deg]C. In contrast, at 18[deg]C, RT-PCR analysis revealed increased amounts of vitellogenin- and estrogen receptor-mRNA after 12 and 24 hr of exposure to 17[beta]-estradiol, if compared to

14[deg]C. Owing to increased vitellogenin gene expression at 18[deg]C, the sensitivity of primary hepatocytes to 17[beta]-estradiol and bisphenol-A could be increased.

Pichowski, J. S., M. Cumberbatch, et al. (2000). "Investigation of induced changes in interleukin 1[beta] mRNA expression by cultured human dendritic cells as an in vitro approach to skin sensitization testing." Toxicology in Vitro **14**(4): 351.

http://www.sciencedirect.com/science/article/B6TCP-40SFFYB-8/2/cac096480d04ea1da0a1560ee674dd2d

It has been reported previously that in vitro treatment of human blood derived dendritic cells (DC) with contact allergens provokes the elevated expression of mRNA for interleukin (IL) 1[beta], under conditions where similar treatment of cells with the non-sensitizing skin irritant sodium lauryl sulfate (SLS) did not alter IL-1[beta] mRNA levels (Reutter et al., 1997). The purpose of the present investigation was to evaluate further this phenomenon and to explore the potential utility of this approach for the purpose of skin sensitization testing. Human peripheral blood progenitor cells prepared from healthy adult volunteers were cultured in the presence of IL-4 and granulocyte/macrophage colony stimulating factor. After 5 days of culture, the majority of cells had a Langerhans cell-like phenotype, with characteristic dendritic morphology and cell surface expression of CD83, major histocompatibility complex class II and CD1a determinants. These blood-derived DC were cultured in the presence of the contact allergen 2,4-dinitrofluorobenzene (DNFB), SLS or vehicle alone and mRNA expression for IL-1[beta], IL-6 and IL-18 was analysed by semiquantitative reverse transcriptase polymerase chain reaction. Constitutive expression of all three cytokines was observed for DC isolated from all donors examined. Exposure to DNFB resulted in upregulation of IL-1[beta] mRNA (two- to threefold) in cells derived from three out of eight donors whereas IL-6 and IL-18 were largely unaffected by allergen exposure. In contrast, SLS treatment did not induce IL-1[beta] mRNA expression in any of the donors investigated. Analysis of cytokine mRNA expression using the protocol described by Reutter et al. (1997), did not increase the sensitivity of measurement of induced cytokine expression. Although selected upregulation of IL-1[beta] by blood derived DC has been confirmed, a wider range of contact allergens and irritants need to be assessed before this approach could be considered for hazard identification.

Reutter, K., D. Jager, et al. (1997). "In vitro model for contact sensitization: II. Induction of IL-1[beta] mRNA in human blood-derived dendritic cells by contact sensitizers." Toxicology in Vitro **11**(5): 619.

http://www.sciencedirect.com/science/article/B6TCP-3RSP2T3-15/2/5423ea258d97109a15cf3901c1a05d42

Epidermal mRNA for interleukin 1[beta] (IL-1[beta]) has been shown to be increased following exposure of mouse skin to sensitizing compounds. In addition, this early upregulation of IL-1[beta] was specific for contact sensitizers, while expression of IL-1[beta] was unaffected by irritants. Langerhans cells are the major source of IL-1[beta] within the epidermis in the induction phase of skin sensitization. Since the isolation of Langerhans cells from skin biopsies results only in low frequencies, we decided to use dendritic cells (DCs) generated from peripheral blood as Langerhans cell equivalents to investigate the ability of five contact sensitizers and one irritant to induce IL-1[beta] gene expression in vitro. For our studies we cultivated DCs in serum-free medium supplemented with granulocyte/macrophage-colony stimulation factor (GM-CSF) and interleukin 4 (IL-4). The DCs showed a typical dendritic morphology, a characteristic expression of surface markers and high stimulatory capacity for autologous T cells. 5-day-old DCs were incubated with subtoxic concentrations of the contact sensitizers pentadecyl-catechol, 2,4,6-

trinitrobenezene sulfonic acid, 2,4-dinitrofluorobenzene, NiSO4, K2Cr2O7 and the irritant sodium dodecyl sulfate. IL-1[beta] mRNA expression was detected by using the reverse transcriptase-polymerase chain reaction (RT-PCR) technique and non-radioactive hybridization procedures. For all contact sensitizers, expression of IL-1[beta] mRNA increased, whereas treatment with the irritant SDS had no significant effect on IL-1[beta] expression. Thus we developed an in vitro system, which may be useful to evaluate allergic potentials of chemicals and products.

Shimizu, M., K. Ohta, et al. (2002). "Sulfation of bisphenol A abolished its estrogenicity based on proliferation and gene expression in human breast cancer MCF-7 cells." Toxicology in Vitro **16**(5): 549.

http://www.sciencedirect.com/science/article/B6TCP-46M1JR0-9/2/585dfd18036804d829dc600bc864ddfb

Bisphenol A, an endocrine-disrupting chemical, is widely used in many consumer products. We previously showed the sulfoconjugation of bisphenol A catalyzed by a human thermostable phenol sulfotransferase, ST1A3. The estrogenic potency of bisphenol A sulfate was compared with that of bisphenol A by an E-screen assay using human breast cancer MCF-7 cells. An increase in the expression level of an estrogen-responsive pS2 gene was also examined using MCF-7 cells after exposure to bisphenol A and its sulfate for their estrogenicity. Bisphenol A sulfate did not exhibit estrogenic effects at 0.1 [mu] (E-screen assay) and 1 m (pS2 gene expression) compared with bisphenol A, which exhibited the effects at 3 n (E-screen assay) and 1 [mu] (pS2 gene expression), respectively. We have therefore evaluated major roles of cytosolic phenol sulfotransferase in the human liver. Bisphenol A sulfation in human liver cytosols was inhibited by more than 90% by p-nitrophenol and quercetin, a typical substrate and specific inhibitor of phenol sulfotransferase, respectively. These results indicated that the estrogenicity of bisphenol A was abolished through its sulfation catalyzed by a human hepatic thermostable phenol sulfotransferase.

zur Nieden, N. I., L. J. Ruf, et al. (2001). "Molecular markers in embryonic stem cells." Toxicology in Vitro **15**(4-5): 455.

http://www.sciencedirect.com/science/article/B6TCP-440BK3X-16/2/1c84c2410e44cd9b2163e1f12559bddd

Embryonic stem cells are pluripotent cells derived from mouse blastocysts, which have the capacity to differentiate in vitro into a wide variety of cell types. Based on this potential the embryonic stem cell test (EST) has been developed, which represents an assay system for the classification of compounds for their teratogenic potential, based on the morphological evaluation of contracting myocard cells compared to the cytotoxic effects on undifferentiated stem cells and adult 3T3 fibroblasts. To expand the EST, the quantitative expression of the [alpha]- and [beta]-myosin heavy chain (MHC) genes under the influence of test compounds was studied employing real-time TaqMan PCR analysis. The molecular evaluation of the MHC genes allows a higher sensitivity for the classification of substances and the transfer of the EST to the molecular level allows to start experimental procedures at day 9 of culture. Thus, the modulated EST holds promise as a new easily quantifiable in vitro screening assay in teratology.

Toxicology Letters (8)

Brauch, H., G. Weirich, et al. (2004). "VHL mutations in renal cell cancer: does occupational exposure to trichloroethylene make a difference?" Toxicology Letters **151**(1): 301.

http://www.sciencedirect.com/science/article/B6TCR-4C47J23-6/2/d4fefa5499232039717baa44b805331c

Occupational exposures have long been suspected to play a role in the incidence of renal cell carcinoma (RCC). Especially, the carcinogenicity of the industrial solvent trichloroethylene (TCE) has been controversially debated, both with respect to the epidemiological and the molecular studies. In order to further elucidate this issue, it appeared important to compare suitable RCC patient groups, i.e., TCE-exposed versus non-TCE-exposed patients. We evaluated RCC from a previous German study that had described differences in RCC risks between TCE-exposed (n=17) and non-exposed patients (n=21). We compared age at diagnosis and histopathologic parameters of tumors as well as somatic mutation characteristics in the kidney cancer causing VHL tumor suppressor gene. RCC did not differ with respect to histopathological characteristics in both patient groups. We noticed a younger age at diagnosis in TCE-exposed patients compared to non-exposed patients (P=0.01). Moreover, the non-TCE-exposed patients did not share the somatic VHL mutation characteristics of TCE-exposed patients such as the previously identified hot spot mutation 454 C > T P81S or multiple mutations. These data support the notion of a putative genotoxic effect of TCE leading to VHL gene damage and subsequent occurrence of RCC in highly exposed subjects.

Garrett, S. H., V. Phillips, et al. (2002). "Transient induction of metallothionein isoform 3 (MT-3), c-fos, c-jun and c-myc in human proximal tubule cells exposed to cadmium." Toxicology Letters **126**(1): 69.

http://www.sciencedirect.com/science/article/B6TCR-44JHY8R-7/2/fd4cbec082de4a2bdfe6ac0d1d7f0139

Cadmium (Cd+2) has been shown to transiently increase the expression of mRNA for the third isoform of the metallothionein (MT-3) gene family in cultured human proximal tubule (HPT) cells. The goal of the present study was to further define the expression of MT-3 in mortal (HPT) and immortal (HK-2) cultures of HPT cells when exposed to lethal and sub-lethal concentrations of Cd+2 under both acute and chronic time periods of exposure. Expression of MT-3 mRNA and protein was determined in cultured HPT cells and HK-2 cells using reverse-transcription-polymerase chain reaction (RT-PCR) and immuno-blotting, and expression of c-fos, c-jun and c-myc mRNA by RT-PCR. The results confirmed that exposure of the HPT cells to Cd+2 induced a transient increase in MT-3 mRNA and extended the induction to include a subsequent transient increase in the level of the MT-3 protein. The induction of MT-3 was rapid and returned to control values within 48 h of exposure despite the continued presence of lethal and sublethal concentrations of Cd+2. It was also demonstrated that the pattern of expression of MT-3 mRNA was similar to that of the early response genes, c-fos, c-jun and c-myc. It was shown that the HK-2 cells did not express MT-3 when exposed to Cd+2, but had similar expression of the c-fos, c-jun and c-myc genes. The results demonstrate that MT-3 expression is metal responsive in HPT cells.

Kojima, M., T. Masui, et al. (2004). "Lead nitrate-induced development of hypercholesterolemia in rats:

sterol-independent gene regulation of hepatic enzymes responsible for cholesterol homeostasis." Toxicology Letters **154**(1-2): 35.

http://www.sciencedirect.com/science/article/B6TCR-4D4VHW9-4/2/f49f52e6eaec345d3ad0250778acb2eb

Changes in the gene expressions of hepatic enzymes responsible for cholesterol homeostasis were examined during the process of lead nitrate (LN)-induced development of hypercholesterolemia in male rats. Total cholesterol levels in the liver and serum were significantly increased at 3-72 h and 12-72 h, respectively, after LN-treatment (100 [mu]mol/kg, i.v.). Despite the development of hypercholesterolemia, the genes for hepatic 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR) and other enzymes (FPPS, farnesyl diphosphate synthase; SQS, squalene synthase; CYP51, lanosterol 14[alpha]-demethylase) responsible for cholesterol biosynthesis were activated at 3-24 h and 12-18 h, respectively. On the other hand, the gene expression of cholesterol 7[alpha]-hydroxylase (CYP7A1), a catabolic enzyme of cholesterol, was remarkably suppressed at 3-72 h. The gene expression levels of cytokines interleukin-1[beta] (IL-1[beta]) and TNF-[alpha], which activate the HMGR gene and suppress the CYP7A1 gene, were significantly increased at 1-3 h and 3-24 h, respectively. Furthermore, gene activation of SREBP-2, a gene activator of several cholesterogenic enzymes, occurred before the gene activations of FPPS, SQS and CYP51. This is the first report demonstrating sterol-independent gene regulation of hepatic enzymes responsible for cholesterol homeostasis in LN-treated male rats. The mechanisms for the altered-gene expressions of hepatic enzymes in LN-treated rats are discussed.

Lafuente, M. J., X. Casterad, et al. (2002). "Pi*S and Pi*Z alpha 1 antitrypsin polymorphism and the risk for asbestosis in occupational exposure to asbestos." Toxicology Letters **136**(1): 9.

http://www.sciencedirect.com/science/article/B6TCR-46W1CF4-2/2/3b1471822f16320970b2fa425a5d6686

Alpha 1 antitrypsin is a highly polymorphic anti-elastase enzyme, especially active in the protection of alveoli and liver. Here we studied the distribution of two deficient alleles Pi*Z and Pi*S, in 194 asbestos workers, of whom 100 were asbestosis cases, and 94 were controls without disease (exposed controls, EC). A second group of controls without asbestos exposure (non-exposed controls, NEC; n=122) was also included. Multivariate analysis adjusted by age and smoking habit showed ninefold risk for asbestosis in Pi*Z heterozygous individuals and 5.9-fold risk for Pi*S homozygous although differences were only significant in the first case (cases vs. EC: OR 8.9; p=0.04). Considering both genotypes (Pi*Z heterozygous, Pi*S homozygous) we obtained an OR of 8 (p=0.01). Our results suggest that the alpha 1 antitrypsin polymorphisms, especially Pi*Z, could help to predict asbestosis risk and confirm the high prevalence of the Pi*S allele in Spain.

Navarra, P., P. Puccetti, et al. (1995). "Anticancer drug toxicity via cytokine production: the hydroxyurea paradigm." Toxicology Letters **82-83**: 167.

http://www.sciencedirect.com/science/article/B6TCR-3VXBPDC-V/2/c3c847fefb17707e3b0f904ac98b5ef5

Our previous observations on the toxic effects of hydroxyurea (HU) in adrenalectomized (ADX) rats prompted us to suggest that these effects might be mediated by an increased synthesis of proinflammatory cytokines. This study was conducted to determine whether HU stimulates

cytokine gene expression in vivo. The polymerase chain reaction (PCR) technique was used to assess levels of mRNA for interleukin-1[alpha] (IL-1[alpha]), tumor necrosis factor (TNF) and interleukin-4 (IL-4) in spleen cells from intact and ADX rats treated with HU or vehicle. In ADX rats, expression of proinflammatory-cytokine mRNA was markedly increased by HU, but no expression of these genes was seen in intact animals after treatment. In the latter rats, cytokine-gene expression seemed to be down-regulated by HU-induced elevations in plasma corticosterone levels, since IL-1[alpha] and TNF transcripts could be detected only after corticosterone levels had returned to normal (24 h after treatment). Interestingly, IL-4 mRNA could not be detected in either treated or untreated ADX rats, indicating that expression of this gene is closely related to circulating levels of corticosterone. These findings strongly suggest that the increased toxicity displayed by HU in ADX animals is mediated by stimulation of cytokine synthesis in vivo.

Seidel, B., L. Jiang, et al. (2000). "Differentially displayed genes in neuroblastoma cells treated with a mitochondrial toxin: evidence for possible involvement of ICAM-1 in 3-nitropropionic acid-mediated neurodegeneration." Toxicology Letters **115**(3): 213.

http://www.sciencedirect.com/science/article/B6TCR-408BJ93-5/2/dff3d60f00a9885cd5eab36f451c52a8

The mitochondrial toxin 3-nitropropionic acid (3-NPA) causes neurodegeneration in the basal ganglia and neurological symptoms resembling Huntington's disease (HD) when applied to primates or rodents, and therefore might be used as an animal model for this disorder. For that reason, the molecular mechanisms involved in 3-NPA-induced neurodegeneration are of considerable interest. In our model, murine neuroblastoma cells (Neuro-2a) were treated with different doses of 3-NPA, and changes in gene expression were analyzed by means of mRNA differential display (DDRT-PCR). Using 18 primer combinations, we have identified a set of 33 candidate cDNAs deriving from 29 excised DDRT bands whose expression appeared to be changed in response to the 3-NPA insult (mostly elevated). DNA sequencing revealed that novel, as well as previously described genes, are included in this panel. Amongst the known cDNAs, the differential mRNA expression of the ribosomal proteins S6 and L40, of the protein kinase A (PKA) catalytic beta subunit and of the intercellular adhesion molecule ICAM-1 could be verified using Northern hybridization and RT-PCR, respectively. Furthermore, ICAM-1 expression could also be shown to increase at the protein level, which points to a possible function for this molecule in neuronal cells in the course of neurodegeneration. The results may prove useful in elucidating the multiple processes causing neurodegeneration subsequent to lesions by mitochondrial toxins and excitotoxins as well.

Somji, S., M. Ann Sens, et al. (2002). "Expression of hsp 90 in the human kidney and in proximal tubule cells exposed to heat, sodium arsenite and cadmium chloride." Toxicology Letters **133**(2-3): 241.

http://www.sciencedirect.com/science/article/B6TCR-460DFXH-1/2/f2249d82b2c2600a64d52706a2d75616

The expression of heat shock protein (hsp) 90[alpha] and [beta] mRNA and protein were determined in the human kidney and in human proximal tubule (HPT) cells exposed to lethal and sub-lethal concentrations of Cd+2 under both acute and extended conditions of exposure. Using immunohistochemical analysis, it was demonstrated that hsp 90 was widely distributed in the human adult and fetal kidney. Moderate to strong staining was observed in the straight portions of the distal and proximal tubules, the distal convoluted tubule, the collecting ducts and the parietal epithelium of Bowmans capsule in the glomerulus. Moderate staining was observed in the proximal convoluted tubule of the cortex and the thick loops of Henle within the medulla. In

addition, the fetal kidney demonstrated strong staining of the blastema, the 'S-shaped' bodies, and the developing glomeruli. Analysis of hsp 90[alpha] and [beta] mRNA expression in total RNA isolated from in situ microdissected proximal tubules or HPT cells demonstrated similar expression levels of both the [alpha] and [beta] isoforms in this tubule segment. It was demonstrated that HPT cells exhibited the classic heat shock response when subjected to a physical (heat) or chemical stress (NaAsO2). Heat stress, elevated temperature at 42.5 [deg]C for 1 h, caused a modest increase in both hsp 90[alpha] and [beta] mRNA and protein. Similar results were obtained when the cells were subjected to a classic chemical stress of exposure to 100 [mu]M NaAsO2 for 4 h. In contrast, acute exposure of HPT cells to 53.4 [mu]M CdCl2 for 4 h resulted in no consistent increase in hsp 90[alpha] and [beta] mRNA or protein. Chronic exposure to Cd+2 likewise failed to increase either hsp 90 mRNA or protein expression, even at concentrations of Cd+2 that were lethal to the cells during the time course. This study shows that the HPT has a high basal expression of hsp 90, which is not induced by Cd+2 exposure.

Somji, S., J. H. Todd, et al. (2000). "Expression of heat shock protein 60 in human proximal tubule cells exposed to heat, sodium arsenite and CdCl2." Toxicology Letters **115**(2): 127.

http://www.sciencedirect.com/science/article/B6TCR-408BJC1-5/2/e344ad1d859faea13b8624a03df873dd

The expression of hsp 60 mRNA and protein were determined in human proximal tubule cells (HPT) exposed to lethal and sub-lethal concentrations of Cd2+ under both acute and extended conditions of exposure. It was demonstrated that HPT cells exhibited the classic heat shock response when subjected to a physical (heat) or chemical stress (sodium arsenite). Heat stress, elevated temperature at 42.5[deg]C for 1 h, caused an increase in both hsp 60 mRNA and protein following removal of the stress. Similar results were obtained when the cells were subjected to a classic chemical stress of exposure to 100 [mu]M sodium arsenite for 4 h. Acute exposure of HPT cells to 53.4 [mu]M CdCl2 for 4 h also resulted in an increase in hsp 60 mRNA and protein following removal of the metal. An extended exposure to Cd2+ was modeled by treating the cells continuously with Cd2+ at both lethal and sub-lethal levels over a 16-day time course. It was demonstrated that chronic exposure to Cd2+ failed to increase either hsp 60 mRNA or protein expression in HPT cells, even at concentrations of Cd2+ that were lethal to the cells during the time course. In fact, hsp 60 protein levels were decreased compared to controls at lethal levels of Cd2+ exposure. These findings suggest that hsp 60 expression may have two distinct roles when the human proximal tubule cell is exposed to Cd2+. A protective role through hsp 60 induction when the proximal tubule cell is acutely exposed to Cd2+ and a deleterious role when hsp 60 protein is down-regulated during extended exposure to Cd2+.

Toxicon (4)

Clissa, P. B., G. D. Laing, et al. (2001). "The effect of jararhagin, a metalloproteinase from Bothrops jararaca venom, on pro-inflammatory cytokines released by murine peritoneal adherent cells." Toxicon **39**(10): 1567.

http://www.sciencedirect.com/science/article/B6TCS-43K2G13-G/2/04a86f0b5a781c7f1a11bb747bdd3512

The release of pro-inflammatory cytokines (IL-1[beta], IL-6 and TNF-[alpha]) from murine peritoneal adherent cells (MPAC) was studied after exposure to jararhagin, a metalloproteinase/disintegrin isolated from Bothrops jararaca venom. MPACs were treated with LPS (lipopolysaccharide), jararhagin, or EDTA-inactivated jararhagin for up to 24 h. Following incubation, the culture supernatant was assayed by ELISA for the presence of cytokines, while the cells were analysed for viability and cytokine mRNA expression. The cells exposed to native jararhagin released TNF-[alpha] and IL-1[beta] after 4 and 24 h respectively. When MPACs were exposed to Jararhagin treated with EDTA, TNF-[alpha] and IL-1[beta] production was sustained throughout the culture period and IL-6 production was observed. TNF-[alpha], IL-6 and IL-1[beta] mRNA were detected 4 h after stimulation with either native or EDTA-treated jararhagin. Addition of jararhagin to LPS stimulated cells resulted in a dramatic decrease in the release of IL-6 and TNF-[alpha]. RT-PCR showed that this inhibition does not occur at the transcriptional level and further experiments showed that jararhagin degraded soluble cytokines by proteolytic activity. This study suggests that jararhagin induces TNF-[alpha], IL-1[beta] and IL-6 expression, which may be rapidly degraded by its proteolytic activity.

DaSilva, L., D. Cote, et al. (2003). "Pulmonary gene expression profiling of inhaled ricin." Toxicon **41**(7): 813.

http://www.sciencedirect.com/science/article/B6TCS-48KF64N-1/2/4d3374021a5e286c7c37ba59eb8ef6e1

Aerosol exposure to ricin causes irreversible pathological changes of the respiratory tract resulting in epithelial necrosis, pulmonary edema and ultimately death. The pulmonary genomic profile of BALB/c mice inhalationally exposed to a lethal dose of ricin was examined using cDNA arrays. The expression profile of 1178 mRNA species was determined for ricin-exposed lung tissue, in which 34 genes had statistically significant changes in gene expression. Transcripts identified by the assay included those that facilitate tissue healing (early growth response gene (egr)-1), regulate inflammation (interleukin (IL)-6, tristetraproline (ttp)), cell growth (c-myc, cytokine-inducible SH2-containing protein (cish)- 3), apoptosis (T-cell death associated protein (tdag)51, pim-1) and DNA repair (ephrin type A receptor 2 (ephA2)). Manipulation of these gene products may provide a means of limiting the severe lung damage occurring at the cellular level. Transcriptional activation of egr-1, cish-3, c-myc and thrombospondin (tsp)-1 was already apparent when pathological and physiological changes were observed in the lungs at 12 h postexposure. These genes may well serve as markers for ricin-induced pulmonary toxicity. Ongoing studies are evaluating this aspect of the array data and the potential of several genes for clinical intervention.

Ivanovski, G., F. Gubensek, et al. (2002). "mRNA secondary structure can greatly affect production of recombinant phospholipase A2 toxins in bacteria." Toxicon **40**(5): 543.

http://www.sciencedirect.com/science/article/B6TCS-44HTMX5-1/2/07784f5c6b087787f50076c99156cd40

The neurotoxic activity of ammodytoxin A (AtxA), a phospholipase A2 from Vipera ammodytes ammodytes venom, has been investigated by protein engineering. With the aim of obtaining AtxA as a non-fused protein in the bacterial cytoplasm and avoiding problems with incomplete cleavage in vivo of the initial Met preceding the first residue (Ser1), a double mutant (S1A/E4Q) was prepared and expressed in Escherichia coli. Immunoblotting of the bacterial lysate showed that the mutant was synthesized at a low level not exceeding 0.5% of total cell protein. Analysis of the potential secondary structure of the mutant mRNA in the translation initiation region suggested that the Ala1 (GCC) and Leu2 (CUG) codons used are likely to be involved in a hairpin

structure with the Thr13 (ACG) and Gly14 (GGG) codons, hindering effective translation at the ribosome. To weaken this structure (by [Delta]G of about 20 kJ/mol) the same double mutant was prepared using another mutagenic oligonucleotide with silent mutations in the Ala1 (GCU) and Leu2 (UUG) codons. The mutant was successfully produced at a level of approximately 15% of total protein, with the initial Met completely removed in the bacterial cell. Such an approach could be important in solving similar problems in bacterial production of other toxic proteins.

Lima, C., P. Bianca Clissa, et al. (2003). "Characterisation of local inflammatory response induced by Thalassophryne nattereri fish venom in a mouse model of tissue injury." Toxicon **42**(5): 499.

http://www.sciencedirect.com/science/article/B6TCS-49HMS1P-1/2/1204fc67d1441720fc61488796a69425

The Thalassophryne nattereri fish venom induces a severe burning pain, oedema, and necrosis observed both clinically and experimentally. The present study was carried out in order to describe the pattern of local acute inflammatory response after T. nattereri venom injection. Our findings show that the edematogenic response induced by T. nattereri venom in footpad of mice was dose- and time dependent, and remained significantly elevated over 48 h after injection. Analysis of footpad homogenates were tested for the presence of TNF-[alpha], IL-1[beta] and IL-6, and demonstrated augmented levels of these cytokines. Our results showed that the injection of venom developed an inadequate cellular inflammatory response evidenced by poor infiltration of mononuclear cells, preceded by decreased number of these cells in peripheral blood. In contrast, we observed an early intense recruitment of neutrophil to peritoneal cavity, accompanied by a significant decrease in the number of mononuclear cells. A drastic increase in the total amount of cells, mainly in neutrophils, followed by mononuclear cell recruitment was observed 24 h. In addition, we also demonstrated that T. nattereri venom affects the viability of mononuclear cells (J774A1) in culture. We conclude that the scarcity of inflammatory cellular influx into local lesions (intraplantar) induced by T. nattereri venom could be a consequence of an impaired blood flow in venules at injured tissue and cytotoxic effect of the venom on inflammatory cells can contribute to this impairment.

Transactions of the Royal Society of Tropical Medicine and Hygiene (8)

Brown, A. E., K. C. Kain, et al. (1992). "Demonstration by the polymerase chain reaction of mixed Plasmodium falciparum and P. vivax infections undetected by conventional microscopy." Transactions of the Royal Society of Tropical Medicine and Hygiene **86**(6): 609.

http://www.sciencedirect.com/science/article/B75GP-4C0DW4J-26P/2/adab96c3a385f65ecfb3488c9d224ecb

Mixed malaria infections (Plasmodium falciparum and P. vivax) are suspected to occur at a greater frequency than is detected by conventional light microscopy. To determine this frequency we carried out a prospective 'blinded' comparison of diagnosis by conventional light microscopy and enzymatic amplification of the circumsporozoite gene extracted from dried spotted blood samples. Patients were previously healthy, active duty Thai soldiers assigned to a malaria risk area presenting with malaria. Microscopy (oil immersion objective at 1000 x magnification) involved examination of Giemsa-stained thick and thin blood films by an experienced

microscopist. Whole blood samples (25 [mu]l) dried on filter paper were used for species-specific parasite deoxyribonueleic acid (DNA) amplification by the polymerase chain reaction (PCR) and hybridization with radiolabelled P. falciparum and P. vivax probes. Of 137 Consecutive cases of malaria studied, 9% (3/32) of microscopically diagnosed P. falciparum infections and 5% (5/104) of microscopically diagnosed P. vivax infections were found to be mixed by the PCR/DNA probe systems, While 1 Case was diagnosed as mixed by both microscopy and PCR. The possibility that malaria patients may have undetected mixed infections should be kept in mind because of the specific therapy required both for P. falciparum and for radical cure of P. vivax.

Checchi, F., R. Durand, et al. (2002). "High Plasmodium falciparum resistance to chloroquine and sulfadoxine-pyrimethamine in Harper, Liberia: results in vivo and analysis of point mutations." Transactions of the Royal Society of Tropical Medicine and Hygiene **96**(6): 664.

http://www.sciencedirect.com/science/article/B75GP-4DKTH96-GK/2/0600cc8c157753876fcd336255bf667b

In Liberia, little information is available on the efficacy of antimalarials against Plasmodium falciparum malaria. We measured parasitological resistance to chloroquine and sulfadoxine-pyrimethamine (SP) in Harper, south-west Liberia in a 28-d study in vivo. A total of 50 patients completed follow-up in the chloroquine group, and 66 in the SP group. The chloroquine failure rate was 74.0% (95% confidence interval [95% CI] 59.7-85.4%) after 14 d of follow-up and 84.0% (95% CI 70.9-92.8%) after 28 d (no polymerase chain reaction [PCR] analysis was performed to detect reinfections in this group). In the SP group, the failure rate was 48.5% (95% CI 36.2-61.0%) after 14 d and 69.7% (95% CI 57.1-80.4%) after 28 d, readjusted to 51.5% (95% CI 38.9-64.0%) after taking into account reinfections detected by PCR. Genomic analysis of parasite isolates was also performed to look for point mutations associated with resistance. Genotyping of parasite isolates revealed that all carried chloroquine-resistant K-76T mutations at gene pfcrt, whereas the triple mutation (S108N, N511, C59R) at dhfr and the A437G mutation at dhps, both associated with resistance to SP, were present in 84% and 79% of pretreatment isolates respectively. These results seriously question the continued use of chloroquine and SP in Harper and highlight the urgency of making alternative antimalarial therapies available. Our study confirms that resistance to chloroquine may be high in Liberia and yields hitherto missing information on SP.

Chen, S. C. A., B. J. Currie, et al. (1997). "Cryptococcus neoformans var. gattii infection in northern Australia: existence of an environmental source other than known host eucalypts." Transactions of the Royal Society of Tropical Medicine and Hygiene **91**(5): 547.

http://www.sciencedirect.com/science/article/B75GP-4BY314W-8G/2/8789e2d32136f97178d1d223c830e62d

The 2 known host trees of Cryptococcus neoformans var. gattii, Eucalyptus camaldulensis and E. tereticornis, do not occur naturally in the 'Top End' of the Northern Territory (NT) of Australia. Nine clinical isolates of C. neoformans var. gattii from the NT were analysed by random amplification of polymorphic deoxyribonucleic acid and polymerase chain reaction 'fingerprinting'.Two isolates were assigned to profile VGI, previously established as the common RAPD profile. The remaining 7 were assigned to profile VGII; 6 of these isolates were recovered from individuals living in the 'Top End'. The results strongly support the existence of an alternative environmental niche for C. neoformans var. gattii, as all isolates from Eucalyptus spp. in Australia to date have been of profile VGI.

Farnert, A., A. P. Arez, et al. (1999). "Sampling and storage of blood and the detection of malaria parasites by polymerase chain reaction." Transactions of the Royal Society of Tropical Medicine and Hygiene **93**(1): 50.

http://www.sciencedirect.com/science/article/B75GP-4D33HMY-2H/2/bbde7e340d57e65e76435b3ddb51af87

Polymerase chain reaction (PCR) is now widely used in malaria research for analysis of field samples. However, little has been reported regarding loss of sensitivity due to field methodology. Therefore, studies were carried out in relation to blood sampling (anticoagulants, culture medium, filter paper), storage (temperature, time and immediate lysis) and handling (repeated thawing and freezing). The PCR was unaffected by citrate and EDTA but partly inhibited by heparin (inhibition was reversed by heparinase at optimal concentrations). Samples collected on filter paper showed a significant 100-fold lower sensitivity (compared to control samples frozen immediately after collection) when stored at 30 [deg]C and 60% humidity; and the paper quality appeared to be critical. Storage of unprocessed whole blood at 4 [deg]C, 20 [deg]C or 30 [deg]C rarely resulted in any loss of sensitivity. Repeated thawing generally resulted in 10-fold loss of sensitivity compared to blood kept frozen until DNA extraction. The presence of antimalarial drug did not apparently affect sensitivity. We conclude that the mode of collection and storage of blood samples may influence the sensitivity of detection of malaria parasites by PCR. This may be critical in studies including individuals with low parasitaemia, mixed infections and comparison of data from different settings.

Fernandas, O., S. S. Santos, et al. (2001). "A mini-exon multiplex polymerase chain reaction to distinguish the major groups of Trypanosoma cruzi and T. rangeli in the Brazilian Amazon." Transactions of the Royal Society of Tropical Medicine and Hygiene **95**(1): 97.

http://www.sciencedirect.com/science/article/B75GP-4C41S0H-2V/2/f5ad6d83e144a5a361660e3fe42c8554

Kane, R. A., J. Bartley, et al. (2002). "Application of single strand conformational polymorphism (SSCP) analysis with fluorescent primers for differentiation of Schistosoma haematobium group species." Transactions of the Royal Society of Tropical Medicine and Hygiene **96**(Supplement 1): S235.

http://www.sciencedirect.com/science/article/B75GP-4BY30RJ-1D/2/84efd49314b26afdf7cb7fbf214139f3

To assess the utility of single-stranded conformational polymorphism (SSCP) analysis for the differentiation of schistosomes, using methods adapted for a Perkin Elmer ABI Prism 377(TM) automated sequencer, 3 isolates of Schistosoma haematobium, 2 of S. intercalatum and single isolates of S. curassoni and S. bovis were selected for study. Two fluorescently labelled, double-stranded polymerase chain reaction products, amplified from the mitochondrial cytochrome oxidase subunit 1 (CO1) gene and the nuclear ribosomal second internal transcribed spacer (ITS2), were generated from single male and female worms. Changes in electrophoretic mobility of fragments within an SSCP profile revealed variation at individual, isolate and species levels. The mutational basis between representative SSCP profiles was confirmed by direct sequencing, demonstrating that single point substitutions were detectable. SSCP analysis has considerable potential as an alternative molecular method of identification and characterization of schistosomes. More broadly, fluorescence-based SSCP analysis is applicable to almost any gene

target from any species of parasite and is a powerful molecular tool for genetic profiling.

Ramirez, J., S. Agudelo, et al. (2002). "The method used to sample ulcers influences the diagnosis of cutaneous leishmaniasis." Transactions of the Royal Society of Tropical Medicine and Hygiene **96**(Supplement 1): S169.

http://www.sciencedirect.com/science/article/B75GP-4BY30RJ-11/2/0010d4b3d390b8be4c69468529104c73

Before beginning treatment for cutaneous leishmaniasis, parasitological confirmation of the disease is required. The most commonly used diagnostic procedures are microscopy and culture of samples taken from the active edge of the lesion. In this study, we compared the sensitivity of previous diagnostic procedures with the polymerase chain reaction (PCR), using smears taken from the edge of the lesion and its centre. The sensitivity was greater with smears taken from the centre of the lesion, both for microscopical examination (85%) and for PCR (81%), compared to those obtained from the edge of the lesion (69% and 58% respectively). When PCR was carried out on biopsy material from the edge of the lesion the sensitivity was 63%.

Zhang, L., R. B. Gasser, et al. (1999). "Screening for different genotypes of Echinococcus granulosus within China and Argentina by single-strand conformation polymorphism (SSCP) analysis." Transactions of the Royal Society of Tropical Medicine and Hygiene **93**(3): 329.

http://www.sciencedirect.com/science/article/B75GP-4D33HTG-50/2/c09c32004f9d8437647d735daa482b76

Single-strand conformation polymorphism (SSCP) analysis was employed for the direct visual display of genetic variability in mitochondrial DNA (mtDNA) fragments within and among populations of Echinococcus granulosus from the People's Republic of China and from Argentina. Fragments of the NADH dehydrogenase I gene (NDI) and the cytochrome c oxidase subunit I (COI) were individually amplified from parasite DNA by polymerase chain reaction, denatured and subjected to SSCP analysis. Using NDI and COI fragments, samples representing different genotypes could be readily identified based on characteristic SSCP profiles. The results demonstrate the utility of SSCP for the direct visual display of nucleotide variation in mtDNA of E. granulosus prior to DNA sequence analysis. The approach compares favourably with existing genotyping procedures and provides a reliable and technically reproducible method for the routine laboratory identification of Echinococcus isolates.

Trends in Genetics (2)

Czank, A. (1996). "One-tube direct PCR from whole Drosophila melanogaster adults." Trends in Genetics **12**(11): 457.

http://www.sciencedirect.com/science/article/B6TCY-4C89G52-D/2/4caa610d0886ded94e7e6386a1ba5188

Illuxley, C., E. D. Green, et al. (1990). "Rapid assessment of S. cerevisiae mating type by PCR." Trends in Genetics **6**: 236.

http://www.sciencedirect.com/science/article/B6TCY-47DVCJN-6W/2/7a3fced38382e85a1427fa1c35366ec1

Vet Rec. (1)

Camenisch, U., Z. H. Lu, et al. (2004). "Diagnostic investigation into the role of Chlamydiae in cases of increased rates of return to oestrus in pigs." Vet Rec. **155**(19): 593-596.

http://veterinaryrecord.bvapublications.com/cgi/content/abstract/155/19/593

Cervical swabs and serum samples were taken from Swiss herds of sows with high rates of irregular return to oestrus (group A) and from control herds without reproductive problems (group B. The genital tracts of 21 slaughtered sows of group A were also examined. The swabs and genital tracts were screened for Chlamydiae by a new 16S rRNA PCR and the sera by an ELISA for Chlamydiaceae lipopolysaccharide. Chlamydophila (Cp) abortus was isolated from seven of the 65 swabs taken from group A but from none of the 128 swabs taken from group B. Chlamydia suis was present in swabs from both groups A (1.5 per cent) and B (2.3 per cent). In addition, Cp abortus was detected in 33.3 per cent of the genital tracts. Of the 193 sera tested, 61.7 per cent were positive, with no significant difference between group A (52.3 per cent) and group B (66.4 per cent). Chlamydia-like organisms were detected in 28.2 per cent of the swabs from group A and in 22 per cent of those from group B.

Vet. Pathol. (4)

Foreman, O., J. Sykes, et al. (2004). "Disseminated Infection with Balamuthia mandrillaris in a Dog." Vet. Pathol. **41**(5): 506-510.

http://www.vetpathology.org/cgi/content/abstract/41/5/506

Bilateral chronic granulomatous nephritis and meningoencephalitis were diagnosed on necropsy of a 2-year-old male Great Dane dog. The causative agent was identified as Balamuthia mandrillaris, based on morphologic features, immunohistochemical staining, and deoxyribonucleic acid detection using the polymerase chain reaction with newly designed primer pairs. Trophozoite and cystic forms of the amoeba were evident within the kidneys and brain parenchyma. This is the first report on a B. mandrillaris infection in a dog.

Huntley, J. F. J., R. H. Whitlock, et al. (2005). "Comparison of Diagnostic Detection Methods for Mycobacterium avium subsp. paratuberculosis in North American Bison." Vet. Pathol. **42**(1): 42-51.

http://www.vetpathology.org/cgi/content/abstract/42/1/42

Tissues and fecal material were collected from 14 North American bison (Bison bison) that were suspected of having Johne's disease and analyzed for the presence of Mycobacterium avium subsp. paratuberculosis (M. paratuberculosis). Sections of ileum, ileal-cecal lymph node, and three sequential sections of jejunum with their associated mesenteric lymph nodes were taken from each animal. Fecal culture indicated that 5 of 14 (35.7%) animals were infected, whereas cultures from tissues detected 12 of 14 (85.7%) animals as infected and 59 of 111 (53.2%) of the tissues as positive for M. paratuberculosis. Polymerase chain reaction analysis identified infection in 14 of 14 (100%) animals and in 91 of 112 (81.2%) tissues. In addition, tissues were processed for Ziehl-Neelsen acid-fast staining, auramine O/acridine orange fluorescent staining, and immunohistochemical staining. Ziehl-Neelsen and auramine O staining identified 7 of 14 (50%) and 5 of 14 (35.7%) animals as infected and 24 of 112 (21.4%) and 28 of 112 (25%) tissues as positive, respectively. Immunohistochemical analyses of bison tissues, using antisera collected from rabbits immunized with four different preparations of M. paratuberculosis, identified a greater percentage of infected animals (ranging from 57 to 93%) and positive tissues (ranging from 28 to 46%). Collectively, these data indicate that DNA-based detection of M. paratuberculosis was more sensitive than bacterial culture or staining, identified infection in all the bison, and detected the greatest number of positive tissues within each animal.

Kramer, J. W., P. J. Venta, et al. (2004). "A von Willebrand's Factor Genomic Nucleotide Variant and Polymerase Chain Reaction Diagnostic Test Associated with Inheritable Type-2 von Willebrand's Disease in a Line of German Shorthaired Pointer Dogs." Vet. Pathol. **41**(3): 221-228.

http://www.vetpathology.org/cgi/content/abstract/41/3/221

Heritable, type-2 von Willebrand's disease (vWD) was studied in a line of German Shorthaired Pointers (GSPs) in which some members had a nucleotide variant in exon 28 of the von Willebrand factor (VWF) gene. A polymerase chain reaction (PCR) diagnostic test for the nucleotide variant was developed to establish the disorder's mode of inheritance and to eliminate it from the line. Thirty-six of the 49 GSPs in the line, 14 unrelated GSP controls, and 71 unrelated dogs of various breeds were tested for the presence of the variant nucleotide. All the dogs with a vWF antigen deficiency (<70% of normal) were either homozygous or heterozygous for the nucleotide variant. The variant was not located in any tested dog in the line or outside of the line with a vWF antigen value greater than 68%. Of the GSPs in the line tested, two were homozygous for the variant, 15 were heterozygous, and 19 were variant free. The collective evidence of this and other studies is consistent with the variant nucleotide being the cause of the type-2 vWD in this line of GSPs and German Wirehaired Pointers. The PCR diagnostic test for the variant nucleotide was successfully used to select and produce progeny that were variant free and vWD free. This test should be effective in the subsequent elimination of this same variant from other lines of dogs.

Weiss, D. J., O. A. Evanson, et al. (2004). "Gene Expression and Antimicrobial Activity of Bovine Macrophages in Response to Mycobacterium avium subsp. paratuberculosis." Vet. Pathol. **41**(4): 326-337.

http://www.vetpathology.org/cgi/content/abstract/41/4/326

We evaluated gene expression and antimicrobial responses of bovine monocyte-derived macrophages incubated with Mycobacterium avium subsp. paratuberculosis (M. a. ptb), the causative agent of Johne's disease. Gene expression was evaluated by the use of human noncompetitive high-density oligonucleotide microarrays. Bovine messenger RNA hybridized with 14.2-18.2% of the 12,600 oligonucleotide probe sets. When macrophages incubated with M. a. ptb were compared with nonactivated control macrophages, macrophages activated by addition of interferon-{gamma} and lipopolysaccharide, and macrophages incubated with Mycobacterium avium subspecies avium (M. a. a), 47, 79, and 27 genes, respectively, were differentially expressed. Differential expression of six of these genes was confirmed using reverse transcriptase polymerase chain reaction. Several functional assays were performed to evaluate the potential relevance of differentially expressed genes to host defense. Macrophages phagocytizing M. a. a had a greater capacity to kill the organisms and to acidify phagosomes and a greater degree of apoptosis than did macrophages incubated with M. a. ptb. The results of these studies indicate that multiple genes and metabolic pathways are differentially expressed by macrophages ingesting mycobacterial organisms. Although the intracellular fate of mycobacterial organisms appears to be dependent on a complex interaction between macrophage and organism, phagosome acidification and apoptosis may play central roles in organism survival.

Veterinary Immunology and Immunopathology (51)

Abdalla, S. A., H. Horiuchi, et al. (2004). "Molecular study on chicken tumor necrosis factor receptor-II and tumor necrosis factor receptor-associated factor-5." Veterinary Immunology and Immunopathology **98**(1-2): 31.

http://www.sciencedirect.com/science/article/B6TD5-4BBHBMW-2/2/08c9b850e658a6209668819994711ddc

Tumor necrosis factor (TNF) receptor-associated factors (TRAFs) were identified as signal transducers for the tumor necrosis factor receptor (TNFR) superfamily. In this study, we cloned and characterized two genes that encode chicken TNFR-II and TRAF5. The initial cDNA fragments were obtained by suppressive subtractive hybridization (SSH) of chicken spleen cells with or without lipopolysaccharide stimulation (Salmonella typhimurium SL1181 (RE-mutant)). The results showed that chicken TNFR-II is 1518 bp in length with an open reading frame (ORF) of 1386 bp having 31% homology with human TNFR-II. Expression analysis of chicken TNFR-II revealed that it is highly expressed in the spleen and bursa of Fabricius. The chicken cell lines IN24, MSB1 and 1104B express TNFR-II abundantly. The time course analysis of expression in spleen, bursa of Fabricius and IN24 cell line showed that TNFR-II is maximally expressed at 6 h after stimulation in bursa of Fabricius and after 8 h stimulation in the IN24 cell line. With regard to TRAF5, the complete sequence was 1936 bp in length with an ORF of 1671 bp that showed 71.3% homology with human TRAF5. Expression analysis showed that, among the tissues examined, TRAF5 was strongly expressed in spleen and bursa of Fabricius, while among the cell lines examined, it was maximally expressed in IN24. Thus, both genes were expressed in the same tissues and cell line among examined materials. These results suggest that chicken TNFR-II may interact with TRAF5 adaptor protein to complete its signal transduction pathway.

Barnes, A., A. Bee, et al. (2000). "Immunological and inflammatory characterisation of three canine cell lines: K1, K6 and DH82." Veterinary Immunology and Immunopathology **75**(1-2): 9.

http://www.sciencedirect.com/science/article/B6TD5-40NMSK9-2/2/6a7b6e0c5d2dbcdb42c3545d8cb10012

Three canine cell lines, K1, K6 and DH82, derived from canine malignant neoplasms, were characterised. They were examined for expression of surface antigens, cytokines, neuropeptide receptors, matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs). The growth characteristics of the cell lines were established and bioassays used to detect production of TNF-[alpha], IL-1 and IL-6. In the DH82 cell line, production of TNF-[alpha] and IL-6 was readily detected. Neither K1 or K6 cell lines produced any measurable amounts of TNF-[alpha], IL-1 or IL-6. At a molecular level, using reverse transcription-polymerase chain reaction (RT-PCR) to detect specific mRNA, the DH82 cell line expressed TNF-[alpha], IL-1 and IL-6, whereas the K1 and K6 cell lines expressed TNF-[alpha]. Canine IL-5, IL-8 and IL-10 mRNA were detected in the DH82 cell line but only IL-5 and IL-8 mRNA were detected in the K1 and K6 cell lines. Gelatin zymography was used for the detection of MMP-2 and MMP-9 and all three cell lines produced MMP-2 but only the DH82 cell line produced MMP-9. Reverse zymography was used to detect TIMP-1 and TIMP-2 and all three cell lines produced both proteins. The presence of these MMPs and TIMPs was confirmed at a molecular level using RT-PCR. Canine MMP-14 mRNA was detected in all three cell lines. For this investigation several genes for canine inflammatory molecules were cloned and sequenced for molecular detection; these included IL-1, IL-6, IL-8, TNF-[alpha], MMP-9, MMP-14, TIMP-1, TIMP-2 and [beta]-actin. Of all the cell surface antigens tested, only CD14 was expressed on the DH82 cell line although CD5 and CD45 was partially expressed. The K1 and K6 cell lines were negative for all of the CD markers tested. K1 and K6 were negative for Neurokinin 1 receptor (NK1-R) but positive for Calcitonin gene related peptide receptor type 1 (CGRP-1R) and Calcitonin gene related peptide receptor component protein (CGRP-RCP). The DH82 cell line expressed neither NK1-R or CGRP-1R; however, it did express CGRP-RCP. Generally the DH82 cell line exhibited considerable similarity to canine monocytes, but all three cell lines will be useful as standards and for the purification of various immunological and inflammatory mediators in the dog.

Beckman, M. J., J. J. Rejman, et al. (1999). "cDNA cloning and gene expression of the type 1 bovine interleukin-1 receptor." Veterinary Immunology and Immunopathology **71**(3-4): 245.

http://www.sciencedirect.com/science/article/B6TD5-3Y9HGF9-8/2/5bd66a91b390aecc1b03eed22a620615

Regulation of interleukin-1 (IL-1) mediated biological responses is complicated by the multiple ligands and receptors of the IL-1 family. Most studies of IL-1 receptors have used human or rodent cells. Here, we report that the coding region of the bovine type 1 interleukin-1 receptor (type 1 IL-1R) cDNA extends 1719 bp in length. Northern analysis of specific bovine cell and tissue RNA demonstrated a 4.5 kb transcript. Overall, the bovine type 1 IL-1R coding region exhibits approximately 81 and 76% similarity with the human type 1 IL-1R at the nucleotide and amino acid level, respectively, and somewhat less similarity with the mouse and rat sequences. Type 1 IL-1R transcripts were confirmed by RT-PCR in several bovine cell types, including peripheral blood mononuclear cells (PBMCs), neutrophils (PMNs), and fibroblast, peritoneal macrophage, and arterial endothelial cell lines. It is expected that molecular clones for the bovine type 1 and 2 IL-1 receptors will provide us with the tools needed to decipher species-and cell-specific regulation of IL-1 action in the bovine.

Bull, M. E., T. W. Vahlenkamp, et al. (2004). "Spontaneous T cell apoptosis in feline immunodeficiency virus (FIV)-infected cats is inhibited by IL2 and anti-B7.1 antibodies." Veterinary Immunology and Immunopathology **99**(1-2): 25.

http://www.sciencedirect.com/science/article/B6TD5-4C0V78C-1/2/574de5538a6093ef3f05765aef3a4401

Lymph node (LN) T cells from feline immunodeficiency virus (FIV)-infected cats have an increased expression of B7 co-stimulatory molecules as well as their ligand CTLA4, resembling an activation phenotype shown to induce anergy and apoptosis in activated T cells. In addition, LN T cells from FIV-infected cats also show increased spontaneous apoptosis compared to uninfected animals. The apoptosis observed in these animals occurs primarily in T cells expressing B7 and CTLA4, suggesting a role for B7 and CTLA4 interactions in the induction of anergy/apoptosis. In order to investigate the role of B7 and CTLA4 interactions on T cell apoptosis in LN T cells from FIV-infected cats, we performed blocking experiments by measuring T cell apoptosis in LN T cell cultures treated with anti-feline B7.1, B7.2, and CTLA4 specific antibodies, as well as interleukin (IL)-2. The addition of IL2, the primary cytokine produced by B7/CD28 interactions, resulted in a significant decrease of T cell apoptosis in cultured LN cells as assessed by two-color flow cytometry and TUNEL assay. The addition of anti-B7.1 antibodies significantly inhibited T cell apoptosis in FIV-infected cats with low-level plasma viremia, while addition of anti-B7.2 and anti-CTLA4 antibodies had no affect. These results suggest a role of B7 signaling in the increased spontaneous apoptosis observed in LN T cells from FIV-infected animals.

Byrne, K. M., W. C. Davis, et al. (1997). "Cytokine RNA expression in an equine CD4 + subset differentiated by expression of a novel 46-kDa surface protein." Veterinary Immunology and Immunopathology **56**(3-4): 191.

http://www.sciencedirect.com/science/article/B6TD5-3RH6HB7-1/2/ad9fc0aebd4ca2834fc28346076b7bbf

Two monoclonal antibodies (MAb), HB65A (IgG2a) and HB86A (IgGI), recognize a unique cell surface molecule on equine T-lymphocytes. The molecule, designated EqWC4, identified by these MAbs is present on a subpopulation of CD4+ equine lymphocytes (6.3-10.2% of Arabian lymphocytes CD4+ WC4+) and a smaller population of CD8+ lymphocytes (0.5% to 1.2% of Arabian lymphocytes CD8+ WC4+). EqWC4 is absent from B-lymphocytes, granulocytes, and macrophages. Both MAbs bound to a 46-kDa protein following immunoprecipitation reactions with lysates of surface labeled thymocytes. Immunoaffinity purification using HB65A yielded two molecules of 46 kDa and 52 kDa under reducing conditions and a third 92-kDa molecule was present in nonreduced conditions. Activation by mitogen did not increase expression of EqWC4 on equine lymphocytes. Lymphocytes from Arabian, Pony, and Thoroughbred breeds showed a common distribution of EqWC4 among leukocytes. However, there were significantly fewer Pony lymphocytes bound to HB65A and HB86A when compared to Arabian and Thoroughbred breeds. Using reverse transcriptase-polymerase chain reaction (RT-PCR), magnetically enriched populations (to 80% of cells isolated) of EqWC4+ lymphocytes expressed a cytokine RNA profile dominated by -interleukin2 (IL-2) and interferon-gamma (IFN-[gamma]) for unstimulated cells. Upon mitogen stimulation, IL-4 was also expressed at low levels while the IL-2 levels decreased and the IFN-[gamma] levels increased relative to unstimulated cells. EqWC4 is similar to CD28 in molecular weight and its formation of dimers and could therefore be the equine orthologue. However, because of the differences in CD28 expression, EqWC4 probably represents a previously uncharacterized equine lymphocyte marker.

Clarke, R. A., A. L. Burn, et al. (2001). "Molecular analysis and nematode resistance association of a polymorphism at the 5' end of the sheep IgE gene." Veterinary Immunology and Immunopathology **79**(1-2): 15.

http://www.sciencedirect.com/science/article/B6TD5-430WX9F-2/2/b2ab6a0cf16309f288fa95ba649f51ee

Previous work using Southern analysis of genomic DNA detected a polymorphism at the 5' end of the sheep and cattle IgE gene. Identical length differences found between fragments following digestion with restriction enzymes indicated that the basis for the polymorphism was an insertion/deletion event. To characterise the polymorphism, the entire cattle and sheep C[epsiv] genes were sequenced including 668 bp of 5' untranslated DNA. Sequence comparison revealed a high degree of similarity between the ovine and bovine genes at both the nucleotide and amino acid level. A feature of the 5' untranslated DNA was the presence of an 87 bp repeat starting at -365 upstream of the C[epsiv] start site. PCR primers were designed to span most of the 5' untranslated sequence, including the repeat unit, and used to amplify genomic DNA from a panel of 40 sheep. Three alleles were found with frequencies of 0.7, 0.29, 0.01 which were identical to the Southern analysis results. Sequencing of the two commonest alleles revealed the basis for the polymorphism was a 36 bp deletion from the 87 bp repeat.Association studies in a sheep selection flock phenotypically assessed for parasite resistance found a highly significant association between one of the IgE alleles and resistance to the intestinal nematode parasite Trichostrongylus colubriformis (P=0.005). Attempts to confirm this finding in two other flocks using linkage analysis and genotype association failed to find any significant associations between the IgE polymorphism and resistance to either T. colubriformis or Haemonchus contortus.

Collins, R. A., C. J. Howard, et al. (1999). "Bovine interleukin-12 and modulation of IFN[gamma] production." Veterinary Immunology and Immunopathology **68**(2-4): 193.

http://www.sciencedirect.com/science/article/B6TD5-3WM5540-B/2/0b31ec0454faf4794f423743f8e100b1

The effects of IL-12 on the responses of cattle peripheral blood mononuclear cells (PBMC) to bovine respiratory syncytial virus (BRSV) antigen and ovalbumin (OVA) were tested, in vitro. IL-12 did not affect the proliferative responses of PBMC to these antigens but markedly accelerated and augmented the level of IFN[gamma] secreted. When tested on lymphoblasts rather than resting T-cells IL-12 also enhanced proliferation. In contrast IL-4 and, to greater extent, IL-10 inhibited the response. The effect of IL-12 on IFN[gamma] synthesis was confirmed at the level of IFN[gamma]. mRNA expression using Taqman(R) PCR. CD4 and CD8 T-cell populations produced IFN[gamma], however, CD4 T-cells comprised the largest contributors to the IFN[gamma] production. [gamma]/[delta] T-cells did not contribute markedly. A comparison of the species cross-reactivity showed bovine IL-12 was also active in the human system. This study shows that antigen-driven responses in cattle can be significantly influenced by exogenous cytokines and suggests the IL-12/IL-10 balance is crucial for regulation of IFN[gamma].

Cox, E., J. Mast, et al. (1997). "Expression of [beta]2 integrins on blood leukocytes of cows with or without bovine leukocyte adhesion deficiency." Veterinary Immunology and Immunopathology **58**(3-4): 249.

http://www.sciencedirect.com/science/article/B6TD5-3S130WJ-6/2/dbb15f9346b55701e596bc9bf84065ca

Peripheral blood leukocytes of 11 normal cows, 7 cows heterozygous and 2 heifers homozygous for bovine leukocyte adhesion deficiency (BLAD) were analysed by flow cytometry for the intensity of their [beta]2 integrin expression (LFA-1(CD11a/CD18), CR3 (CD11b/CD18) and CR4 (CD11c/CD18)). BLAD-homozygotes revealed no or a very weak expression of the [beta]2

integrins and had a 10-fold and 4- to 5-fold increase in absolute number of neutrophils and monocytes, respectively, whereas the absolute number of lymphocytes remained normal. The mean fluorescence intensity (MFI) of the [beta]2 integrins (CD18) in heterozygous animals was 56 to 90% of this in the normal cows (MFI between 14 and 512). The difference in the expression level was most pronounced for LFA-1 on the small cluster of lymphocytes with the highest MFI for LFA-1. Repeated analysis and phorbol myristate acetate stimulation revealed that the LFA-1 expression on this high-expressing cell population of the peripheral blood allowed a ready identification of BLAD-heterozygotes by flow cytometry.

Creevy, K. E., J. T. R. Bauer, et al. (2003). "Canine leukocyte adhesion deficiency colony for investigation of novel hematopoietic therapies." Veterinary Immunology and Immunopathology **94**(1-2): 11.

http://www.sciencedirect.com/science/article/B6TD5-48M7X5H-1/2/3a6ffc85399c98e668c7ee58ad0af856

The genetic immunodeficiency disease canine leukocyte adhesion deficiency (CLAD) was originally described in juvenile Irish Setters with severe, recurrent bacterial infections. CLAD was subsequently shown to result from a mutation in the leukocyte integrin CD18 subunit which prevents leukocyte surface expression of the CD11/CD18 complex. We describe the development of a mixed-breed CLAD colony with clinical features that closely parallel those described in Irish Setters. We demonstrate that the early identification of CLAD heterozygotes and CLAD-affected dogs by a combination of flow cytometry and DNA sequencing allows the CLAD-affected animals to receive life-saving antibiotic therapy. The distinct clinical phenotype in CLAD, the ability to detect CD18 on the leukocyte surface by flow cytometry, and the history of the canine model in marrow transplantation, enable CLAD to serve as an attractive large-animal model for the investigation of novel hematopoietic stem cell and gene therapy strategies.

Davis, E. G., Y. Sang, et al. (2004). "Equine [beta]-defensin-1: full-length cDNA sequence and tissue expression." Veterinary Immunology and Immunopathology **99**(1-2): 127.

http://www.sciencedirect.com/science/article/B6TD5-4BS0FTF-2/2/443489947d000d8ed0b218aeee1bbb3d

[beta]-Defensins are cysteine-rich endogenously produced antimicrobial peptides that play an important role in innate immune defense. Although, previous investigations have identified [beta]-defensins in several mammalian species, no reports have identified equine [beta]-defensins. Using a strategy of database searching for expressed sequence tags (EST) we identified putative expression of equine [beta]-defensins in hepatic tissue. Based on this information, sequence specific primers were designed for the equine gene enabling the identification of the full-length cDNA sequence of equine [beta]-defensin-1. Comparative analyses showed that equine [beta]-defensin-1 has 46-52% amino-acid identity with other [beta]-defensins, sharing the greatest identity with porcine [beta]-defensin-1. Complete conservation of cysteine residues was maintained between the species evaluated, and RT-PCR analysis revealed diverse mRNA tissue expression for equine [beta]-defensin-1. These data extend the repertoire of equine antimicrobial peptides and expand our understanding of equine innate immunity.

Davis, E. G., Y. Sang, et al. (2005). "Molecular cloning and characterization of equine NK-lysin." Veterinary Immunology and Immunopathology **105**(1-2): 163.

http://www.sciencedirect.com/science/article/B6TD5-4F924G1-2/2/c1463dd522c5c160852179b155703b3e

NK-lysin is an antimicrobial peptide of cytotoxic and NK lymphocytes that has powerful antibacterial properties as well as antitumoral activity. Here we report the full-length cDNA and deduced amino acid sequence for equine NK-lysin. Equine NK-lysin is 67% identical to porcine NK-lysin, 53% identical to bovine NK-lysin and 41% identical to granulysin in amino acid sequence. Complete conservation of cysteine residues between equine, bovine and porcine NK-lysin suggests similar disulfide bonding patterns among these peptides. Equine NK-lysin has the most positive surface charge when compared with other homologues. Similar to expression profiles in other species, equine NK-lysin is constitutively transcribed in various lymphocytes that include CD4+ and CD8+ staining cells. These findings suggest that equine NK-lysin, similar in cDNA sequence to the porcine, bovine and human homologues may play a role in antimicrobial defense.

Endo, Y., Y. Goto, et al. (2000). "Inhibitory effect of stromal cell derived factor-1 on the replication of divergent strains of feline immunodeficiency virus in a feline T-lymphoid cell line." Veterinary Immunology and Immunopathology **74**(3-4): 303.

http://www.sciencedirect.com/science/article/B6TD5-408BJFM-C/2/885fff6f31c0ba8c6a9f4585b9d576b6

The effect of a CXC-chemokine, stromal cell derived factor-1 (SDF-1), on the replication of divergent strains of feline immunodeficiency virus (FIV) was examined in order to identify the mechanism of cell entry of FIV. A chemotaxis assay, using a modified Boyden chamber method, confirmed the biological activity of recombinant human (rh) SDF-1 for a feline T-lymphoid cell line (Kumi-1). The viral replication of FIV, as measured by the reverse transcriptase (RT) activity in the culture supernatant, was significantly suppressed by addition of rhSDF-1 in a dose-dependent manner in Kumi-1 cells. Furthermore, PCR analysis of the FIV proviral genome indicated that the inhibitory effect of rhSDF-1 on the replication of FIV in Kumi-1 cells was due to the inhibitory effect in the early event of replication. The inhibitory effect on viral replication by exogenous rhSDF-1 was shown for four divergent FIV isolates of subtypes A, B, and D in Kumi-1 cells.

Fujiwara, S., S. Yasunaga, et al. (2003). "Cytokine profiles of peripheral blood mononuclear cells from dogs experimentally sensitized to Japanese cedar pollen." Veterinary Immunology and Immunopathology **93**(1-2): 9.

http://www.sciencedirect.com/science/article/B6TD5-48B01MX-3/2/2bb20b2b105d472184cd5e5ba345daf2

Japanese cedar (Cryptomeria japonica, CJ) pollinosis is mediated by type-I hypersensitivity and induces seasonal rhinitis and conjunctivitis in humans. Previous studies showed that dogs could be experimentally sensitized with CJ pollen. In this study, we carried out quantitative analysis of mRNA levels of various cytokines in the peripheral blood mononuclear cells (PBMC) of 12 dogs experimentally sensitized to Japanese cedar pollen. Experimental sensitization was carried out by injection of crude CJ pollen extract with aluminium hydroxide gel. The expression levels of interleukin (IL)-1[beta], IL-2, IL-4, IL-6, IL-8, IL-10, IL-18, interferon (IFN)-[gamma], transforming growth factor (TGF)-[beta]1, and tumor necrosis factor (TNF)-[alpha] mRNAs in the PBMC were quantified using a real-time sequence detection system. In the PBMC tested without culture, the expression levels of IL-8 and TNF-[alpha] mRNAs in experimentally sensitized dogs were significantly higher than those in control dogs. The expression level of IFN-[gamma] mRNA in the

sensitized group was significantly lower than that in the control group. When the PBMCs were cultured in the presence of CJ pollen extract, the level of IL-4 mRNA expression was markedly increased in the PBMC from the experimentally sensitized dogs. In the PBMC stimulated with the CJ pollen extract, the expression level of IL-2 mRNA in the sensitized group was also significantly higher than that in the control group. Our data indicated that a Th2 response and proliferation of PBMC occur in response to the sensitizing antigen in dogs experimentally sensitized with CJ pollen, and revealed the presence of antigen-specific Th2 cells in this canine model. In addition, the expression levels of the mRNAs encoding proinflammatory cytokines were shown to be elevated after CJ pollen sensitization, indicating the activation of monocytes and macrophages.

Giguere, S. and J. F. Prescott (1999). "Quantitation of equine cytokine mRNA expression by reverse transcription-competitive polymerase chain reaction." Veterinary Immunology and Immunopathology **67**(1): 1.

http://www.sciencedirect.com/science/article/B6TD5-3VJ3DKH-1/2/9844aec300dbcab520eb551ccf31e714

A reverse transcription-competitive polymerase chain reaction (RT-cPCR) method was developed to quantitate equine interleukin (IL)-1[alpha], IL-1[beta], IL-2, IL-4, IL-5, IL-6, IL-10, IL-12 p35, IL-12 p40, interferon-[gamma] (INF-[gamma]), tumor necrosis factor-[alpha] (TNF-[alpha]), and [beta]-actin mRNA expression. Using primers based on equine-specific sequences, these cytokines could be detected in concanavalin A-stimulated peripheral blood mononuclear cells. The specificity of the amplified product was confirmed by sequencing. For each cytokine, the assay was made quantitative by generating competitor DNA fragments (mimic) containing the same primer template as a equine cytokine, but differing in size to make them distinguishable on an agarose gel. Serial dilutions of the mimic were added to PCR reactions containing constant amount of equine cDNA. Following gel electrophoresis and ethidium bromide staining, densitometric analysis of the bands corresponding to the target and mimic were used to construct a standard curve from which the amount of target cDNA was derived. Quantitation of IL-6 gene expression from a cDNA sample on four different days gave a coefficient of variation or 6.6%. Sample-to-sample variation in the efficiency of the reverse transcription as well as in the quantity of quality of starting RNA was considerably attenuated by normalizing the results to [beta]-actin mRNA expression used as a house-keeping gene. Small differences (2-fold) in cytokine mRNA expression were reliably detected. The sensitivity and reproducibility of this technique will make it valuable in following changes in equine cytokine gene expression in vitro and in vivo. In addition, the RT-cPCR technique described will have broad applicability for quantitation of cytokine gene expression in other animal species of veterinary interest.

Giguere, S., L. Viel, et al. (2002). "Cytokine induction in pulmonary airways of horses with heaves and effect of therapy with inhaled fluticasone propionate." Veterinary Immunology and Immunopathology **85**(3-4): 147.

http://www.sciencedirect.com/science/article/B6TD5-44SK852-1/2/98134b202f844a8928f61cfbc80f7af1

Work in humans and laboratory animals has identified a central role for cytokines and chemokines in development and persistence of lower airway inflammation. The objectives of this study were to determine interleukin (IL)-1[beta], IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, interferon (IFN)-[gamma] and tumor necrosis factor (TNF)-[alpha] induction in bronchoalveolar lavage (BAL) of control horses and horses with heaves both during remission and exacerbation of the disease, and to determine the effect of therapy with inhaled fluticasone propionate on the cytokine profile of horses with heaves. IL-1[beta] and TNF-[alpha] mRNA expression was significantly higher in

horses with heaves after exposure to moldy hay compared to either values obtained during clinical remission or to healthy controls. IL-8 mRNA expression and protein concentrations were significantly higher in horses with heaves than in controls. Both IL-4 and IFN-[gamma] mRNA expression was increased at various times in heaves-susceptible horses compared to controls. IL-2, IL-5 and IL-10 mRNA expression was not detected in BAL cells of either group. Therapy with inhaled fluticasone propionate after induction of a severe heaves exacerbation resulted in complete resolution of clinical signs, normalization of pulmonary function tests, and significant decrease in BAL neutrophilia. This was associated with a significant decrease in IL-4 mRNA expression and increase in IFN-[gamma]/IL-4 ratio in horses with heaves. These results demonstrate the clinical efficacy of inhaled fluticasone propionate for the treatment of heaves and suggest a role for cytokines in the development of lower airway inflammation in heaves-susceptible horses.

Govaerts, M. M. and B. M. Goddeeris (2001). "Homologues of natural killer cell receptors NKG2-D and NKR-P1 expressed in cattle." Veterinary Immunology and Immunopathology **80**(3-4): 339.

http://www.sciencedirect.com/science/article/B6TD5-43G3056-D/2/17a35e9b335e6f3edbab17856947fe4c

Partial transcripts of the homologues in cattle, of the genes encoding the NKR-P1 and NKG2-D natural killer cell lectin-like receptor families, were cloned by reverse transcriptase-PCR from bovine spleen. Three different cDNAs were partially sequenced for the NKG2-D homologue, and two for the NKR-P1 homologue. Identity to human nucleotide sequences was of 90 and 75%, respectively, and all structural residues of C-type lectin carbohydrate recognition domains were conserved. The identification of two of its members allows to hypothesise the existence of a bovine NK gene complex, prospectively located on chromosome 5.

Grell, S. N., T. Kirsten, et al. (2005). "Marked induction of IL-6, haptoglobin and IFN[gamma] following experimental BRSV infection in young calves." Veterinary Immunology and Immunopathology **103**(3-4): 235.

http://www.sciencedirect.com/science/article/B6TD5-4DS805H-2/2/6c63e804bf4ef095359b0ea360a67dbc

Bovine respiratory syncytial virus (BRSV) has been identified worldwide as an important pathogen associated with acute respiratory disease in calves. An infection model has been developed reflecting accurately the clinical course and the development of pathological signs during a natural BRSV-infection. In the experiments described in the present study, calves were infected at 13-21 weeks of age and reinfected 14 weeks later. Blood samples from the entire infection period were analysed for acute phase protein (haptoglobin) by ELISA and for expression (mRNA level in peripheral blood mononuclear cells) of the cytokines interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-6 (IL-6) and interferon-[gamma] (IFN[gamma]) by quantitative real-time reverse transcribed polymerase chain reaction (RT-PCR). IFN[gamma], interleukin-6 and haptoglobin were markedly induced together with development of clinical signs in response to the first infection with BRSV. The IFN[gamma] response was biphasic, with an early peak at day 1-3 post infection (p.i.) and a later increase between day 5 and 8 p.i. Reinfection also resulted in an induction of IFN[gamma], but without induction of clinical signs, IL-6 and haptoglobin. These results indicate that early mediators connected with the innate responses are induced on a first encounter with the pathogen, but not on a second encounter (reinfection) where the adaptive immune system may act as the first line defence.

Gunn-Moore, D. A., S. M. A. Caney, et al. (1998). "Antibody and cytokine responses in kittens during the development of feline infectious peritonitis (FIP)." Veterinary Immunology and Immunopathology **65**(2-4): 221.

http://www.sciencedirect.com/science/article/B6TD5-3V7JFMB-D/2/57480a2e56d750bb2d3bbccd90363840

Two recombinant FIPV spike proteins were assessed for their immunogenic properties in 8-week-old kittens, which were then challenged intranasally with FIPV 79-1146. Humoral responses were assessed by ELISA and serum neutralisation test. Changes in PBMC cytokine mRNA levels were detected by a reverse transcription, semiquantitative polymerase chain reaction assay (RT-sqPCR), assessing IL-2, IL-4, IL-6, IL-10, IL-12 and IFN[gamma]. All of the kittens developed clinical signs typical of FIP, which were confirmed on gross post mortem examination. The recombinant proteins induced little or no specific antibody response prior to challenge, and failed to alter the course of disease compared to controls. One week after virus challenge, the stimulated PBMCs showed small increases in the expression of IL-6 and IFN[gamma] mRNA, which correlated with a transient pyrexia. After this time expression of IL-6 mRNA remained unaltered but, as FIP developed, mRNA levels of IL-2, IL-4, IL-10, IL-12 and IFN[gamma] became markedly depressed.

Hutchinson, L. E., M. G. Stevens, et al. (1994). "Cloning bovine cytokine cDNA fragments and measuring bovine cytokine mRNA using the reverse transcription-polymerase chain reaction." Veterinary Immunology and Immunopathology **44**(1): 13.

http://www.sciencedirect.com/science/article/B6TD5-47DD8FF-C/2/6f2ef730d3065d403bdb512c345df5df

Bovine cytokine-specific primers and the reverse transcription-polymerase chain reaction (RT-PCR) were used to clone cDNA fragments that were specific for bovine IL-1[alpha], IL-1[beta], IL-2, and IFN-[gamma]. Specificity of the cDNA fragments was verified by sequence analysis based on known bovine IL-1[alpha], IL-1[beta], IL-2, and IFN-[gamma] gene sequences. In addition, RT-PCR was used to monitor cytokine mRNA expression in concanavalin A (Con A) and lipopolysaccharide (LPS)-stimulated bovine peripheral blood mononuclear cells (PBMC), and the results were compared with those obtained by measuring PBMC cytokine secretion using biologic assays. IL-1 activity in LPS-stimulated PBMC cultures was similar at 12 h and 24 h, although the activity decreased by approximately 40% at 48 h. IL-2 and IFN-[gamma] activity in supernatants of Con A-stimulated PBMC cultures was low at 12 h and reached maximum levels at 48 h. RT-PCR transcript analysis detected an increase in IL-1[alpha], IL-1[beta], IL-2, and IFN-[gamma] mRNA expression that was usually correlated with the detection of these soluble cytokines by the bioassays. These results indicate that RT-PCR is a sensitive and effective method of obtaining cDNA probes and that this technique can be used to monitor bovine cytokine mRNA expression.

Ishizaka, T., A. Setoguchi, et al. (2001). "Molecular cloning of feline interferon-[gamma]-inducing factor (interleukin-18) and its expression in various tissues." Veterinary Immunology and Immunopathology **79**(3-4): 209.

http://www.sciencedirect.com/science/article/B6TD5-435CRHC-5/2/b409880bb322e3dbedc6be58f44ce93a

Interleukin-18 (IL-18) is a cytokine with potent interferon-[gamma]-inducing activity, and plays an important biologic role in the enhancement of the activity of natural killer cells and cytotoxic T-lymphocytes. In this study, feline IL-18 cDNA was cloned and characterized to establish a basis for the prospective cytokine therapy in small animal practice. The nucleotide sequence of feline IL-18 cDNA obtained in this study was 712 bp long and contained its entire open reading frame encoding 192 amino acid residues. The predicted amino acid sequence of feline IL-18 cDNA showed 77.2, 84.8, 60.2 and 62.6% similarity with those of human, dog, rat and mouse counterparts, respectively. The feline IL-18 cDNA included a putative cleavage site of IL-1[beta]-converting enzyme (ICE) and IL-1 signature-like sequences identified in human and mouse IL-18 cDNAs. Expression of IL-18 mRNA was detected in various tissues including spleen, liver and cerebrum in the cat.

Johnsen, C. K., A. N. Jensen, et al. (2003). "The porcine skin associated T-cell homing chemokine CCL27: molecular cloning and mRNA expression in piglets infected experimentally with Staphylococcus hyicus." Veterinary Immunology and Immunopathology **96**(1-2): 13.

http://www.sciencedirect.com/science/article/B6TD5-49CT10T-9/2/c511049afccf97e682b053f17b516a67

CCL27 (also named CTACK, ALP, ILC and ESkine) is a CC chemokine primarily expressed by keratinocytes of the skin. The cognate receptor of CCL27 named CCR10 (GPR-2), is also expressed in skin-derived cells, and in addition by a subset of peripheral blood T-cells and in a variety of other tissues. In this paper, we report the cloning of porcine CCL27 cDNA and investigation of CCL27 mRNA expression in Staphylococcus hyicus infected piglets. At the protein level, 77 and 74% homology was found to human and mouse CCL27 sequences, respectively. The results of the expression analyses show that CCL27 mRNA is upregulated in the skin of infected piglets and to a lesser extent in piglets recovered from disease and without clinical signs of infection, indicating a role for CCL27 both during inflammation and after recovery from an infection.

Johnson, C. M., S. Yang, et al. (2004). "Selection of HPRT primers as controls for determination of mRNA expression in dogs by RT-PCR." Veterinary Immunology and Immunopathology **99**(1-2): 47.

http://www.sciencedirect.com/science/article/B6TD5-4C4W519-1/2/b4f831cf5e18d6e3f698284387a297c8

Reliable housekeeping gene controls are critical for measuring and comparing gene expression at the transcription level by Northern blot and RT-PCR. In order to develop such controls for studying cytokine mRNA expression in dogs, DNA sequence encoding a full-length canine HPRT protein has been obtained. Numerous primer pairs derived from the canine HPRT sequence have been tested on canine genomic DNA as well as cDNA. The data from the present study suggest that there may be processed HPRT pseudogenes in dogs. Three pairs of canine HPRT primers designed and tested in the present study were able to differentiate between cDNA and genomic DNA under specific PCR conditions. These primers would be useful controls for measurement of mRNA expression by RT-PCR in the dog.

Khalifeh, M. S. and J. R. Stabel (2004). "Upregulation of transforming growth factor-beta and interleukin-10 in cows with clinical Johne's disease." Veterinary Immunology and Immunopathology **99**(1-2): 39.

http://www.sciencedirect.com/science/article/B6TD5-4C04W24-1/2/6ea54717df13e1e8c33bf291975e8863

Johne's disease progresses through distinct stages including a protracted subclinical stage in which the infection appears to be controlled; followed by a more acute stage in which the host animal demonstrates clinical signs such as diarrhea and weight loss. Little is known about the dynamics of the host immune response during these two phases of disease, however, it is possible that immune modulation in the early stages of disease may play an important role in disease progression. We hypothesized that the clinical stage of Johne's disease is mediated by the expression of cytokines such as transforming growth factor-beta (TGF-[beta]) and interleukin-10 (IL-10) that may be accompanied by the downregulation of IFN-[gamma] gene expression. In the present study, tissue samples were collected from the ileum, ileocecal junction, ileocecal lymph node, and mesenteric lymph nodes of healthy, subclinically or clinically infected cows. The expression of TGF-[beta], IL-10, and IFN-[gamma] genes in these tissues was determined by quantitative competitive RT-PCR. The results demonstrate that TGF-[beta] and IL-10 mRNA levels are higher in cows that have progressed to the clinical stage of disease compared to subclinically infected or healthy cows. In contrast, IFN-[gamma] gene expression was significantly higher in subclinically infected cows. These results suggest that a change in the balance of cytokines at the site of infection may contribute to the ability of the host to control Mycobacterium avium subsp. paratuberculosis infection.

Kipar, A., C. M. Leutenegger, et al. (2001). "Cytokine mRNA levels in isolated feline monocytes." Veterinary Immunology and Immunopathology **78**(3-4): 305.

http://www.sciencedirect.com/science/article/B6TD5-42PBYFW-7/2/166730adc5e0a180c9d50a3792d2b8c0

Real-time PCR systems were developed to quantitate cytokine expression in short-time cultivated feline monocytes. Feline-specific interleukin-1[beta] (IL-1[beta]), IL-6, and tumor necrosis factor-[alpha] (TNF-[alpha]) primers as well as TaqMan probes were designed and were adapted to a quantitative PCR system which had been previously established for feline IL-10 and IL-12 p40. Quantitative analysis of cytokine messenger RNA (mRNA) transcription based on the comparison of the cytokine with the housekeeping gene feline glyceraldehyde-3-phosphate dehydrogenase (GAPDH), providing universally expressed mRNA. GAPDH mRNA was readily detectable in cDNA prepared from short-time cultivated peripheral blood monocytes. Cytokine mRNA was demonstrated in all samples at variable amounts. IL-1[beta] and TNF-[alpha] mRNA was constitutively expressed whereas IL-6, IL-10 and IL-12 p40 mRNA was generally expressed at a lower level and was occasionally not detected. There was a great variability of cytokine production between individual cats and at different time points in the same cat.

Leutenegger, C. M., A. M. Alluwaimi, et al. (2000). "Quantitation of bovine cytokine mRNA in milk cells of healthy cattle by real-time TaqMan(R) polymerase chain reaction." Veterinary Immunology and Immunopathology **77**(3-4): 275.

http://www.sciencedirect.com/science/article/B6TD5-41Y868D-B/2/72d5af9104cfd22fe3cb66b319d357da

Here we present a novel methodology to quantitate bovine cytokines and growth factors contributing to immunity against bacterial infections of the mammary gland in cattle. Real-time TaqMan(R) PCR systems were developed to overcome limitations of conventional quantitative PCR methods. The TaqMan(R) method is based on the cleavage of fluorescent dye-labeled

probes by the 5'-3' exonuclease activity of the Taq DNA polymerase during PCR and measurement of fluorescence intensity by an automated spectrophotometer integrated in a sequence detection system (Applied Biosystems, Foster City, CA). The bovine-specific TaqMan(R) probes were designed to encompass an intron, thus allowing differentiation between complementary DNA (cDNA) and genomic DNA (gDNA) amplification products. Quantitative analysis of cytokine cDNA was performed in comparison to bovine glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Messenger RNA (mRNA) from the universally expressed housekeeping gene GAPDH proved to be useful as an amplification control and allowed for correction of variations in different numbers of cells in the starting material, in the efficiencies of RNA extraction and reverse transcription. With this method, high-throughput analysis of large numbers of samples was possible within a short time. In addition, decreasing the numbers of working steps shortened the time for analysis and increased accuracy. Profiles of cytokines (interleukin (IL)-2, IL-6, IL-8, IL-12 p40, TNF-[alpha], IFN-[gamma]) and granulocyte-macrophage colony stimulating factor (GM-CSF) were established in normal lactating cattle. Differences of cytokine profiles obtained with the real-time TaqMan(R) PCR system and conventional methods are discussed.

Leutenegger, C. M., C. N. Mislin, et al. (1999). "Quantitative real-time PCR for the measurement of feline cytokine mRNA." Veterinary Immunology and Immunopathology **71**(3-4): 291.

http://www.sciencedirect.com/science/article/B6TD5-3Y9HGF9-D/2/f621b091ad022739ce5eafee7e4a20f8

We have developed real-time PCR systems to quantitate feline cytokine gene expression. The method is based on the cleavage of fluorescent dye-labelled probes by the 5'-3' exonuclease activity of the Taq DNA polymerase during PCR and measurement of fluorescence intensity by a Sequence Detection System. The feline-specific TaqMan probes were designed to encompass an intron, thus allowing differentiation of complementary DNA versus genomic DNA amplification products. Quantitative analysis of cytokine cDNA concentrations was performed in comparison to feline GAPDH. Messenger RNA (mRNA) from the universally expressed housekeeping gene GAPDH proved to be useful as an amplification control and allowed for correction of variations in the efficiencies of RNA extraction and reverse transcription. GAPDH mRNAs were readily detectable in cDNAs prepared from unstimulated feline peripheral blood mononuclear cells (PBMCs) and from frozen cell pellets, while cytokines (Interleukin (IL)-4, IL-10, IL-12 p35, IL-12 p40, IFN[gamma], IL-16) were expressed at variable amounts. IFN[gamma] transcription was found to be upregulated in stimulated PBMCs and feline cell lines. The synthesis of cDNA and the performance of the PCR in separate tubes proved to be of superior sensitivity compared to a single-tube based system. The assays described are highly reproducible, require no post-PCR manipulation of the amplicons and permit the analysis of several hundred PCR reactions per day. With this method it is possible to detect and quantify cytokine mRNA expression reliably in small amounts of cells even after storage of samples for at least 5 years.

Ma, Z., T. S. Khatlani, et al. (2001). "Molecular cloning and expression analysis of feline melanoma antigen (MAGE) obtained from a lymphoma cell line." Veterinary Immunology and Immunopathology **83**(3-4): 241.

http://www.sciencedirect.com/science/article/B6TD5-44HXJY5-8/2/a8ccb86768f88ef4e596ab26d738579a

Melanoma antigens (MAGE) are regarded as inducing tumor-specific immune response and thought to be potential therapeutical agents for cancer immunotherapy. We hereby report the cloning of feline MAGE cDNA obtained from a lymphoma cell line derived from cat malignant

lymphoma, and its expression pattern in tumor and normal tissues. The cDNA encoding the MAGE is 1668 base pairs (bp) in length, and contains an open reading frame (ORF) of 936 bp encoding a protein of 311 amino acids. The predicted amino acid sequence has 29-46% of homology with other MAGE proteins from human and mouse. mRNA transcripts for the feline MAGE were detected in certain tumors, but not in adult cat normal tissues except in testis, by reverse transcription polymerase chain reaction (RT-PCR) analysis. This indicates that the expression pattern of feline MAGE mRNA is similar to those of other MAGE family genes in tumors and normal tissues.

Maeda, S., S. Fujiwara, et al. (2002). "Lesional expression of thymus and activation-regulated chemokine in canine atopic dermatitis." Veterinary Immunology and Immunopathology **88**(1-2): 79.

http://www.sciencedirect.com/science/article/B6TD5-460DF4D-1/2/e7e902273a910b512a5a7546fd26b0fc

In this study, we investigated the mRNA expression of a chemokine, thymus and activation-regulated chemokine (TARC), and cytokines including IL-1[beta], IL-4, IFN-[gamma] and TNF-[alpha] in skin samples obtained from both dogs with atopic dermatitis (AD) and healthy dogs. TARC mRNA was found to be selectively expressed in lesional skin of the dogs with AD, but not in non-lesional skin of the dogs with AD or the normal skin of the healthy dogs. The expression levels of IL-1[beta], IFN-[gamma] and TNF-[alpha] in the lesional skin were also significantly higher than those in the non-lesional skin of the dogs with AD. However, IL-4 mRNA was not detected in any of the skin samples in this study. The present results suggest that TARC and inflammatory cytokines such as IL-1[beta], IFN-[gamma] and TNF-[alpha] may play roles in the pathogenesis of canine AD as well as that of human AD.

Mansfield, L. S., J. F. Urban, et al. (1998). "Construction of internal cDNA competitors for measuring IL-10 and IL-12 cytokine gene expression in swine." Veterinary Immunology and Immunopathology **65**(1): 63.

http://www.sciencedirect.com/science/article/B6TD5-3TW96JF-6/2/3972c3216ef2b6dbfe2b58c0889aedad

A competitive PCR assay (cPCR) was used to quantify swine cytokine responses to parasite infection. Internal standards (deleted cDNA competitor molecules [DcDNA mimics]) were produced and tested for swine interleukin-12 (IL-12), interleukin-10 (IL-10) and hypoxanthine phosphoribosyltransferase (HPRT) from PCR generated cDNA cloned in plasmid vectors. Deletion clones for the cDNA competitor molecules (DcDNA mimics) were generated for IL-10, IL-12 and HPRT by PCR in a single step and verified by (1) amplification of the expected smaller PCR product with the original primers, (2) appropriate fragment size released by restriction digestion of the deleted clone, and (3) correct sequence of the new DcDNA insert. DcDNA mimics were used to quantitate cytokine gene mRNA production during experimental and natural infections of swine with the gastrointestinal nematode parasite Trichuris suis. Mesenteric lymph node cells were collected from control and infected pigs at the time of maximal pathogenicity (35 days after infection) and snap frozen. After RNA extraction, samples were reverse transcribed (RT) to cDNA. cPCR was performed using the housekeeping gene HPRT DcDNA mimic and HPRT specific primers to insure RNA integrity and concentration. Cytokine cDNA content in these samples was then quantitated using cytokine mimics and gene specific primers. IL-10 gene expression in MLN draining the colon of pigs experimentally infected with T. suis increased 10-20 fold at day 35 compared to control pigs. IL-12 gene expression was not detectable in MLN of these pigs, but was detectable in MLN of pigs exposed naturally to T. suis on a contaminated dirt lot that also exhibited signs of secondary bacterial invasion. Swine IL-10 and IL-12 gene

expression can be quantitated in local mesenteric tissues. This cPCR assay will enable scientists to quantitate cytokine gene expression in swine and determine the nature of immune responses to important infectious diseases.

Marti, E., G. Szalai, et al. (1995). "Partial sequence of the equine immunoglobulin epsilon heavy chain cDNA." Veterinary Immunology and Immunopathology **47**(3-4): 363.

http://www.sciencedirect.com/science/article/B6TD5-3YXBC9M-H/2/1faedd3a36aab0f3faf63ef129d093bb

In order to isolate a part of the immunoglobulin E (IgE) heavy chain cDNA of the horse, primers have been designed based upon well conserved sequences in humans, sheep and rats. The PCR resulted in a 500 bp fragment which hybridised with a human IgE constant region probe. The fragment was cloned and sequenced and its derived protein sequence compared with the corresponding sequences in humans, sheep and mice. Most amino acids common to these three species are also shared by the horse.

McDonald, T. L., M. A. Larson, et al. (2001). "Elevated extrahepatic expression and secretion of mammary-associated serum amyloid A 3 (M-SAA3) into colostrum." Veterinary Immunology and Immunopathology **83**(3-4): 203.

http://www.sciencedirect.com/science/article/B6TD5-44HXJY5-6/2/b2049b0913fa0030354df1029e360402

Mammary-associated serum amyloid A 3 (M-SAA3) was secreted at highly elevated levels in bovine, equine and ovine colostrum and found at lower levels in milk 4 days postparturition. N-terminal sequencing of the mature M-SAA3 protein from all the three species revealed a conserved four amino acid motif (TFLK) within the first eight residues. This motif has not been reported to be present in any of the hepatically-produced acute phase SAA (A-SAA) isoforms. Cloning of the bovine M-Saa3 cDNA from mammary gland epithelial cells revealed an open reading frame that encoded a precursor protein of 131 amino acids which included an 18 amino acid signal peptide. The predicted 113 residue mature M-SAA3 protein had a theoretical molecular mass of 12,826 Da that corresponded with the observed 12.8 kDa molecular mass obtained for M-SAA3 in immunoblot analysis. The high abundance of this extrahepatically produced SAA3 isoform in the colostrum of healthy animals suggests that M-SAA3 may play an important functional role associated with newborn adaptation to extrauterine life and possibly mammary tissue remodeling.

Menne, S., Y. Wang, et al. (2002). "Real-time polymerase chain reaction assays for leukocyte CD and cytokine mRNAs of the Eastern woodchuck (Marmota monax)." Veterinary Immunology and Immunopathology **87**(1-2): 97.

http://www.sciencedirect.com/science/article/B6TD5-45RFMX0-1/2/b11ecc62cd0715c1f329c5ab06f3ad41

Real-time polymerase chain reaction (PCR) assays were developed for woodchuck leukocyte cluster of differentiation (CD) and cytokine mRNA expression. Plasmid DNA standards of each marker (CD3, CD4, CD8, IL-2, IFN-[gamma], TNF-[alpha], IL-4, IL-10), and RNA standards from mitogen-stimulated woodchuck peripheral blood mononuclear cells (PBMCs) were used to

validate and optimize the assays for TaqMan 7700(R) and iCycler(R) PCR instruments. The complementary DNAs (cDNAs) produced by reverse transcription (RT) of RNA were quantified by real-time PCR against the plasmid DNA standards (6-8 log range) with detection of as few as 10-50 copies of amplicon cDNA per reaction. Analysis of unstimulated and concanavalin A-stimulated woodchuck PBMC demonstrated increased CD and cytokine mRNA expression following mitogenic activation. A liver sample from a woodchuck hepatitis virus (WHV) infected woodchuck with histologically confirmed acute hepatitis had increased intrahepatic CD and cytokine mRNAs compared to liver from an uninfected control woodchuck. The real-time PCR assays were highly specific for the woodchuck markers in PBMC and liver samples and were equally applicable for use in alternate real-time PCR instrumentation. These assays will enable the high-throughput analyses of mRNA markers during WHV infection, and thereby facilitate continued modelling of the immunopathogenesis and immunotherapy of human hepatitis B virus (HBV) infection.

Mizuno, T., Y. Goto, et al. (2003). "Quantitative analysis of Fas and Fas ligand mRNAs in a feline T-lymphoid cell line after infection with feline immunodeficiency virus and primary peripheral blood mononuclear cells obtained from cats infected with the virus." Veterinary Immunology and Immunopathology **93**(3-4): 117.

http://www.sciencedirect.com/science/article/B6TD5-48S4NPC-5/2/9eab09e7beead23dde93591ce43344cb

Apoptosis is frequently observed in feline lymphocytes in association with feline immunodeficiency virus (FIV) infection. In this study, to investigate the mechanism of FIV-induced apoptosis, levels of Fas and Fas ligand mRNAs were measured by real-time reverse transcription-PCR. In a feline T-lymphoid cell line the amounts of Fas ligand mRNA increased along with the induction of apoptosis after in vitro infection with FIV. In PBMC collected from 10 cats naturally infected with FIV, Fas ligand mRNA levels were significantly higher than those in PBMC from five uninfected cats. These results indicate that the increased expression of Fas ligand may be involved in the induction of apoptosis of lymphocytes in FIV infection.

Mukamoto, M. and H. Kodama (2000). "Regulation of early chicken thymocyte proliferation by transforming growth factor-[beta] from thymic stromal cells and thymocytes." Veterinary Immunology and Immunopathology **77**(1-2): 121.

http://www.sciencedirect.com/science/article/B6TD5-41JTNY3-9/2/8b00da064e2c9c5bc119b8d4f439f29f

We examined expression of TGF-[beta]s in chicken thymic stromal cells and thymocytes and roles of TGF-[beta]s in thymocyte development within the thymus. Thymic stromal cells expressed TGF-[beta] 2 and 3 genes but not TGF-[beta] 4 gene. Thymocytes showed expressions of TGF-[beta] 2, 3 and 4 genes and each TGF-[beta] gene was expressed more strongly in CD3- than CD3+ thymocytes. When anti-TGF-[beta] antibody was added with supernatants of stromal cells into thymocyte culture, only proliferative activity of CD3- thymocytes was enhanced and the cell in S and G2/M compartments of cell cycle increased. These results suggest that TGF-[beta] which is expressed in the thymus may regulate the ability of immature thymocytes to progress through the cells cycle and to differentiate to CD3+ thymocytes.

Mukamoto, M., H. Kodama, et al. (1999). "Effects of cytokines from thymocytes and thymic stromal cells

on chicken intrathymic T cell development." Veterinary Immunology and Immunopathology **67**(3): 223.

http://www.sciencedirect.com/science/article/B6TD5-3VXYR2M-3/2/b446b53e91ea4163d9b2a8c6c34e9230

We have studied the ability of thymic stromal cells (TSC) and thymocytes to produce cytokines and the involvement of cytokines in intrathymic T cell development. When thymocytes were co-cultured with thymic stromal cells in absence of direct contact and mitogenic stimulation, induction of thymocyte proliferation was observed. Supernatants of cultured stromal cells (TSC-CS) promoted a high proliferative response on CD3- thymocytes but had little effect on CD3+ thymocytes. These results indicate that stromal cells have produced a cytokine which can induce immature thymocyte proliferation. Moreover, stromal cells express the MRNA for stem cell factor (SCF) and c-kit (the receptor for SCF) was detected on CD3- thymocytes but not on CD3+ thymocytes. Since SCF can enhance the proliferation of immature thymocytes in synergy with IL-7 in mammals, there is a possibility that chicken stromal cells may produce a IL-7-like factor. Thymocytes have clearly expressed interferon (IFN)-[gamma]. In contrast, thymic stromal cells showed no detectable expression of IFN-[gamma]. CD3+ thymocytes express IFN-[gamma] MRNA more strongly than CD3- thymocytes, suggesting that IFN-[gamma] from thymocytes may operate on stromal cells and then may indirectly induce clonal elimination of CD3+ cells on stromal cells. The expression of these cytokines and receptors by thymic stromal cells and thymocyte subpopulations suggests that these cytokines participate in paracrine interactions between these cell populations during thymocyte differentiation.

Muller, C., T. J. Coffey, et al. (2003). "Lack of TNF alpha supports persistence of a plasmid encoding the bovine leukaemia virus in TNF-/- mice." Veterinary Immunology and Immunopathology **92**(1-2): 15.

http://www.sciencedirect.com/science/article/B6TD5-47XSY12-1/2/3255233245fbdf30258dd2156bf83309

Tumour necrosis factor (TNF) is well recognised for its role in mediating innate immune responses. However, the mechanisms of TNF that influence the adaptive immune response to viral infections are poorly understood. Over recent years, there has been evidence to suggest a role for TNF in the early phase of infection of ruminants with bovine leukaemia virus (BLV). In this study, we infected TNF-/- mice with a plasmid encoding infectious BLV to further elucidate the role of TNF in BLV infection. TaqMan quantitative PCR showed that proviral DNA was present in genomic DNA isolated from spleen cells of TNF-/- mice 4 weeks post-infection, whereas it was not detected in wild-type mice. We were not able to detect differences in serum IgM or IgG levels between the TNF-/- and wild-type mice, or antibodies to BLV after this short period. In showing that the lack of TNF enables the plasmid encoded BLV to persist longer, and therefore rendering the mice more susceptible to an infection with BLV, the data suggest an important defence function of TNF in the early phase of BLV infection.

Oleksiewicz, M. B., B. Kristensen, et al. (2002). "Development of a rapid in vitro protein refolding assay which discriminates between peptide-bound and peptide-free forms of recombinant porcine major histocompatibility class I complex (SLA-I)." Veterinary Immunology and Immunopathology **86**(1-2): 55.

http://www.sciencedirect.com/science/article/B6TD5-45CN9YP-2/2/ca289494e3d43a4e1f63730219503967

The extracellular domains of swine leukocyte antigen class I (SLA-I, major histocompatibility complex protein class I) were cloned and sequenced for two haplotypes (H4 and H7) which do not share any alleles based on serological typing, and which are the most important in Danish farmed pigs. The extracellular domain of SLA-I was connected to porcine [beta]2 microglobulin by glycine-rich linkers. The engineered single-chain proteins, consisting of fused SLA-I and [beta]2 microglobulin, were overexpressed as inclusion bodies in Escherichia coli. Also, variants were made of the single-chain proteins, by linking them through glycine-rich linkers to peptides representing T-cell epitopes from classical swine fever virus (CSFV) and foot-and-mouth disease virus (FMDV). An in vitro refold assay was developed, using a monoclonal anti-SLA antibody (PT85A) to gauge refolding. The single best-defined, SLA-I restricted porcine CD8+ T-cell epitope currently known is a 9-residue peptide from the polyprotein of CSFV (J. Gen. Virol. 76 (1995) 3039). Based on results with the CSFV epitope and two porcine haplotypes (H4 and H7), the in vitro refold assay appeared able to discriminate between peptide-free and peptide-occupied forms of SLA-I. It remains to be seen whether the rapid and technically very simple in vitro refold assay described here will prove generally applicable for the screening of virus-derived peptides for SLA-I binding.

Pappalardo, B. L., T. Brown, et al. (2000). "Cyclic CD8+ lymphopenia in dogs experimentally infected with Bartonella vinsonii subsp. berkhoffii." Veterinary Immunology and Immunopathology **75**(1-2): 43.

http://www.sciencedirect.com/science/article/B6TD5-40NMSK9-4/2/e0227e1add71562479dd3333430c3b97

Until recently, it was presumed that Bartonella vinsonii only infected voles, a species of North American rodents. In April of 1993, however, our laboratory isolated a novel subspecies of B. vinsonii (B. vinsonii subsp. berkhoffii) from the blood of a dog diagnosed with vegetative valvular endocarditis. Subsequently, based on a seroepidemiologic survey of dogs from North Carolina and Virginia presenting for a variety of medical problems, we found evidence supporting a potentially important association between B. vinsonii and Ehrlichia canis co-infection in dogs. In the following study, eight dogs were infected with B. vinsonii: four specific pathogen free dogs and four dogs that had previously been infected with E. canis. Flow cytometric analysis of peripheral blood lymphocytes revealed a cyclic elevation of the CD4/CD8 T-cell ratio that correlated with cyclic CD8+ lymphopenia in all dogs infected with B. vinsonii, regardless of prior exposure to E. canis.

Pedersen, N. C., C. M. Leutenegger, et al. (2001). "Virulence differences between two field isolates of feline immunodeficiency virus (FIV-Aetaluma and FIV-Cammar) in young adult specific pathogen free cats." Veterinary Immunology and Immunopathology **79**(1-2): 53.

http://www.sciencedirect.com/science/article/B6TD5-430WX9F-5/2/23e6e22bab241b943b05766a906088e7

The goal of this study was to identify a strain of feline immunodeficiency virus (FIV) that would be more virulent for adult cats than the prototype FIV-Aetaluma and, thereby, enhance the FIV infection model for HIV-1 related research. Diehl et al. reported that one clade C strain of FIV, FIV-Cammar, was more virulent than other known FIV isolates. Mortalities from 58 to 100% were reported for kittens 12 weeks of age and less following intravenous inoculation. A more variable and somewhat less virulent disease course was observed in neonatal to 8-10-week-old kittens infected orally, intravaginally or intrarectally with this same isolate (Obert and Hoover, 2000). However, no studies have been done with FIV-Cammar in adult cats. Therefore, the virulence of FIV-Cammar for young adult cats was compared to that of FIV-Aetalulma, the original FIV isolate. One group of five cats were inoculated intraperitoneally with 470 TCID50 of FIV-Cammar in the

form of pooled plasma from acutely infected cats, while a second group was infected with plasma containing the 750 TCID50 of FIV-Aetaluma. The cats were observed for 20 weeks for gross signs of disease, hematologic abnormalities, time of antibody appearance, and plasma and peripheral blood mononuclear cell (PBMC) associated virus levels. Viral RNA and proviral DNA were measured by a real-time PCR, sensitive to 50 copies per milliliter. The only outward sign of disease was lymphadenopathy, which occurred at a similar time and intensity in both groups of cats. Cats infected with FIV-Cammar were more likely to be neutropenic and lymphopenic during the first 10-12 weeks of infection than cats infected with FIV-Aetaluma. Both groups of cats showed similar overall declines in absolute mean CD4 cell counts and identical concomitant increases in CD8 cells. CD4/CD8 cell ratios were also similar. Antibody, as measured by an ELISA against recombinant FIV-TM antigen, appeared in all cats by 4 weeks post-infection. The most significant differences were in plasma viral RNA and PBMC proviral DNA levels. Cats infected with FIV-Cammar had up to 100 times higher mean levels of viral RNA during the first few weeks of infection than cats infected with FIV-Aetaluma. This difference was also mirrored in levels of proviral DNA in PBMC, which were significantly higher in the FIV-Cammar infected cats. Plasma viral RNA and PBMC proviral DNA levels were virtually identical in both groups of cats at 20 weeks post-infection. However, proviral DNA in tissues such as thymus and popliteal lymph nodes was 10-fold or so higher in FIV-Cammar infected cats at 20 weeks and histopathologic lesions were more severe. Based on these various parameters, we concluded that FIV-Cammar was more virulent than FIV-Aetaluma in young adult cats during the 20-week study period. However, we were not able to recreate the severe and rapidly progressive disease previously reported for kittens, suggesting an age-related resistance similar to that observed previously for FIV-Aetaluma (George et al., 1993).

Philpott, M. S., J. P. Ebner, et al. (1992). "Evaluation of 9-(2-phosphonylmethoxyethyl) adenine therapy for feline immunodeficiency virus using a quantitative polymerase chain reaction." Veterinary Immunology and Immunopathology **35**(1-2): 155.

http://www.sciencedirect.com/science/article/B6TD5-476TWCF-8Y/2/f0eab0e0ca821d2149b38cbd741a0679

To determine the efficacy of 9-(2-phosphonylmethoxyethyl)adenine (PMEA) as a prophylactic chemotherapeutic agent for the treatment of lentivirus infections, three groups of specific pathogen free cats were treated with 0, 3, or 6 mg kg-1 twice daily doses of PMEA beginning 24 h prior to virus challenge with feline immunodeficiency virus Petaluma strain. Treatment was continued for 7 weeks post challenge. During this time cats were monitored for drug toxicity, virus specific antibody response, circulating viral antigen and infectious recoverable virus. To determine the long-term influence of PMEA therapy the cats were monitored for 1 year following the cessation of treatment. The low levels of infectious virus present in blood prompted the development of quantitative polymerase chain reaction assay to enumerate viral DNA burdens in the peripheral blood mononuclear cells of the infected cats and thereby assess drug efficacy. The results indicate that, although prophylactic PMEA did not prevent infection, it did substantially limit feline immunodeficiency virus replication. Furthermore, viral DNA levels remained low in the cats receiving drug a full year (the duration of the study) after cessation of treatment.

Sarli, G., L. Mandrioli, et al. (2001). "Immunohistochemical characterisation of the lymph node reaction in pig post-weaning multisystemic wasting syndrome (PMWS)." Veterinary Immunology and Immunopathology **83**(1-2): 53.

http://www.sciencedirect.com/science/article/B6TD5-445RJM3-5/2/92f235d75d29a90058141376e9acea76

The superficial inguinal lymph nodes of 10 piglets which had died spontaneously of post-weaning multisystemic wasting syndrome (PMWS), in which the porcine circovirus type II (PCV-II) genome was revealed by PCR, were submitted to immunohistochemical investigation for CD4, CD8, IgM, MAC387, S-100 protein, vimentin and F-VIII-RA and compared with three normal cases. The lymph node reaction was graded as initial, intermediate and end stage according to histological criteria. In the initial and intermediate stages, absence of follicles and depletion of lymphocytes were evident. Associated with this was a reduction in numbers of interfollicular dendritic cells and interdigitating cells and a reduction/absence of B cells and mainly CD4+ T lymphocytes. In the end stage the reduced expression of high endothelial venules and the prevalence of the stromal component of the lymph node was prominent, as well as the above changes. It is concluded that more than one mechanism is involved in the immunosuppressive ability of PCV-II: reduction of the antigen presenting ability and reduction of B cells and CD4+ T cell function.

Seow, H. F., J. S. Rothel, et al. (1995). "Expression, biological activity and kinetics of production of recombinant ovine TNF-[alpha]." Veterinary Immunology and Immunopathology **44**(3-4): 279.

http://www.sciencedirect.com/science/article/B6TD5-3YS90B5-6/2/0009eec8ba91fafe4dbb124b508dde46

Ovine tumour necrosis factor-alpha (OvTNF-[alpha]) was cloned by reverse transcription-polymerase reaction using RNA isolated from lipopolysaccharide (LPS)-stimulated alveolar macrophages and primers based on the human TNF-[alpha] cDNA sequence. An expression vector carrying the coding sequence of the mature form of ovine TNF was constructed. The recombinant Ov-TNF[alpha] (rOvTNF-[alpha]) was expressed as a glutathione-S-transferase (GST) fusion protein. It was cleaved with thrombin to yield rOvTNF free of the GST moiety. Growth at a lower temperature of 30[deg]C and use of Escherichia coli strains AM207, AM305, E392 and NM522 did not improve the recovery of rOvTNF-[alpha] from the soluble fraction to a significant extent. Purification of recombinant proteins was achieved rapidly and easily by affinity chromatography using glutathione-Sepharose. Yields of pure rOvTNF-[alpha] achieved in E. coli JM109 and AM207 were approximately 1 mg L-1. Both rOvTNF-[alpha] and recombinant human TNF-[alpha] (rhTNF-[alpha]) exerted cytotoxicity on L929 cells. However, rOvTNF-[alpha] but not rhTNF-[alpha] stimulated proliferation of ovine thymocytes. Maximum levels of TNF-[alpha] mRNA expression by LPS-stimulated ovine alveolar macrophages were detected at approximately 4 h post-stimulation.

Sondgeroth, K., C. Leutenegger, et al. (2005). "Development and validation of puma (Felis concolor) cytokine and lentivirus real-time PCR detection systems." Veterinary Immunology and Immunopathology **104**(3-4): 205.

http://www.sciencedirect.com/science/article/B6TD5-4F83PJP-1/2/1d632446c1a145e23508c5b9eb5171fc

Studies of immune correlates of disease outcome associate humoral immune response mediated by T-helper 2 cytokines (IL-4, IL-10) with more virulent disease relative to a cell-mediated response driven by T-helper 1 cytokines (IL-2, IFN-gamma), particularly in viral and other intra-cellular infections. Specifically, the kinetics of both human immunodeficiency virus (HIV) and feline immunodeficiency virus (FIV) infection are closely associated with Type 1 versus Type 2 cytokine profiles. Puma (Felis concolor) lentivirus (PLV) is closely related to FIV, but based on phylogenetic and clinical studies, is more ancient and less pathogenic. The aims of this study were to validate feline real-time PCR primer/probe systems for puma cytokines and PLV as sensitive, quantitative assays for use in investigations of PLV pathogenicity. We demonstrate that primer/probe systems for IL-4, IL-10, IFN-gamma, TNF-alpha, GAPDH, and the pol region of

PLV-1695 amplify puma cytokines and PLV-1695 with high amplification efficiency and sensitivity. Detection of PLV-1695 provirus in experimentally inoculated domestic cats proved to be of equivalent sensitivity, specificity, and positive and negative predictive value to co-culture of one million peripheral blood mononuclear cells (PBMC). Evaluation of cytokine induction during naturally occurring PLV infection will allow insight into mechanisms of host control associated with apathogenic infection. In addition, determination of viral loads during different stages of PLV infection or in different tissues from domestic cats or pumas will further elucidate capacity of these viruses to replicate and establish infection.

Tang, L. (2001). "Molecular cloning of canine IL-13 receptor [alpha] chain ([alpha]1 and [alpha]2) cDNAs and detection of corresponding mRNAs in canine tissues." Veterinary Immunology and Immunopathology **79**(3-4): 181.

http://www.sciencedirect.com/science/article/B6TD5-435CRHC-3/2/1099cdb50fa7d53bfc1c4a6d559a8835

This communication reports the cloning of cDNAs encoding two canine IL-13 receptor [alpha] chains (caIL-13R[alpha]1 and caIL-13R[alpha]2). As described for the members of type-I cytokine receptors, both caIL-13R[alpha]1 and caIL-13R[alpha]2 were found to contain the highly conserved motifs, such as cysteine and tryptophan residues in their N-terminal portion and the WSXWS at C-terminus. The isolated caIL-13R[alpha]1 cDNA contains 1547 nucleotides with an open reading frame that encodes 405 amino acid residues. Canine IL-13R[alpha]1 is 82.0 and 69.3% identical to human and mouse IL-13R[alpha]1s, respectively, at the amino acid level. Canine IL-13R[alpha]1 has an almost identical cytoplasmic domain to its human and mouse counterparts. The isolated caIL-13R[alpha]2 cDNA contains 1454 nucleotides and encodes an open reading frame of 386 amino acid residues. Canine IL-13R[alpha]2 is 62.6 and 47.5% identical to its human and mouse counterparts, respectively, at the amino acid level. Using RT-PCR with caIL-13R[alpha]1 and caIL-13R[alpha]2 specific primers, mRNAs of caIL-13R[alpha]1 and caIL-13R[alpha]2 were detected in most dog tissues. In addition, RT-PCR detected caIL-13R[alpha]1 mRNA in one of two canine mastocytoma (C2 but not Br) cell lines and in a canine macrophage-derived cell line (DH82). CaIL-13R[alpha]2 mRNA was detected in all three canine cell lines.

Tang, L., C. Sampson, et al. (2001). "Cloning and characterization of cDNAs encoding four different canine immunoglobulin [gamma] chains." Veterinary Immunology and Immunopathology **80**(3-4): 259.

http://www.sciencedirect.com/science/article/B6TD5-43G3056-5/2/9cea06a5afa391cc60b4250f3d5eb604

cDNAs encoding four different canine immunoglobulin G (caIgG) [gamma] chains were identified in this study. One of these IgG [gamma] chain cDNAs, (caIgG-A), represents 92.5% of the IgG [gamma] chain cDNAs in a dog spleen cell cDNA library; a second partial IgG [gamma] chain cDNA (caIgG-B) was also identified in the library. The other two IgG [gamma] chain cDNAs (caIgG-C and caIgG-D) were RT-PCR amplified from canine lymphoma samples. Comparison of the four different canine IgG [gamma] chain cDNAs showed homologies from 83.6 to 89.2% and from 73.1 to 81.8% at nucleotide and amino acid sequence levels, respectively. Despite the high similarity in CH1, CH2 and CH3 domains among the different caIgG [gamma] chains, the hinge regions were distinct, sharing only 19.0-35.2% homology at the amino acid level. No multiple duplication of the hinge region, as reported for human IgG1 and IgG3, was detected in any of the canine IgG [gamma] chains. The numbers of cysteines in the putative hinge regions were found to be 3, 2, 7 and 3 for the four canine IgG heavy [gamma] chains (A, B, C and D), respectively.

Specific primers were designed based on caIgG [gamma] chain hinge region DNA sequences and were used in RT-PCR for measuring different caIgG [gamma] chain mRNA levels in canine PBMC samples.

Waldvogel, A. S., B. M. Hediger-Weithaler, et al. (2000). "Interferon-[gamma] and Interleukin-4 mRNA expression by peripheral blood mononuclear cells from pregnant and non-pregnant cattle seropositive for bovine viral diarrhea virus." Veterinary Immunology and Immunopathology **77**(3-4): 201.

http://www.sciencedirect.com/science/article/B6TD5-41Y868D-4/2/36810a61527b76d401c32a60a7f7c4b3

The acceptance of the fetal allograft by pregnant women and mice seems to be associated with a shift from a Th 1 dominated to a Th 2 dominated immune response to certain infectious agents. The goal of this study was to examine cytokine expression in peripheral blood mononuclear cells (PBMCs) from cattle immune to bovine viral diarrhea virus (BVDV) to determine whether pregnancy also has an influence on the type of immune response in this species. Forty-six heifers and cows between 14 months and 13 years of age were included in this study. Twenty-four were seropositive and 22 seronegative for BVDV. Eleven of the seropositive animals and 11 of the seronegative animals were in the eighth month of gestation, the remaining animals were virgin heifers. PBMC from these animals were analyzed for Interferon (IFN)-[gamma] and Interleukin (IL)-4 mRNA expression by real-time RT-PCR after stimulation with a non-cytopathic strain of BVDV. Additionally, an ELISA was performed to measure IFN-[gamma] in the supernatants of stimulated cell cultures. In BVDV seropositive animals, IFN-[gamma] mRNA levels were significantly higher than in BVDV seronegative animals and there was a significant positive correlation between the changes in IFN-[gamma] and IL-4 mRNA expression. There was, however, no significant difference in IFN-[gamma] and IL-4 mRNA levels between pregnant and non-pregnant animals. These results are inconsistent with BVDV inducing a Th1 or Th2 biased immune response. Furthermore, a shift in the cytokine pattern during bovine pregnancy was not evident.

Wang, Y., D. S. Zarlenga, et al. (2002). "Recombinant bovine soluble CD14 sensitizes the mammary gland to lipopolysaccharide." Veterinary Immunology and Immunopathology **86**(1-2): 115.

http://www.sciencedirect.com/science/article/B6TD5-458PB2S-1/2/f102e0d0d1a06fc14e29de98ffc17383

Standard therapies including administration of potent antibiotics, aggressive fluid resuscitation and metabolic support have not been successful in relieving symptoms and reducing mortality associated with acute coliform mastitis. It is important to understand the pathophysiological response of the mammary gland to coliform infections when designing preventive or therapeutic regimens for controlling coliform mastitis. Our laboratory has previously shown that macrophages and polymorphonuclear neutrophils in milk express CD14 on their cell surface. In this study, we found that soluble CD14 (sCD14) is present in milk whey as a 46 kDa protein reacted with anti-ovine CD14 antibody. Additional functional studies found that: (1) under serum-free condition, complexes of LPS-recombinant bovine soluble CD14 (rbosCD14) induced activation of mammary ductal epithelial cells (as measured by changes in interleukin-8 (IL-8) mRNA level by competitive RT-PCR) at low concentrations of LPS after 6 or 24 h incubation (1-1000 ng/ml), whereas LPS alone did not induce activation of mammary ductal epithelial cells at the same concentrations, and (2) intramammary injection of low concentrations of LPS did not increase concentration of leukocytes in milk. In contrast, LPS-rbosCD14 complex containing the same concentration of LPS increased the concentration of leukocytes in the injected mammary gland at 12 and 24 h post-

injection. These results indicate that rbosCD14 sensitizes mammary epithelial cells to low concentrations of LPS in vitro and in vivo. Endogenous sCD14 in milk may be important in initiating host responses to Gram-negative bacterial infections.

Weber, E. R., C. R. Helps, et al. (2000). "Molecular cloning and phylogenetic analysis of a cDNA encoding the cat (Felis domesticus) Ig epsilon constant region." Veterinary Immunology and Immunopathology **76**(3-4): 299.

http://www.sciencedirect.com/science/article/B6TD5-41FTRYG-C/2/a5cf2939bf42000ae5fed538139d7184

A feline splenic cDNA library was screened with a 32P-labelled cDNA probe encoding the canine IgE epsilon heavy chain subunit. A cDNA sequence of 1614 nucleotides encoding the complete feline IgE heavy chain, as well as a portion of a variable region, was identified. A search of the GenBank database revealed an identity of 82% at the nucleotide level and 76% at the amino acid level between the feline epsilon heavy chain sequence and the canine homologue. In a separate study, feline genomic DNA, isolated from whole feline embryo cells, was subjected to PCR amplification using primers based on known partial genomic DNA sequences for the feline C[epsiv] gene. Following removal of an intron from the 683 bp PCR product, the coding sequence yielded an ORF of 506 bp. The DNA sequence of this PCR clone differed by a single nucleotide from the cDNA clone. This difference is silent, and therefore the proteins encoded by the two sequences are identical over the regions cloned and sequenced. Phylogenetic analysis of the constant regions of nine immunoglobulin epsilon genes revealed that the feline cDNA is most similar to the canine homologue.

Wonderling, R., T. Powell, et al. (2002). "Cloning, expression, purification, and biological activity of five feline type I interferons." Veterinary Immunology and Immunopathology **89**(1-2): 13.

http://www.sciencedirect.com/science/article/B6TD5-46MBHFW-2/2/4e33fbdc4cb75064e8d501acfab93aa0

Type I interferons (IFN) are important mediators of the host defense against viral infections in mammals. In humans multiple subtypes of IFN-[alpha] exist, most of which possess antiviral activity. Little is known about the type I IFN genes in cats and the role they may play in feline immunological responses to viruses. We have isolated cDNAs encoding five feline IFN-[alpha] (feIFN) subtypes that share from 95 to 99% amino acid sequence identity. FeIFN-[alpha]5 has five additional amino acids inserted at position 139, which are not present in the other four subtypes. Sequence identity of the feIFN proteins encoded by the five clones compared to human IFN-[alpha]2 is approximately 60%. Unlike most of the human subtypes, each of the five feline IFN sequences has an N-glycosylation recognition site. Expression of all five feIFN-[alpha] subtypes in Chinese hamster ovary (CHO) cells was confirmed by Western blot analysis, and all resulting proteins were glycosylated. The antiviral activity of each feIFN-[alpha] subtype produced in transiently transfected CHO cell cultures was tested in vitro. In addition, subtype feIFN-[alpha]6 was expressed in the yeast, Pichia pastoris. The resulting secreted mature recombinant protein was purified and demonstrated significant antiviral activity and induction of 2',5'-oligoadenylate synthetase activity in vitro.

Yoshihara, K., S. Inumaru, et al. (1998). "Cloning and sequencing of cDNA encoding bovine macrophage colony-stimulating factor (bM-CSF) and expression of recombinant bM-CSF using baculovirus."

Veterinary Immunology and Immunopathology **63**(4): 381.

http://www.sciencedirect.com/science/article/B6TD5-3T176MD-6/2/51c37cfd13ab5653e806931f83c3f893

The cDNAs encoding bovine macrophage colony-stimulating factors [alpha] and [beta] (M-CSF[alpha] and M-CSF[beta]) were cloned and recombinant bovine M-CSF[alpha] (rbM-CSF[beta]) in its dimeric form was expressed by using a recombinant baculovirus/insect cell system. The predicted amino acid sequence of rbM-CSF[alpha] and rbM-CSF[beta] shared 83.3 and 75.9% ([alpha]), 75.3 and 65.9% ([beta]) similarity with the sequence for human and murine M-CSFs, respectively. The biological activity of rbM-CSF[beta] was confirmed by the colony-forming assay using mouse bone marrow cells. SDS-PAGE under a reducing condition showed that the molecular weight of rbM-CSF[beta] was approximately 34 kDa. On the other hand, Western blot analysis under a non-reducing condition revealed that this rbM-CSF[beta] was secreted in dimeric form into the cell supernatant.

Zawitkowski, M., G. Russ, et al. (2002). "Cloning and expression of the ovine CD40 molecule and the inhibition of the mixed lymphocyte reaction by the ovine CD40e-EGFP fusion protein." Veterinary Immunology and Immunopathology **89**(1-2): 37.

http://www.sciencedirect.com/science/article/B6TD5-46MBHFW-4/2/b28599b08da9c94cd1d94aee4524b1ab

The CD40 molecule is a member of the tumour necrosis factor receptor (TNFR)-like supergene family and plays a major role as a co-stimulatory molecule in the activation of T cells in response to antigens presented by dendritic cells. In this study, reverse transcription-PCR cloning was used to derive the sequence encoding ovine CD40. The ovine CD40 sequence demonstrated a similarity of 97, 76 and 64% with the bovine, human and murine sequences, respectively, at the nucleic acid level. The cysteine residues characteristic of the TNFR family and N-linked glycosylation sites are conserved. Furthermore, RNA analysis confirmed expression of CD40 mRNA in both ovine dendritic cells from lymphatic drainage and dermal fibroblasts in culture. In addition, cDNA encompassing the extracellular region of ovine CD40 (CD40e) was fused 'in-frame' with the enhanced green fluorescent protein (EGFP) to generate a fusion protein upon the transfection of Chinese hamster ovary (CHO) cells.Immunoprecipitation with an anti-GFP monoclonal antibody of a 78 kD a protein from conditioned medium of CHO transfectants confirmed that the CD40e-EGFP was secreted in the supernatant. All experiments were controlled with a pEGFP-N1 vector-blank construct. Moreover, the biological activity of ovine CD40e-EGFP was demonstrated by its ability to inhibit a two-way mixed lymphocyte reaction. Thus these observations confirm that ovine CD40 blockade inhibits co-stimulation mediated by CD40-CD40L (CD154) interactions as has been reported in murine and human studies.

Veterinary Microbiology (68)

Angen, O., P. Ahrens, et al. (1998). "Development of a PCR test for identification of Haemophilus somnus in pure and mixed cultures." Veterinary Microbiology **63**(1): 39.

http://www.sciencedirect.com/science/article/B6TD6-3V7WKX0-

4/2/c37e5e3bbd738a17da74eec3b6ab22d5

Based on the 16S rRNA sequences of a collection of well-characterized strains of Haemophilus somnus a set of primers was selected as candidates for a species-specific PCR test. All investigated H. somnus strains were found positive in the test, including 12 strains earlier found to represent H. somnus by DNA-DNA hybridization as well as representatives of the 16 ribotypes previously described within this species. The specificity of the test was evaluated on a broad collection of strains within the family Pasteurellaceae and on other Gram positive and negative species. None of these strains gave rise to an amplicon in the PCR test. The performance of the test on mixed cultures was evaluated by adding P. multocida to serial dilutions of H. somnus and incubating the agarplates for 1 and 2 days. This showed that the PCR test applied to the harvest from an agarplate can be expected to detect a single colony of H. somnus in the presence of 109 CFU of P. multocida even after 2 days of incubation. In conclusion, the present PCR test has been shown to represent a specific test for identification of H. somnus both in pure and mixed cultures. It represents a quick, sensitive and reliable method for identification of bacteria belonging to this phenotypically heterogeneous and often slow growing species.

Atyeo, R. F., T. B. Stanton, et al. (1999). "Differentiation of Serpulina species by NADH oxidase gene (nox) sequence comparisons and nox-based polymerase chain reaction tests." Veterinary Microbiology **67**(1): 47.

http://www.sciencedirect.com/science/article/B6TD6-3WM557T-6/2/6eb8d123cb9fea4dd3c70bda9878bc4e

The NADH oxidase genes (nox) of 18 strains of intestinal spirochaetes were partially sequenced over 1246 bases. Strains examined included 17 representatives from six species of the genus Serpulina, and the type strain 513AT of the human intestinal spirochaete Brachyspira aalborgi. Sequences were aligned and used to investigate phylogenetic relationships between the organisms. Nox sequence identities between strains within the genus Serpulina were within the range 86.3-100%, whilst the nox gene of B. aalborgi shared between 78.8-83.0% sequence identity with the nox sequences of the various Serpulina strains. A phenogram produced based on sequence dissimilarities was in good agreement with the current classification of species in the genus Serpulina, although an atypical strongly beta-haemolytic porcine strain (P280/1), previously thought to be S. innocens, appeared distinct from other members of this species. Primer pairs were developed from the nox sequence alignments for use in polymerase chain reaction (PCR) identification of the pathogenic species S. hyodysenteriae (NOX1), S. intermedia (NOX2), and S. pilosicoli (NOX3), and for the combined non-pathogenic species S. innocens and S. murdochii (NOX4). The PCRs were optimised using 80 strains representing all currently described species in the genus Serpulina, as well as the type strain of B. aalborgi. Tests NOX1 and NOX4 specifically amplified DNA from all members of their respective target species, whilst tests NOX2 and NOX3 were less sensitive. NOX2 amplified DNA from all 10 strains of S. intermedia from pigs but from only 4 of 10 strains from chickens, whilst NOX3 amplified DNA from only 18 of 21 S. pilosicoli strains, even at low stringency. Tests NOX1 and NOX4 should prove useful in veterinary diagnostic laboratories, whilst NOX2 and NOX3 require further refinement.

Bhudevi, B. and D. Weinstock (2001). "Fluorogenic RT-PCR assay (TaqMan) for detection and classification of bovine viral diarrhea virus." Veterinary Microbiology **83**(1): 1.

http://www.sciencedirect.com/science/article/B6TD6-43T1NXR-1/2/502b8636708dd0db2c3da5871152271e

A single tube fluorogenic RT-PCR-based 'TaqMan' assay was developed for detection and classification of bovine viral diarrhea virus (BVDV). TaqMan-PCR was optimized to quantify BVD virus using the ABI PRISM 7700 sequence detection system and dual-labeled fluorogenic probes. Two different gene specific labeled fluorogenic probes for the 5' untranslated region (5' UTR) were used to differentiate between BVD types I and II. Sensitivity of the single tube TaqMan assay was compared with two-tube TaqMan assay and standard RT-PCR using 10-fold dilutions of RNA. Single tube TaqMan assay was 10-100-fold more sensitive than the two-tube TaqMan assay and the standardized single tube RT-PCR. Specificity of the assay was evaluated by testing different BVD virus strains and other bovine viruses. A total of 106 BVD positive and negative pooled or single serum samples, field isolates and reference strains were tested. Quantitation of cRNA from types I and II BVD virus was accomplished by a standard curve plotting cycle threshold values (CT) versus copy number. Single tube TaqMan-PCR assay was sensitive, specific and rapid for detection, quantitation and classification of BVD virus.

Boye, M., S. Kamstrup, et al. (1991). "Specific sequence amplification of bovine virus diarrhea virus (BVDV) and hog cholera virus and sequencing of BVDV nucleic acid." Veterinary Microbiology **29**(1): 1.

http://www.sciencedirect.com/science/article/B6TD6-47DD9RX-Y/2/f512e63f4f62e002c0e81e99764896d6

The pestiviruses are small enveloped RNA viruses and are causative agents of economically important animal diseases in cattle, swine, sheep and goats worldwide. We used the polymerase chain reaction to amplify one common fragment of several different strains of both hog cholera virus and bovine virus diarrhea virus (BVDV). The fragment is located at the 5'-end of the genome immediately upstream of the open reading frame. This is a highly conserved region among the different published pestivirus sequences.An internal restriction digest of the amplified fragment with XhoI and PstI was performed in order to confirm specificity of the amplified fragment. The fragment was sequenced for a number of different BVDV strains, and the sequences obtained were compared to those published and used to deduce genetic relationships between strains. Apart from this common fragment we have amplified several other fragments of the Danish BVDV strain Ug59 and obtained specific amplification fragments of the expected size.

Burr, P. D., M. E. M. Campbell, et al. (1996). "Detection of Canine Herpesvirus 1 in a wide range of tissues using the polymerase chain reaction." Veterinary Microbiology **53**(3-4): 227.

http://www.sciencedirect.com/science/article/B6TD6-3W323GD-T/2/7063b9bfaf9f37df66cf6d59edc48ec4

Canine herpesvirus 1 (CHV-1), a member of the alphaherpesvirus sub-family, is known to cause fatal infections in litters of puppies and may also be involved in infertility, abortion, and stillbirths in adult dogs. The purpose of this study was to determine the presence of CHV-1 DNA using the polymerase chain reaction (PCR) in twelve key sites that have been associated with latency for other herpesviruses. A 605 base pair portion of the viral glycoprotein B (gB) gene was amplified using degenerate primers, cloned, and sequenced. Conventional 20mer primers were designed using this sequence information to amplify a 120 bp fragment of gB situated between the original degenerate primers. The specificity of amplification was confirmed by Southern Blot hybridisation using an internal oligonucleotide probe. DNA was extracted from tissue samples taken from twelve dogs at post mortem and from twenty-four blood samples. Nine out of twelve dogs showed evidence of infection with CHV-1; the tissues most commonly affected were lumbo-sacral ganglia (5/12 dogs), tonsil (5/12), parotid salivary gland (4/9), and liver (4/9). No positive results were detected within the twenty-four blood samples. These results indicate that exposure to CHV-1

may be much more common than previously suggested.

Collins, D. M., D. M. Stephens, et al. (1993). "Comparison of polymerase chain reaction tests and faecal culture for detecting Mycobacterium paratuberculosis in bovine faeces." Veterinary Microbiology **36**(3-4): 289.

http://www.sciencedirect.com/science/article/B6TD6-476VHVX-B8/2/4a6faa4ee38776fe8be66dda71998968

A polymerase chain reaction (PCR) test for M. paratuberculosis was developed based on a 218 bp segment of a DNA insertion sequence, IS900, that is specific for this organism. The method involved two consecutive amplification reactions, with the second set of primers being nested inside the first set. The method reliably detected 50 organisms/g faeces. This PCR test was applied to 32 bovine faecal specimens containing high, moderate or low numbers of M. paratuberculosis organisms as determined by culture. The PCR test detected all specimens containing >=1600 colony forming units (cfu)/g faeces, six of ten specimens with 160-480 cfu/g faeces but only two of 13 specimens containing <= 112 cfu/g faeces. The sensitivity of this test was better than that of a commercial PCR test which was carried out on the same faecal specimens.

Criado-Fornelio, A., A. Martinez-Marcos, et al. (2003). "Presence of Mycoplasma haemofelis, Mycoplasma haemominutum and piroplasmids in cats from southern Europe: a molecular study." Veterinary Microbiology **93**(4): 307.

http://www.sciencedirect.com/science/article/B6TD6-48716RD-1/2/e58338f809156386ecd798449a32f242

Clinical symptoms produced by Mycoplasma spp. and piroplasmids in cats are sometimes similar. Diagnosis of these pathogens is difficult by microscopic procedures and molecular methods have been used as an alternative. We present in this work, the development of new molecular procedures for diagnosis of the aforementioned organisms, together with a molecular characterization of isolates found in southern European cats.A single PCR-RFLP procedure was designed for diagnosis of Mycoplasma spp. and a seminested PCR-RFLP was designed for diagnosis of piroplasmids. The 16S or 18S rRNA genes of isolates found in clinical samples were partially sequenced in all positive cases.Mycoplasma spp. was detected in 9 (30%) out of 30 symptomatic cats from Spain. Sequencing indicated that 66.6% of these isolates can be ascribed to Mycoplasma haemofelis and only 33.3% to Mycoplasma haemominutum. Partial 16S rRNA sequences obtained in Spanish isolates were very similar to those previously published from the UK and the USA.The presence of piroplasmids (Babesia and Theileria spp.) was studied in 16 cats from Spain (n=13) and Portugal (n=3). Animals analyzed were 10 cats with immunosuppressive viral infection (either FeLV or FIV), 5 asymptomatic cats and 1 cat with Babesia-compatible symptoms. Asymptomatic cats were all PCR-negative. Partial sequencing of 18S rRNA gene demonstrated that the Babesia-symptomatic cat was infected with Babesia canis canis whereas 3 (30%) out of the 10 cats with immunosuppressive viral infection were coinfected with piroplasmids (1 with B. canis canis, 1 with Theileria annae, and 1 with B. canis canis and T. annae both).

David, D., B. Yakobson, et al. (2002). "Rabies virus detection by RT-PCR in decomposed naturally infected brains." Veterinary Microbiology **87**(2): 111.

http://www.sciencedirect.com/science/article/B6TD6-45JPGT6-1/2/f8d6d4cdd3994a5dd14b38bc0df59594

The warm climate of Israel and mishandling of the cadavers during transit to the laboratory requires an accurate method for diagnosis of rabies in decomposed tissues. By using the reverse transcriptase polymerase chain reaction (RT-PCR) 10 decomposed brain samples that collected between 1998 and 2000 were diagnosed as negative by direct fluorescent antibody test (FAT), were found positive. Three of the 10 decomposed brains were confirmed as positive by isolation of rabies virus in tissue culture and by mouse inoculation (MIT) while the other seven decomposed samples were found positive only by RT-PCR. Direct sequencing and molecular analysis of a 328 bp fragment of the N gene of all the rabies sequences confirmed their geographical origin. These results demonstrated the importance of the RT-PCR in the detection of rabies virus in decomposed naturally infected brains, especially in cases when the sample is not suitable for other laboratory assays. Thus, the RT-PCR can provide a positive diagnosis; however, when a negative result is obtained due to the nature of the decomposed tissue that can be caused by technical reasons and a false negative might be the case.

Decaro, N., G. Elia, et al. (2005). "A real-time PCR assay for rapid detection and quantitation of canine parvovirus type 2 in the feces of dogs." Veterinary Microbiology **105**(1): 19.

http://www.sciencedirect.com/science/article/B6TD6-4F05RD3-1/2/d696d57ecaf39cc9e6bbff16db85461b

We describe a rapid, sensitive and reproducible real-time PCR assay for detecting and quantifying canine parvovirus type 2 (CPV-2) DNA in the feces of dogs with diarrhea. An exogenous internal control was added to control the assay performance from extraction to amplification. The method was demonstrated to be highly specific and sensitive, allowing a precise CPV-2 DNA quantitation over a range of eight orders of magnitude (from 102 to 109 copies of standard DNA). The reproducibility of the CPV-2 real-time PCR assay was assessed by calculating the coefficients of variation (CV) intra-assay and inter-assay for samples containing amounts of CPV-2 DNA spanning the whole range of the real-time PCR standard curve. Then, fecal specimens from diarrheic dogs were analyzed by hemagglutination (HA), conventional PCR and real-time amplification. Comparison between these different techniques revealed that real-time PCR is more sensitive than HA and conventional gel-based PCR, allowing to detect low viral titers of CPV-2 in infected dogs.

Eaves, F. W., J. B. Molloy, et al. (1994). "A field evaluation of the polymerase chain reaction procedure for the detection of bovine leukaemia virus proviral DNA in cattle." Veterinary Microbiology **39**(3-4): 313.

http://www.sciencedirect.com/science/article/B6TD6-476VM9J-191/2/df983d06b737345737f9feed4c7c98be

A polymerase chain reaction (PCR) procedure that detects proviral bovine leukaemia virus (BLV) in peripheral blood mononuclear cell DNA was evaluated. Blood samples from all animals (164) in a commercial dairy herd with a 30% prevalence of BLV infection, and from 194 animals from BLV free herds were tested. The absence of any positive PCR results in animals from BLV free herds confirmed the specificity of the assay. Initial testing of the infected herd using a single amplification PCR (SA-PCR), detected BLV infection in 62 of 72 adult animals that were seropositive by the agar gel immunodiffusion (AGID) test and in one persistently seronegative cow. Infection in this cow was confirmed by sheep bioassay. Subsequent testing of the SA-PCR

negative, seropositive animals using a double amplification PCR (DA-PCR) detected proviral BLV in eight of nine animals that were available for retesting. The PCR assay was also able to distinguish BLV infected calves from uninfected calves that were serologically positive because of the presence of colostral antibody. Lymphocytes from all seropositive animals were cultured for determination of BLV antigen expression. Cultures from 37 of 62 SA-PCR positive animals produced detectable quantities of viral antigens. However, antigen expression was not detected in cultures from seropositive animals that were negative in the SA-PCR. In addition, in experimental transmission tests, inoculation of more than 106 lymphocytes from these cows was required for sheep to become seropositive to BLV. These results suggest that the failure of the PCR assays to detect some seropositive animals was due to a low proportion of lymphocytes being infected with BLV in these animals. The DA-PCR detected BLV infection with a sensitivity comparable to that of the AGID test and the sheep bioassay. PCR assays may be an alternative to the sheep bioassay as an adjunct to serological testing for use in situations where it is essential to detect all infected cattle. However, the stringent precautions found to be essential to prevent false positive results due to contamination of samples with PCR product are likely to preclude the routine use of PCR for diagnosis of BLV infection.

Edmonds, M. D., A. Cloeckaert, et al. (2002). "Brucella species lacking the major outer membrane protein Omp25 are attenuated in mice and protect against Brucella melitensis and Brucella ovis." Veterinary Microbiology **88**(3): 205.

http://www.sciencedirect.com/science/article/B6TD6-46B75TX-8/2/d776e6c53a9afc97c557eda45c3c06b5

To aid in the development of novel efficacious vaccines against brucellosis, Omp25 was examined as a potential candidate. To determine the role of Omp25 in virulence, mutants were created with Brucella abortus (BA25), Brucella melitensis (BM25), and Brucella ovis (BO25) which contain disruptions in the omp25 gene ([Delta]omp25 mutants). Western immunoblot analysis and PCR verified that the Omp25 protein was not expressed and that the omp25 gene was disrupted in each strain. BALB/c mice infected with B. abortus BA25 or B. melitensis BM25 showed a significant decrease in mean CFU/spleen at 18 and 4 weeks post-infection, respectively, when compared to the virulent parental strain (Pn=5). Mice infected with B. ovis BO25 had significantly lower mean CFU/spleen counts from 1 to 8 weeks post-infection, at which point the mutant was cleared from the spleens (Pn=5). Murine vaccination with either BM25 or the current caprine vaccine B. melitensis strain Rev. 1 resulted in more than a 2 log10 reduction in bacterial load following challenge with virulent B. melitensis (Pn=5). Vaccination of mice with the B. ovis mutant resulted in clearance of the challenge strain and provided 2.5 log10 greater protection against virulent B. ovis than vaccine strain Rev. 1. Based on these data, the B. melitensis and B. ovis [Delta]omp25 mutants are interesting vaccine candidates that are currently under study in our laboratory for their safety and efficacy in small ruminants.

Eisenberg, S. W. F., A. J. A. M. van Asten, et al. (2003). "Detection of circovirus with a polymerase chain reaction in the ostrich (Struthio camelus) on a farm in The Netherlands." Veterinary Microbiology **95**(1-2): 27.

http://www.sciencedirect.com/science/article/B6TD6-48XJNR6-4/2/93ee88e285f2c0b0b8ed674c6b4d1cd0

This study describes for the first time the presence of circoviruses in ostrich tissue including embryos. A polymerase chain reaction (PCR) was used for the detection of the virus in liver samples. The use of a polymerase for low copy detection significantly increased the sensitivity of the test as well as a Southern blot. Viral DNA could be detected in chicks and eggs that did not

hatch. For localisation of the virus in the liver in situ hybridisation was performed on a selection of positive liver tissues.

Engstrom, B. E., C. Fermer, et al. (2003). "Molecular typing of isolates of Clostridium perfringens from healthy and diseased poultry." Veterinary Microbiology **94**(3): 225.

http://www.sciencedirect.com/science/article/B6TD6-48KFGND-3/2/b03de9e7ecff9c766576b2027a19ab96

The bacterium Clostridium perfringens can cause both clinical and subclinical disease in poultry. To study the pathogenesis and epidemiology of disease caused by C. perfringens, methods for typing its various strains need to be evaluated. C. perfringens isolates from healthy and diseased poultry from different parts of Sweden were analysed by polymerase chain reaction (PCR) in order to establish the presence of [alpha]-, [beta]-, [beta]2-, [epsiv]-, [iota]- and enterotoxin genes. In order to subtype C. perfringens isolates, the two methods amplified fragment length polymorphism (AFLP) and pulsed field gel electrophoresis (PFGE) were compared on 21 C. perfringens isolates from 10 different farms. In a second study, 32 isolates of C. perfringens type A from three broilers from a healthy flock reared without ionophorous anticoccidials were subtyped by PFGE. All 53 isolates analysed with PCR belonged to the toxin type A of C. perfringens, with the gene coding for [alpha]-toxin production. Two isolates possessed the [beta]2-gene as well, but none had the other toxin genes. Both AFLP and PFGE differentiated 21 strains into 10 different subtypes. This differentiation correlated closely with the origins of the isolates. Unique subtypes were isolated from seven farms. Only isolates from birds of one farm demonstrated more than one subtype of C. perfringens. The subtyping of the isolates from a healthy flock showed that each bird carried two to three different subtypes and two different subtypes were found in the same kind of tissue sample in four cases. Three of the four different subtypes found in this study were new, compared with the first study. AFLP and PFGE were found to be equally suitable for subtyping of C. perfringens isolates. The wide variation in subtypes in the healthy broilers could be the result of the antibiotic-free rearing of these birds.

Fickel, J., D. Lieckfeldt, et al. (2003). "Comparison of glycoprotein B (gB) variants of the elephant endotheliotropic herpesvirus (EEHV) isolated from Asian elephants (Elephas maximus)." Veterinary Microbiology **91**(1): 11.

http://www.sciencedirect.com/science/article/B6TD6-46YJ2PV-1/2/7ed6dba11ded470ffe7e59f3b2223f4a

The recently described elephant endotheliotropic herpesviruses (EEHV) have been associated with the deaths of numerous captive elephants. A proposed tool for the detection of EEHV infection in elephants is the PCR-based screening for EEHV-DNA in whole blood samples. Unfortunately, this detection method has only been successful in post-mortem analyses or in animals already displaying clinical signs of EEHV disease, thus rendering this method unsuitable for identification of carrier elephants. Here, we focus on glycoprotein B (gB) for serologic assay development, since gB is an envelope protein known to induce a neutralising antibody response in other herpesvirus infections. We sequenced the entire gB gene from five Asian elephants with EEHV, representing four different gB variants. Computer-aided methods were used to predict functionally important regions within EEHVgB. An extra-cytoplasmic region of 153 amino acids was predicted to be under positive selection and may potentially contain antigenic determinants that will be useful for future serologic assay development.

Frydendahl, K., T. Kare Jensen, et al. (2003). "Association between the porcine Escherichia coli F18 receptor genotype and phenotype and susceptibility to colonisation and postweaning diarrhoea caused by E. coli O138:F18." Veterinary Microbiology **93**(1): 39.

http://www.sciencedirect.com/science/article/B6TD6-47RJMC6-1/2/2aa0ce4cb4c99377860327e4370c0f04

Porcine postweaning Escherichia coli enteritis is a cause of significant morbidity and mortality in pigs worldwide, and effective prevention remains an unsolved problem. This study examined the correlation between susceptibility of pigs to experimental infection with an E. coli F18 strain and the porcine intestinal F18 receptor genotypes. Thirty-one pigs classified as either belonging to the susceptible or the resistant genotype were inoculated with cultures of an E. coli O138:F18 isolated from a pig with postweaning diarrhoea. Susceptibility to colonisation and diarrhoea was assessed by clinical observations, faecal shedding of the challenge strain, histopathology and microscopic adhesion tests. Ten of 14 (71.4%) genetically susceptible pigs and one of 17 (5.9%) resistant pigs developed diarrhoea attributable to the challenge strain. There was no difference in susceptibility between homozygotic and heterozygotic susceptible pigs. Faecal shedding of the challenge strain correlated with the genetic receptor profile. Twenty pigs examined immunohistochemically revealed focal to extensive small intestinal mucosal colonisation by E. coli O138:F18 in nine of 10 susceptible and three of 10 resistant pigs. Results of in vitro adhesion assays performed with F18 cells on enterocyte preparations from 24 pigs, showed complete concordance with the F18 genotypes. In conclusion, this study showed a high correlation between the porcine intestinal F18 receptor genotypes and susceptibility to disease. However, pigs of the resistant F18 receptor genotype were not entirely protected against intestinal colonisation by E. coli F18.

Galosi, C. M., C. G. Barbeito, et al. (2004). "Argentine strain of equine herpesvirus 1 isolated from an aborted foetus shows low virulence in mouse respiratory and abortion models." Veterinary Microbiology **103**(1-2): 1.

http://www.sciencedirect.com/science/article/B6TD6-4D99SY5-1/2/5a31317fbb3ff73e6c7559b1c980a00f

The equine herpesvirus 1 (EHV-1) was isolated in Argentina from an aborted equine foetus in 1979. This virus (SPv) has special restriction patterns (RP) in comparison with other Argentine isolates. In addition, SPv could be distinguished on the basis of its pathogenicity in baby mice inoculated intracerebrally. We studied the growth properties of the SPv in cell culture and its effects in a mouse respiratory and abortion model. We observed that SPv did not modify its capacity to grow in cell culture with respect to reference HH1 strain. Nevertheless, we found significant differences between the titres of the two strains at 8-14 h post-infection (PI). In this work we demonstrated that SPv showed low virulence in female at different stages of gestation, consistently, with results found in the mouse respiratory model. We considered that this low virulence of SPv could be related to its RP because the RP of HH1 strain are similar to those of the HVS25A strain and both showed effect on pregnant mice. More specific studies about genomic alterations to the SPv are necessary for identifying, more clearly, if the intra-strain variations have relation with the low virulence in the mouse respiratory and abortion model.

Girjes, A. A., A. Hugall, et al. (1993). "Comparison of type I and type II Chlamydia psittaci strains infecting koalas (Phascolarctos cinereus)." Veterinary Microbiology **37**(1-2): 65.

http://www.sciencedirect.com/science/article/B6TD6-476F474-

7/2/92a4399988269b12d7d056bbb547dc5a

The native Australian marsupial Phascolarctos cinereus, otherwise known as the koala, is prone to infection by the obligate intracellular parasite Chlamydia psittaci, which causes ocular 'pink eye' and urogenital 'dirty tail' diseases. Several chlamydial DNA probes to both chromosomal and plasmid sequences were used to type by Southern blot analysis 51 samples taken from wild and captive koalas from habitats on the eastern seaboard of Australia as far apart as Queensland and Victoria. Two types of C. psittaci were observed and called types I and II. Type II was found more frequently than type I and occurred in both ocular and urogenital samples, while type I showed a strong but not absolute preference for ocular sites.Cross-hybridization analyses indicated that type I and type II had about 10% DNA sequence identity to each other. DNA analyses showed that type II was very closely related to some ovine and bovine chlamydiae but type I could not be related to any other C. psittaci strain available. Light and electron microscopic analyses of infected BGM monolayers revealed that the two strains were similar in morphological ch characteristics. The type I strain was considerably more infectious than the type II strain in BGM cells and in the yolk sacs of embryonated eggs.A PCR based assay detected both type I and type II koala chlamydiae in samples that had been negative by Southern blot and tissue culture and provided the first evidence that both types can occur simultaneously at the one site of infection.

Gradil, C., M. Sampath, et al. (1994). "Detection of verotoxigenic Escherichia coli in bull semen using the polymerase chain reaction." Veterinary Microbiology **42**(2-3): 239.

http://www.sciencedirect.com/science/article/B6TD6-476TTW8-G/2/21e1d28a50e2303893510d1c51162351

Oligonucleotide primers used in a polymerase chain reaction (PCR) protocol detected the verotoxin 2 (VT2) gene in E. coli present in experimentally contaminated bull semen. The VT2 (Shiga-like toxin II [SLT-II]) primers targeted a 346-bp fragment of the gene coding for the A subunit of the toxin. PCR products, corresponding to the VT2 gene sequence, were amplified from template E. coli nucleic acid extracted from 18-h broth culture and from E. coli in contaminated semen in the undiluted state, diluted in egg yolk-Tris and diluted in milk. The sensitivity of the assay to detect E. coli was determined to be 1 pg of nucleic acid, and as few as 10-20 E. coli organisms/ml could be detected in raw and diluted semen. Preliminary confirmation of the PCR product was accomplished by slot blot hybridization to a radiolabeled specific oligoprobe.Sequencing of the PCR products identifying VT2 gene sequence revealed 99.7% homology with published gene sequences for VT2. This study demonstrates the feasibility of applying PCR technology for the detection of E. coli in bovine semen. This technique may find wide application for the detection of other pathogens that may be present in semen.

Gram, T., P. Ahrens, et al. (2000). "An Actinobacillus pleuropneumoniae PCR typing system based on the apx and omlA genes -- evaluation of isolates from lungs and tonsils of pigs." Veterinary Microbiology **75**(1): 43.

http://www.sciencedirect.com/science/article/B6TD6-40J1DVM-5/2/f861aeb4f02e9681867e88361b77d96e

The genetic variability of a gene coding for an outer membrane lipoprotein (omlA) was used to develop a PCR typing system for Actinobacillus pleuropneumoniae. Sequence differences in the middle region of the gene divided the A. pleuropneumoniae serotypes in five distinct groups. Group I included serotypes 1, 9, 11 and 12 (omlA I), Group II consisted of serotypes 2 and 8 (omlA II), Group III included serotypes 3, 6 and 7 (omlA III), Group IV (omlA IV) consisted of

serotype 4 and Group V of serotypes 5a, 5b and 10 (omlA V). The sequence differences were utilized to construct PCR primers specific for each group, except of Group IV, as the amplicon of serotype 4 could be separated from Group III by size. Together with a PCR apx typing system, the omlA PCR typing system could discriminate the majority of A. pleuropneumoniae serotypes of biovar 1 except of serotypes 1, 9 and 11 and serotypes 2 and 8. The PCR typing system was tested on 102 field strains of A. pleuropneumoniae isolated from lungs of diseased pigs. The serotyping results of the investigated field strains were in agreement with the apx and omlA gene patterns found in the reference strains of the bacteria, with the exception of the omlA gene of five strains of serotype 8. To examine the apx and omlA gene pattern of tonsil isolates, the PCR typing system was tested on a total of 280 A. pleuropneumoniae field strains isolated from tonsils of pigs. Agreement between serotyping and DNA typing was found in 96% of the isolates using the apx gene patterns and in 89% of the isolates using the omlA gene. The same serotype specific apx/omlA gene pattern was thus found in the majority of the tonsil isolates and in isolates from diseased lungs. Most of the differences in the omlA gene were found in 18 tonsil isolates of serotype 12. The omlA/apx PCR typing system described in the present study makes it possible to determine the type specificity of the majority of A. pleuropneumoniae isolates by simple PCR technique and enables phenotype independent characterization of isolates non-typable by serotyping.

Gram, T., P. Ahrens, et al. (1996). "Evaluation of a PCR for detection of Actinobacillus pleuropneumoniae in mixed bacterial cultures from tonsils." Veterinary Microbiology **51**(1-2): 95.

http://www.sciencedirect.com/science/article/B6TD6-3W0FBDG-B/2/d92518ae253c6f1038f7707ca3438b04

A PCR for the detection of Actinobacillus pleuropneumoniae was evaluated. All of 102 field isolates of A. pleuropneumoniae reacted in the PCR by amplification of a 985 bp product. No PCR amplification product was observed when examining strains of A. wreae, A. capsulatus, A. hominis, A. equuli, A. rossii, A. suis, Escherichia coli, Bordetella bronchiseptica, Streptococcus suis, Pasteurella haemolytica, Pasteurella multocida, Haemophilus parasuis, Haemophilus taxon Minor group, Haemophilus taxon D/E and Haemophilus taxon F. Amplification of a 985 bp product was, however, observed when testing strains of A. lignieresii. The lower detection limit of the PCR test was 103 A. pleuropneumoniae CFU/PCR test tube and was not affected by addition of 106 E. coli CFU/PCR test tube. Mixed bacterial cultures from tonsils of 101 pigs from 9 different herds were tested by culture and by PCR using four different bacteriological media. While 65% reacted positive in the PCR only 23% were positive by culture, thereby suggesting a superior sensitivity of the PCR test to that of culture. The use of selective media, large inoculum and incubation for 48 h gave the highest number of positive PCR reactions from mixed bacterial cultures. Tonsil cultures from 50 pigs from an A. pleuropneumoniae-negative herd did not react in the PCR. The results show that PCR on mixed bacterial cultures from tonsils may be a highly sensitive method for the detection of A. pleuropneumoniae in pig herds.

Grom, J. and D. Barlic-Maganja (1999). "Bovine viral diarrhoea (BVD) infections - control and eradication programme in breeding herds in Slovenia." Veterinary Microbiology **64**(2-3): 259.

http://www.sciencedirect.com/science/article/B6TD6-3VNR6P1-J/2/2c5d350d786a21fabcc7028b4f892bcd

A Slovenian BVD control and eradication programme was initiated in 1994, and the results from testing of bovine herds for antigen and antibodies in 1996 are presented. Samples originating from breeding herds, breeding herds for young bulls, and insemination stations were tested by antigen or antibody ELISA, or by PCR. Out of 7968 samples from 354 herds we found 18% of the

animals antibody-positive. In one region situated in the north-east of Slovenia we found the herds to be almost nearly free of BVDV infections (5% prevalence). No positive antigen ELISA findings were done in 374 blood samples from recruitment herds for young bulls, whereas two out of 206 sera were investigated by PCR-reacted positive. The differences in seroprevalence found between regions is thought to be caused by differences in summer pasturing and husbandry practices.

Guliani, S., G. A. Smith, et al. (1999). "Reactivation of a macropodid herpesvirus from the eastern grey kangaroo (Macropus giganteus) following corticosteroid treatment." Veterinary Microbiology **68**(1-2): 59.

http://www.sciencedirect.com/science/article/B6TD6-3XMPK3F-7/2/a208af37c16e05a1f515f49a230e316f

The family Herpesviridae is a large group of viruses which contain double stranded DNA genomes. Biological characteristics, such as host signs, site of replication and site of latency have been used to describe three major subfamilies, Alphaherpesvirinae, Betaherpesvirinae and Gammaherpesvirinae within the family Herpesviridae. Macropodid herpesviruses (MaHV) have been implicated in fatal outbreaks amongst the captive marsupial populations of Australia. These outbreaks have resulted in the isolation of nine MaHV strains which have been classified into two species called macropodid herpesvirus 1 and 2 (MaHV-1 and MaHV-2). Biological characteristics have been used to place MaHV-1 and -2 within the subfamily Alphaherpesvirinae. Molecular phylogenetic reconstructions indicate an unusual position for MaHV-1 and -2 within the alphaherpesviruses. Current isolates of MaHVs have all been obtained from marsupials exhibiting clinical disease. A common biological characteristic of herpesviruses is the establishment of latent infections in nervous tissue. We have determined that MaHV are able to latently infect eastern grey kangaroos through reactivating and isolating a herpesvirus by inducing immunosuppression. We have investigated the possible sites of latency for MaHV-1 using molecular techniques. Detection of herpesvirus DNA in the trigeminal ganglia taken from two naturally infected eastern grey kangaroos indicates dissemination via a respiratory route.

Gunn-Moore, D. A., T. J. Gruffydd-Jones, et al. (1998). "Detection of feline coronaviruses by culture and reverse transcriptase-polymerase chain reaction of blood samples from healthy cats and cats with clinical feline infectious peritonitis." Veterinary Microbiology **62**(3): 193.

http://www.sciencedirect.com/science/article/B6TD6-3TNYWT9-3/2/ae9ad7cd4e4b652c0f977f248732f1ae

A reverse transcriptase-polymerase chain reaction (RT-PCR) assay for the detection of the feline coronavirus (FCoV) genome and a co-cultivation method for the isolation of field strains of FCoV are described. Using the RT-PCR assay to assess blood samples from cats with feline infectious peritonitis (FIP) (n=47) and healthy cats from households with endemic FCoV (n=69) it was shown that approximately 80% of the cats were viraemic, irrespective of their health status. It was also shown that, over a 12-month period, a similar percentage of healthy cats remained viraemic, and that the presence of viraemia did not appear to predispose the cats to the development of FIP. The co-cultivation system proved to be a suitable method for the culture of field strains of FCoV from blood samples, so long as the cultures were maintained for at least 4 weeks. Using this system, followed by the RT-PCR, viraemia was detected as frequently as by RT-PCR on RNA extracted directly from peripheral blood mononuclear cells.

Hassanein, R., T. Sawada, et al. (2003). "Molecular identification of Erysipelothrix isolates from the tonsils of healthy cattle by PCR." Veterinary Microbiology **95**(4): 239.

http://www.sciencedirect.com/science/article/B6TD6-496NMHH-3/2/a13ed83e7586933d92be673910134ac5

For 79 isolates from the tonsils of healthy cattle identified as Erysipelothrix by cultivation, biochemical and serological tests, genotypic identification was performed by polymerase chain reaction (PCR) using four species-specific sets of oligonucleotide primers (ER1F-ER1R, ER2F-ER2R, ER3F-ER3R and ER4F-ER4R). The results of PCR for 79 bovine isolates were compared with those of serological typing. For 19 isolates, serotyping and genotyping results were the same. PCR allowed for the identification of 36 untypable isolates as Erysipelothrix species, strain 1. Serotyping and genotyping results of the remaining 24 isolates were different. Supplemental tests are frequently needed for Erysipelothrix identification.

Hewinson, R. G., P. C. Griffiths, et al. (1997). "Detection of Chlamydia psittaci DNA in avian clinical samples by polymerase chain reaction." Veterinary Microbiology **54**(2): 155.

http://www.sciencedirect.com/science/article/B6TD6-3RH11R6-G/2/ec10531407407d0dce7f1895b9d45e90

A polymerase chain reaction (PCR) assay was developed to detect Chlamydia psittaci DNA in faeces and tissue samples from avian species. Primers were designed to amplify a 264 bp product derived from part of the 5' non-translated region and part of the coding region of the ompA gene which encodes the major outer membrane protein. Amplified sequences were confirmed by Southern hybridization using an internal probe. The sensitivity of the combined assay was found to be between 60 to 600 fg of chlamydial DNA (approximately 6 to 60 genome copies). The specificity of the assay was confirmed since PCR product was not obtained from samples containing several serotypes of C. trachomatis, strains of C. pneumoniae, the type strain of C. pecorum, nor from samples containing microorganisms commonly found in the avian gut flora. In this study, 404 avian faeces and 141 avian tissue samples received by the Central Veterinary Laboratory over a 6 month period were analysed by PCR, antigen detection ELISA and where possible, cell culture isolation. PCR performed favourably compared with ELISA and cell culture, or with ELISA alone. The PCR assay was especially suited to the detection of C. psittaci DNA in avian faeces samples. The test was also useful when applied to tissue samples from small contact birds associated with a case of human psittacosis where ELISA results were negative and chlamydial isolation was a less favourable method due to the need for rapid diagnosis.

Hirasawa, T., T. Kaneshige, et al. (1994). "Sensitive detection of canine parvovirus DNA by the nested polymerase chain reaction." Veterinary Microbiology **41**(1-2): 135.

http://www.sciencedirect.com/science/article/B6TD6-476VK43-TY/2/e43e77c6eb336358e0314272f80dad6f

A polymerase chain raction (PCR) for the detection of canine parvovirus (CPV) was developed. To increase the sensitibity and specificity of the reaction, the nested PCR with a double-nested primer pair (inner primer pair) was designed. The sequences of the PCR primer pairs were selected from the conserved region in the CPV VP1/VP2 gene. The PCR with the outer or inner primer pair alone (single PCR) could detect 10 fg of viral replicative form (RF) DNA on agarose gel electrophoresis; whereas as little as 100 ag of the RF DNA was detected by the nested PCR,

which was shown to be 100 times more sensitive than the single PCR. Samples prepared from feline panleukopenia virus and mink enteritis virus, both having a very close antigenic relationship to CPV, were also amplified by the nested PCR. The specificity of the reaction was confirmed by restriction enzyme analysis and Southern hybridization. Next, fecal samples were examined by the nested PCR. All 10 samples suspected of CPV infection were positive, and two restriction sites (HaeIII and HindIII sites) in the PCR product were conserved among them. On the other hand, specific amplification was not observed in the samples derived from normal dogs. The number of the genome copy in positive samples was estimated about 109-1011/g by the single PCR and 1011-1013/g by the nested PCR. The assay can be completed in 1-1.5 days, and does not need radioisotopes. Thus, the nested PCR seems to be a sensitive, specific and practical method for the detection of CPV in fecal samples.

Huang, B., S. Subramaniam, et al. (2002). "Vaccination of ducks with recombinant outer membrane protein (OmpA) and a 41 kDa partial protein (P45N') of Riemerella anatipestifer." Veterinary Microbiology **84**(3): 219.

http://www.sciencedirect.com/science/article/B6TD6-449V2NF-1/2/e08033c518bf236da7cd8900f8e827d8

The generation of protective immunity against Riemerella anatipestifer infection in ducks were investigated by immunizations with recombinant glutathione sulfatransferase (GST) fusion's proteins of OmpA, a 42 kDa major outer membrane protein, and P45N', a 41 kDa N-terminal fragment of a newly identified 45 kDa potential surface protein from R. anatipestifer. The DNA encoding OmpA and P45N' were isolated from R. anatipestifer serotype 15 (field strain 110/89) and serotype 19 (reference strain 30/90), respectively. Immunoblotting and ELISA results showed that the purified recombinant proteins induced the production of antibodies in immunized ducks. However, neither was protective against subsequent challenge with the virulent serotype 15 strain, 34/90. All the five ducks immunized with formalinized R. anatipestiferstrain 34/90 survived the challenge with the homologous strain whereas six out of seven ducks in the non-immunized control group died within a week following the challenge.

Imberechts, H., H. U. Bertschinger, et al. (1994). "Prevalence of F107 fimbriae on Escherichia coli isolated from pigs with oedema disease or postweaning diarrhoea." Veterinary Microbiology **40**(3-4): 219.

http://www.sciencedirect.com/science/article/B6TD6-476VKWD-141/2/8f5d69e54fbbd1b31390817346d5a444

The study comprises fifty 4 to 12 weeks old pigs that died from oedema disease or severe diarrhoea. Smears were prepared from the mucosa of duodenum, jejunum and ileum, and by immunofluorescence F107 fimbrial antigens were detected. E. coli. strains were isolated from the intestines and were characterised by slide agglutination (serogroup and F107 fimbriae production), by their cytotoxicity for Vero cells, and by gene amplification (genes coding for the major F107 subunit FedA, the toxin causing oedema disease SLT-IIv, and enterotoxins LTI, STIa and STII). F107 fimbriae were demonstrated in association with E. coli of serogroups O139:K12 and O141:K85a,b but not of serogroup O149:K91:F4a,c. Expression in culture of F107 fimbriae by some isolates gave additional evidence for production of these fimbriae by ETEC strains. The genetic determinant of SLT-IIv was found in association with F107, and could not be detected in serogroup O149:K91:F4a,c. Gene fedA was demonstrated in two isolates which were devoid of SLT-IIv. Most isolates from cases of oedema disease belonged to serogroup O139:K12 and did not contain enterotoxin genes. Isolates from pigs that suffered from diarrhoea were serotyped O141:K85a,b or O149:K91:F4a,c, and carried at least two enterotoxin genes in their genomes. In

a small proportion of the cases F107 antigens were demonstrated in intestinal smears although gene fedA was not detected in the corresponding isolates. The results confirm the importance of F107 fimbriae as virulence factor in oedema disease E coli strains, but also demonstrate that F107 fimbriae can be found in association with postweaning diarrhoea isolates. In these latter strauns enterotoxins were always demonstrated, irrespective of the presence of toxin SLT-IIv.

Jacobson, M., A. Aspan, et al. (2004). "Routine diagnostics of Lawsonia intracellularis performed by PCR, serological and post mortem examination, with special emphasis on sample preparation methods for PCR." Veterinary Microbiology **102**(3-4): 189.

http://www.sciencedirect.com/science/article/B6TD6-4D3WHJ1-1/2/f1594a63f8f47f0bf2543e3aa1b0e966

The aim of this study was to find suitable and reliable tools for demonstrating Lawsonia intracellularis in routine clinical diagnosis. Firstly, a method to prepare tissue samples before a polymerase chain reaction (PCR) was evaluated in pigs submitted for necropsy. Secondly, seven different faecal preparation methods and four different DNA polymerases were tested in single or nested PCR, with co-amplification of a mimic molecule. Thirdly, in selected pigs submitted for necropsy, tissue and faecal samples were examined histopathologically and by PCR, and blood samples were analysed serologically. Detection of L. intracellularis in tissue preparations by PCR showed good specificity and correlated to lesions found at necropsy. The sensitivity in spiked tissue samples was 101-102 mimic molecules per tube. In faecal samples, nested PCR on boiled lysate gave the best result with a sensitivity of 102-103 mimic molecules per reaction tube. However, because of the time-consuming procedure and the increased risk for contamination, a commercially available kit was preferred for routine diagnoses, despite a somewhat lower detection rate in subclinically infected pigs. In a few cases, the serological results differed from those obtained by PCR and by necropsy but the reason for this is not clear. This study indicates that the best method for diagnosis of acute enteritis in growers is PCR on faecal or tissue samples. To determine the presence of the bacteria in a herd, serology or repeated faecal sampling for PCR from target animals, or both, should be used.

Johansson, A., C. Greko, et al. (2004). "Antimicrobial susceptibility of Swedish, Norwegian and Danish isolates of Clostridium perfringens from poultry, and distribution of tetracycline resistance genes." Veterinary Microbiology **99**(3-4): 251.

http://www.sciencedirect.com/science/article/B6TD6-4BVPTPT-1/2/cb5985a4d0e3b2049a502980879df8a1

This study was undertaken to determine the in vitro susceptibility of Clostridium perfringens, isolated from poultry to antimicrobials used in poultry production. The minimal inhibitory concentration (MIC) of eight antimicrobials, including the ionophoric coccidiostat narasin, was determined for 102 C. perfringens isolates, 58 from Sweden, 24 from Norway and 20 from Denmark. Susceptibility to each antimicrobial compound was determined by broth microdilution. The isolates were obtained from broilers (89), laying hens (9) and turkeys (4), affected by necrotic enteritis (NE) or by C. perfringens associated hepatitis (CPH), and from healthy broilers. All strains, regardless of origin, proved inherently susceptible to ampicillin, narasin, avilamycin, erythromycin and vancomycin. A low frequency of resistance to virginiamycin and bacitracin was also found. Resistance to tetracycline was found in strains isolated in all three countries; Sweden (76%), Denmark (10%) and Norway (29%). In 80% of the tetracycline-resistant isolates, the two resistance genes tetA(P) and tetB(P) were amplified by PCR whereas in 20% only the tetA(P) gene was detected. No tetM gene amplicon was obtained from any of the tetracycline-resistant isolates. The uniform susceptibility to narasin revealed in this study shows that the substance can

still be used to control clostridiosis. In this study, C. perfringens also showed a low degree of resistance to most other antimicrobials tested. Despite the small amounts of tetracycline used in poultry, a considerable degree of resistance to tetracycline was found in C. perfringens isolates from Swedish broilers.

Jordan, D. M., J. P. Knitted, et al. (2004). "A Lawsonia intracellularis transmission study using a pure culture inoculated seeder-pig sentinel model." Veterinary Microbiology **104**(1-2): 83.

http://www.sciencedirect.com/science/article/B6TD6-4DJ4CM6-2/2/8469b96cc74fc04e18f56a63aaa54bd6

Transmission of Lawsonia intracellularis from experimentally inoculated pigs to naive swine was demonstrated in this study. The study was conducted using conventional pigs divided into three groups as follows: principles inoculated with L. intracellularis, sentinels, and controls. The pigs were inoculated and paired on 13 and 9 days post-inoculation with a sentinel pig for 7 days. Fecal samples and serum samples were collected throughout the study for polymerase chain reaction (PCR) and antibody testing by indirect fluorescent antibody techniques. After co-mingling, the inoculated group was necropsied; sentinel and control pigs were necropsied 7-14 days later. The intestinal tracts were evaluated grossly and microscopically for lesions. PCR was performed on intestinal mucosal scrapings and feces. Warthin-Starry and fluorescent antibody staining procedures were conducted to confirm colonization with L. intracellularis. Gross and microscopic lesions typical of porcine proliferative enteropathy (PPE) were observed in both the inoculated and sentinel groups. Transmission was demonstrated from inoculated principle pigs to sentinel pigs. PCR results detected cyclical shedding of L. intracellularis in the feces. Seroconversion occurred in pigs that were exposed to L. intracellularis. From this study, it was demonstrated that transmission of L. intracellularis can occur easily in an environment with experimentally infected pigs and that PCR can be a useful tool to monitor fecal shedding of the organism.

Keil, D. J. and B. Fenwick (1999). "Evaluation of canine Bordetella bronchiseptica isolates using randomly amplified polymorphic DNA fingerprinting and ribotyping." Veterinary Microbiology **66**(1): 41.

http://www.sciencedirect.com/science/article/B6TD6-3W37Y3C-4/2/b6f509cc2cb7001c1fbd167ce79c7348

Bordetella bronchiseptica is a respiratory tract pathogen in a variety of species. Previous studies suggest little genetic variation among canine B. bronchiseptica isolates. The degree of genetic diversity in 26 canine B. bronchiseptica strains was evaluated using randomly amplified polymorphic DNA (RAPD) fingerprinting and ribotyping. Strains evaluated include historic reference strains (N = 3), vaccine strains (N = 5) and clinical isolates (N = 18). RAPD fingerprinting with the 10-nucleotide primer OPA-4 resulted in four distinct fingerprint patterns. RAPD fingerprinting consistently separated four previously characterized electromorphotype (EMT) 6 strains into two fingerprint types. Ribotyping, using the restriction endonuclease PvuI, resulted in six distinct ribotypes. With the exception of vaccine strains, considerable genetic diversity exists in the canine B. bronchiseptica isolates examined. These findings indicate the genetic variability within canine strains of B. bronchiseptica is greater than appreciated previously. Additionally, OPA-4 RAPD fingerprinting and PvuI ribotyping will be useful tools in epidemiologic studies of canine B. bronchiseptica isolates.

Key, K. F., G. Haqshenas, et al. (2001). "Genetic variation and phylogenetic analyses of the ORF5 gene

of acute porcine reproductive and respiratory syndrome virus isolates." Veterinary Microbiology **83**(3): 249.

http://www.sciencedirect.com/science/article/B6TD6-441H24T-5/2/ece5601046707d9b4fab5af29b98e136

Swine herds in the US have experienced recent outbreaks of a severe form of porcine reproductive and respiratory syndrome (designated acute or atypical PRRS) characterized by abortion and high mortality in pregnant sows. Most of the affected herds had been vaccinated with modified live-vaccines (MLVs) against PRRS. To explore the possible mechanism of the emergence of acute PRRS, the open reading frame 5 (ORF5) gene encoding the major envelope protein (GP5) of acute PRRSV isolates was characterized. The complete ORF5 gene of eight acute PRRSV isolates from herds experiencing acute PRRS outbreaks in Iowa and North Carolina was amplified and sequenced. Sequence analyses revealed that these acute PRRSV isolates shared 88-95% nucleotide and 88-96% amino acid sequence identities to each other, 87-97% nucleotide and 84-96% amino acid sequence identities with other North American PRRSV isolates and the MLVs. Most of the amino acid substitutions locate in the putative signal sequence and two short hypervariable regions at the amino terminus. The ORF5 gene sequence of the acute PRRSV isolate 98-37120-2 from a non-vaccinated swine herd in Iowa is very closely related to that of the RespPRRS MLV, with 97% nucleotide and 96% amino acid sequence identities. Phylogenetic analysis revealed that all eight acute PRRSV isolates are clustered within the North American genotype. Several minor branches that are not associated with geographic origins were also identified within the North American genotype. One acute PRRSV isolate (98-37120-2) is clustered with the RespPRRS MLV and several Danish isolates that were confirmed to be derived from the RespPRRS MLV. The ORF5 gene sequences of other seven acute isolates are more related to those of several earlier PRRSV isolates and the PrimePac MLV than to that of the RespPRRS MLV. Our results showed that the acute PRRSV isolates analyzed in this study differed from each other in ORF5 genes, although they all clustered within the North American genotype. The data from this study do not fully support the hypothesis that the emergence of acute PRRS is due to reversion of MLVs to a pathogenic phenotype, as only one of the eight acute isolates was shown to be very closely related to the RespPRRS MLV.

Larsen, L. E., T. Storgaard, et al. (2001). "Phylogenetic characterisation of the GL sequences of equine arteritis virus isolated from semen of asymptomatic stallions and fatal cases of equine viral arteritis in Denmark." Veterinary Microbiology **80**(4): 339.

http://www.sciencedirect.com/science/article/B6TD6-4325YY9-4/2/f224338dab2aad814905deba3729d6c5

The study describes for the first time the phylogenetic relationship between equine arteritis virus (EAV) isolated from asymptomatic virus-shedding stallions and fatal cases of equine viral arteritis (EVA) in an European country. EAV was isolated from three dead foals and an aborted foetus during three different outbreaks of EVA. From these fatalities, the complete open reading frame 5, encoding the EAV GL protein, was amplified by reverse transcription-polymerase chain reaction and subjected to nucleotide sequence analysis. Furthermore, DNA sequences were obtained from virus isolated from semen samples of seven virus-shedding, but clinically healthy, Danish stallions. DNA sequence alignment revealed an overall divergence of 0-14 and 0-10% at the nucleotide and amino acid levels, respectively. Phylogenetic analysis including 24 previously published sequences revealed that European as well as North American "types" of EAV were present in the semen of asymptomatic carrier stallions and in fatal cases of EVA. Our results reveal that the presence of EAV-shedding stallions in Denmark represents a potential source of severe EVA.

Larsen, L. E., A. Uttenthal, et al. (1998). "Serological and genetic characterisation of bovine respiratory syncytial virus (BRSV) indicates that Danish isolates belong to the intermediate subgroup: no evidence of a selective effect on the variability of G protein nucleotide sequence by prior cell culture adaption and passages in cell culture or calves." Veterinary Microbiology **62**(4): 265.

http://www.sciencedirect.com/science/article/B6TD6-3TTCH9V-2/2/b4ce584f358a07f71bcf65c8d2cbc643

Danish isolates of bovine respiratory syncytial virus (BRSV) were characterised by nucleotide sequencing of the G glycoprotein and by their reactivity with a panel of monoclonal antibodies (MAbs). Among the six Danish isolates, the overall sequence divergence ranged between 0 and 3% at the nucleotide level and between 0 and 5% at the amino acid level. Sequence divergences of 7-8%, 8-9% and 2-3% (nucleotide) and 9-11%, 12-16% and 4-6% (amino acid) were obtained in the comparison made between the group of Danish isolates and the previously sequenced 391-2USA, 127UK and 220-69Bel isolates, respectively. Phylogenetic analysis showed that the Danish isolates formed three lineages within a separate branch of the phylogenetic tree. Nevertheless, the Danish isolates were closely related to the 220-69Bel isolate, the prototype of the intermediate antigenic subgroup. The sequencing of the extracellular part of the G gene of additional 11 field BRSV viruses, processed directly from lung samples without prior adaption to cell culture growth, revealed sequence variabilities in the range obtained with the propagated virus. In addition, several passages in cell culture and in calves had no major impact on the nucleotide sequence of the G protein. These findings indicated that the previously established variabilities of the G protein of RS virus isolates were not attributable to mutations induced during the propagation of the virus. The reactivity of the Danish isolates with G protein-specific MAbs were similar to that of the 220-69Bel isolate. Furthermore, the sequence of the immunodominant region was completely conserved among the Danish isolates on one side and the 220-69Bel isolate on the other. When combined, these data strongly suggested that the Danish isolates belong to the intermediate subgroup.

Madsen, L., F. M. Aarestrup, et al. (2000). "Characterisation of streptomycin resistance determinants in Danish isolates of Salmonella Typhimurium." Veterinary Microbiology **75**(1): 73.

http://www.sciencedirect.com/science/article/B6TD6-40J1DVM-7/2/425b31a9a2087b48989a772126cdc392

Fifty six Danish streptomycin (Sm) resistant isolates of Salmonella enterica serotype Typhimurium from pigs (n=34), calves (n=3) and humans (n=19) were characterised with respect to co-resistances (14 drugs), transferability of Sm-resistance by conjugation, genetic determinants encoding Sm-resistance and diversity with respect to localisation of genes in the genome and DNA-sequences. Forty-six strains carried resistance(s) other than Sm-resistance. Nineteen different co-resistance patterns were observed and tetracycline was the most commonly observed resistance in these patterns. In 22 of the strains, Sm-resistance was transferred by conjugation. Eleven strains contained the gene aadA only, six strains contained aadA+strA+strB, and 35 strains contained strA+strB. Partial sequences of aadA were obtained from four strains. Three strains showed identical sequences to a published aadA sequence from the transposon Tn7, and in one strain the sequence showed one synonymous substitution compared to this sequence. Partial sequences were obtained of strA and strB in seven strains. The sequence of strB was identical to the published sequence of the plasmid RSF1010 in all strains. All seven sequences of strA were identical and differed from the sequence of strA in RSF1010 by two non-synonymous substitutions.

Mahony, T. J., F. M. McCarthy, et al. (2005). "Genetic analysis of bovine viral diarrhoea viruses from

Australia." Veterinary Microbiology **106**(1-2): 1.

http://www.sciencedirect.com/science/article/B6TD6-4FBFPYW-2/2/9e72adbc4ab379211e19c5a081d4368c

Eighty-nine bovine viral diarrhoea viruses (BVDV) from Australia have been genetically typed by sequencing of the 5' untranslated region (5'-UTR) and for selected isolates the Npro region of the viral genome. Phylogenetic reconstructions indicated that all of the samples examined clustered within the BVDV type 1 genotype. Of the 11 previously described genetic groups of BVDV-1, 87 of the samples examined in this study clustered with the BVDV-1c, while two samples clustered with the BVDV-1a. Based on these analyses there appears to be limited genetic variation within the Australian BVDV field isolates. In addition, the phylogenetic reconstructions indicate that the clustering of Australian BVDV in the phylogenetic trees is not a result of geographic isolation.

Manuguerra, J. C., S. Zientara, et al. (2000). "Evidence for evolutionary stasis and genetic drift by genetic analysis of two equine influenza H3 viruses isolated in France." Veterinary Microbiology **74**(1-2): 59.

http://www.sciencedirect.com/science/article/B6TD6-405KDD3-6/2/113e05879cefeaa770960b824e3b5dd7

The amino acid sequences of the HA1 portion of the haemagglutinin of two equine A(H3N8) influenza viruses isolated in France in 1993 and 1998 were analysed to determine their evolutionary relationship with 51 other HA1 amino acid sequences available in databanks. Our data show that the French strain isolated in 1993 belongs to a group of phylogenetically related viruses branched on the main trunk, illustrating the main lineage of evolution of the equine-2 H3 sequences before its split into two distinct lineages in the late 1980s. By contrast, the 1998 French isolate appears to belong to the more recent 'Eurasian' lineage. These data suggest that equine-2 strains antigenically related to old prototype viruses may cocirculate with the more recent 'Eurasian' and 'American' lineages. In conclusion, it may be necessary to include both strains representative of recent equine influenza variants and an older prototype strain in the current equine influenza vaccines.

McKeown, N. E., M. Fenaux, et al. (2004). "Molecular characterization of porcine TT virus, an orphan virus, in pigs from six different countries." Veterinary Microbiology **104**(1-2): 113.

http://www.sciencedirect.com/science/article/B6TD6-4DJ4CM6-6/2/7222a3c1671efc6888a3ca0873adf4df

Human TT virus (TTV), originally isolated from a patient with post-transfusion hepatitis in 1997, is ubiquitous and non-pathogenic. Viruses related to human TTV have since been identified in non-human primates, bovine, ovine, porcine, feline, and canine. The objective of this study was to genetically characterize porcine TTV from pigs in different geographic regions. PCR primers based on the non-coding region of the only available porcine TTV isolate were designed to amplify porcine TTV DNA from sera of pigs in six different countries. Porcine TTV DNA was detected in 66.2% (102/154) of the swine sera. The percentages of positive pigs varied greatly from country to country and even within the same country: 33% in Iowa, USA; 40% in Thailand; 46% in Ontario, Canada; 80% in China; 85% in Korea; 90% in Spain; 100% in Quebec and Saskatchewan, Canada. A total of 40 porcine TTV isolates (five from each geographic region) were sequenced for a 218 bp fragment within the non-coding region. Sequence analyses revealed that porcine TTV isolates from different geographic regions shared 86-100% nucleotide

sequence identity to each other. The prototype Japanese isolate of porcine TTV, Sd-TTV31, shared 90-97% nucleotide sequence identity with porcine TTV isolates reported in this study. Phylogenetic analysis showed that the clustering of the porcine TTV isolates is not associated with geographic origins. Although porcine TTV is not known to be associated with any swine disease, co-infection of pigs with TTV and other known swine pathogens may result in enhanced disease. There are also concerns for risk of potential human infection during xenotransplantation.

Miller, J. M., A. L. Jenny, et al. (2002). "Polymerase chain reaction detection of Mycobacterium tuberculosis complex and Mycobacterium avium organisms in formalin-fixed tissues from culture-negative ruminants." Veterinary Microbiology **87**(1): 15.

http://www.sciencedirect.com/science/article/B6TD6-45FSSJ7-1/2/c1905fd18c803a362b36a51cd4fe3c53

In the US eradication program for bovine tuberculosis, a definitive diagnosis depends on the isolation of Mycobacterium bovis. However, in some cases bacterial culture is unsuccessful, even though the tissue is considered suspicious by histopathology because granulomatous lesions and acid-fast organisms are present. The purpose of this study was to determine if polymerase chain reaction (PCR) tests on formalin-fixed tissue would successfully identify the organisms observed in suspect lesions from culture-negative animals. Diagnostic laboratory records were used to select paraffin blocks of tissue from 102 ruminants that had suspect microscopic lesions but no bacterial isolation. Sections from these blocks were examined with PCR primers for IS6110 to detect Mycobacterium tuberculosis complex infection, or with 16S ribosomal RNA and IS900 primers for detection of Mycobacterium avium. The PCR tests successfully identified a mycobacterial infection in 58 of 102 tissues, including 41 M. tuberculosis complex and 17 M. avium (11 subspecies paratuberculosis). These results demonstrate that PCR testing of formalin-fixed tissue, in combination with bacterial culture, may increase the effectiveness of laboratory diagnostic efforts to detect and identify the most common mycobacterial diseases of ruminants.

Mochizuki, M., R. Harasawa, et al. (1993). "Antigenic and genomic variabilities among recently prevalent parvoviruses of canine and feline origin in Japan." Veterinary Microbiology **38**(1-2): 1.

http://www.sciencedirect.com/science/article/B6TD6-476VKH4-YM/2/0e4ab754f8f1bf69ef95625f9685f3ef

Canine parvovirus type 2 (CPV-2) and feline panleukopenia (FPL) virus (FPLV) are well known and ubiquitous diarrhea-causing pantropic viruses. A "new" antigenic variant of CPV-2 (designated as CPV-2a) has been also prevalent among dogs in Japan. In the present study, 24 canine and 8 feline isolates collected during 1987-1991 were compared with 17 CPV-2 or CPV-2a and 7 FPLV strains that had been characterized previously. Genomic properties were determined by the restriction cleavage patterns of amplified genes encoding the capsid proteins VP1 and VP2 by the polymerase chain reaction. Antigenic properties were determined by hemagglutination-inhibition assay with monoclonal antibodies against an FPLV strain. Growth characteristics in feline CRFK and canine MDCK cells were also examined. Genomic and antigenic properties of the canine isolates were relatively invariable with one exceptional isolate, C27, which was recovered from a typical clinical case of parvovirus infection but possessed properties similar to FPLV rather than CPV-2 and CPV-2a. All isolates from FPL cases possessed the same genomic and antigenic properties as those of reference FPLVs isolated in the 1970s, but three of five strains isolated from the feces of clinically healthy cats were likely to be of canine origin because they possessed very similar properties to CPV-2a. Although species-specificity of these novel isolates could not be determined definitely, the results indicate a possibility that transmission of parvovirus has occurred between these two animal species.

Molia, S., B. B. Chomel, et al. (2004). "Prevalence of Bartonella infection in wild African lions (Panthera leo) and cheetahs (Acinonyx jubatus)." Veterinary Microbiology **100**(1-2): 31.

http://www.sciencedirect.com/science/article/B6TD6-4C0V78T-1/2/1af8cd58d3eeaad030ba2314fe83efec

Bartonella species are emerging pathogens that have been isolated worldwide from humans and other mammals. Our objective was to estimate the prevalence of Bartonella infection in free-ranging African lions (Panthera leo) and cheetahs (Acinonyx jubatus). Blood and/or serum samples were collected from a convenience sample of 113 lions and 74 cheetahs captured in Africa between 1982 and 2002. Whole blood samples available from 58 of the lions and 17 of the cheetahs were cultured for evidence of Bartonella spp., and whole blood from 54 of the 58 lions and 73 of the 74 cheetahs tested for the presence of Bartonella DNA by TaqMan PCR. Serum samples from the 113 lions and 74 cheetahs were tested for the presence of antibodies against Bartonella henselae using an immunofluorescence assay. Three (5.2%) of the 58 lions and one (5.9%) of the 17 cheetahs were bacteremic. Two lions were infected with B. henselae, based on PCR/RFLP of the citrate synthase gene. The third lion and the cheetah were infected with previously unidentified Bartonella strains. Twenty-three percent of the 73 cheetahs and 3.7% of the 54 lions tested by TaqMan PCR were positive for Bartonella spp. B. henselae antibody prevalence was 17% (19/113) for the lions and 31% (23/74) for the cheetahs. The prevalence of seropositivity, bacteremia, and positive TaqMan PCR was not significantly different between sexes and age categories (juvenile versus adult) for both lions and cheetahs. Domestic cats are thus no longer the only known carriers of Bartonella spp. in Africa. Translocation of B. henselae seronegative and TaqMan PCR negative wild felids might be effective in limiting the spread of Bartonella infection.

Nauerby, B., K. Pedersen, et al. (2003). "Analysis by pulsed-field gel electrophoresis of the genetic diversity among Clostridium perfringens isolates from chickens." Veterinary Microbiology **94**(3): 257.

http://www.sciencedirect.com/science/article/B6TD6-48S350K-2/2/06b7d81ce68bbdf9acb27e033414fc27

The aim of this study was to analyse the genetic diversity among Clostridium perfringens isolates from Danish broiler chickens since both sick and presumably healthy animals were investigated. Isolates (n=279) collected from chickens from 25 farms were analysed by pulsed-field gel electrophoresis (PFGE) with the restriction enzyme SmaI. A high genetic diversity was found. Isolates with different PFGE types were toxin typed by PCR and all were found to be of type A. The results showed that healthy broiler chickens carried several different C. perfringens clones both within a flock and even within individual birds, whereas flocks suffering from necrotic enteritis (NE) or cholangio-hepatitis carried only one or two clones.

Noamani, B. N., J. M. Fairbrother, et al. (2003). "Virulence genes of O149 enterotoxigenic Escherichia coli from outbreaks of postweaning diarrhea in pigs." Veterinary Microbiology **97**(1-2): 87.

http://www.sciencedirect.com/science/article/B6TD6-49XPTTK-1/2/8d9bd60bef5253cef966f5eb51a5b5eb

The goal of this research was to determine whether isolates of O149 porcine enterotoxigenic

Escherichia coli (ETEC) recovered from recent outbreaks of severe diarrhea in weaned pigs in Ontario, Canada, had virulence attributes different from those of isolates of the same serogroup from diarrhea of pigs in the 1970s and 1980s. Polymerase chain reaction amplification was used to determine the distribution of 11 virulence-associated genes in recent (100 isolates) and old (35 isolates) Ontario O149 porcine ETEC. These tests demonstrated that 92% of the recent isolates possessed the estA gene for STa enterotoxin, whereas none of the old isolates had this gene. H antigen determination showed that all the isolates which lacked the estA gene (all 35 old isolates plus 8 recent isolates) were H43, whereas isolates which had the estA gene were H10. The astA gene for enteroaggregative heat-stable enterotoxin (EAST1) and the K88ac antigen were present in all 135 isolates. Plasmid analyses identified a cryptic 5.1 kb plasmid in 99% of recent and 60% of old isolates. Suppressive subtractive hybridization associated several types of DNA fragments with the recent O149 ETEC, namely, fragments with no homology to DNA in databases, fragments of LPS biosynthesis genes, and F plasmid DNA. We conclude that the recent outbreaks of PWD in Ontario pigs were associated primarily with a new serotype of O149 ETEC and that isolates of this serotype possessed the estA gene that was not present in old O149 ETEC isolated from pigs in Ontario.

Oleksiewicz, M. B., T. B. Rasmussen, et al. (2003). "Determination of the sequence of the complete open reading frame and the 5'NTR of the Paderborn isolate of classical swine fever virus." Veterinary Microbiology **92**(4): 311.

http://www.sciencedirect.com/science/article/B6TD6-47P1T54-2/2/5d73b40094dc4100fbdbe540ed08e913

The classical swine fever (CSF) epidemic in the Netherlands in 1997-1998 lasted 14 months, during which 429 infected and 1300 at risk herds were culled, at an estimated economical cost of 2 billion US dollars. Despite the overwhelming scale of the epizootic, the CSF virus (CSFV) strain causing the outbreak has remained largely uncharacterized. The Dutch epizootic is epidemiologically linked to a small CSF outbreak in 1997, in Paderborn in Germany. E2 and partial 5' NTR sequencing has shown that the index Paderborn isolate, and several Dutch isolates taken during the 1997-1998 epizootic, are virtually identical, confirming that the Paderborn isolate triggered the Dutch outbreak, and furthermore showing that this single isolate was stable throughout the whole Dutch outbreak (the above reviewed in [C. Terpstra, A. J. de Smit, Veterinary Microbiol. 77 (2000) 3-15]). We determined the nucleotide sequence of the 5' NTR (by 5' RACE) and the complete open reading frame of the Paderborn isolate (GenBank AY072924). Our sequence was identical to previously published partial 5'NTR and E2 sequences for the index Paderborn 1997 and Dutch 1997 (Venhorst) isolates, confirming the identity of the virus we sequenced. Phylogenetic analysis based on the complete open reading frame showed that Paderborn is genetically very different from common European laboratory reference strains. Neutralization studies showed that Paderborn is also antigenically very different from common laboratory strains such as Alfort 187. Paderborn is the only recent European CSFV field isolate for which a complete sequence is available, and given Paderborns genetic and antigenic uniqueness, the Paderborn sequence may have practical use for diagnostic and vaccine antigen development.

Olsen, I., T. B. Johansen, et al. (2004). "A novel IS element, ISMpa1, in Mycobacterium avium subsp. paratuberculosis." Veterinary Microbiology **98**(3-4): 297.

http://www.sciencedirect.com/science/article/B6TD6-4BG3TDW-1/2/1a607c4bf6a8b0f8a4700b911b33dd38

A novel insertion element belonging to the IS110 family was identified in Mycobacterium avium

subsp. paratuberculosis. The IS element, ISMpa1, is 1500 bp and has one ORF encoding a putative transposase. Three copies of ISMpa1 were identified in the M. avium subsp. paratuberculosis genome. The element had inserted into the 3' end of the highly conserved mycobacterial genes prrB and a homologue of M. tuberculosis Rv1593c, and between a putative cytochrome p450 oxygenase and a putative hydrolase. The IS element was present in all (n=11) M. avium subsp. paratuberculosis strains but not detected in most other mycobacterial species examined, including 10 M. avium subsp. avium isolates of human, avian and porcine origin. However two porcine isolates of M. avium subsp. avium and the reference strain IWGMT49 did harbour ISMpa1. These three strains belong to a previously described subgroup of M. avium subsp. avium based on IS1245 restriction fragment length polymorphism (RFLP) pattern and serovars. All of the M. avium subsp. paratuberculosis strains examined had an identical RFLP pattern when probed with sequences corresponding to the 5' end of ISMpa1, whereas a different pattern was seen in the positive M. avium subsp. avium strains. This novel IS element might be a useful tool in strain classification of M. avium subsp. avium and also for the identification of M. avium subsp. paratuberculosis when used in combination with IS900.

Orden, J. A., J. A. Ruiz-Santa-Quiteria, et al. (1998). "Verotoxin-producing Escherichia coli (VTEC) and eae-positive non-VTEC in 1-30-days-old diarrhoeic dairy calves." Veterinary Microbiology **63**(2-4): 239.

http://www.sciencedirect.com/science/article/B6TD6-3V7JFXV-G/2/c9cc35f6e6323241a794838131c7b35f

Faecal samples from 221, 1-30-days-old, diarrhoeic dairy calves were screened for the presence of verotoxin-producing Escherichia coli (VTEC) and eae-positive non-VTEC. Calves were grouped according to their age (1-7, 8-14, 15-21 and 22-30 days) and analyses of prevalences were done by Mantel-Haenzsel [chi]2-test for trend. VTEC and eae-positive non-VTEC were detected in 20 (9.0%) and 18 (8.1%) of the diarrhoeic calves, respectively. A significant age-associated increase in the prevalence of VTEC (p=0.0001), but not in the prevalence of eae-positive non-VTEC (p=0.381), was found. Significant differences in VTEC prevalence were found between the age-group 22-30 days and in all other age-groups. 43 (5.0%) of the 861 E. coli isolates from the 221 diarrhoeic calves were VTEC, and 30 (69.8%) of these strains produced VT1 only. More than one-half of the VTEC strains (55.8%) were positive for the eae gene and all these eae-positive VTEC strains produced VT1 only. A high percentage (76.7%) of VTEC strains belonged to E. coli serogroups (O4, O26, O39, O91, O113, O128 and O145) associated with haemorrhagic colitis and haemolytic uraemic syndrome in humans. 51 (5.9%) of the E. coli strains studied were eae-positive non-VTEC and the serogroups most prevalent among these strains were O4, O14, O26 and O123. Only four of the eae-positive strains were also espB-positive by hybridization with a probe from a human EPEC isolate and none of these strains produced VT.

Orden, J. A., M. Yuste, et al. (2003). "Typing of the eae and espB genes of attaching and effacing Escherichia coli isolates from ruminants." Veterinary Microbiology **96**(2): 203.

http://www.sciencedirect.com/science/article/B6TD6-49H70T5-C/2/9e845db4482746d0cec6486a41164f8b

The types of the eae and espB genes of 178 attaching and effacing Escherichia coli (AEEC) strains isolated from diarrhoeic and healthy ruminants were investigated by PCR. Six types of the eae gene: [beta] (beta), [gamma]1 (gamma-1), [gamma]2 (gamma-2), [epsiv] (epsilon), [zeta] (zeta) and [iota] (iota), and three types of the espB gene: [alpha], [beta] and [gamma] were identified in the strains studied. Moreover, three strains were negative to all the types of the eae gene tested. The types [beta] and [gamma]2 in healthy cattle, [beta], [gamma]2 and [epsiv] in

healthy sheep and goats, and [beta] in diarrhoeic calves, lambs and goat kids were the most frequent types of the eae gene among the strains studied. Although the eae[beta] gene was the most prevalent among AEEC from healthy and diarrhoeic ruminants, the percentages of AEEC strains with this type found in this study in diarrhoeic animals (66.7-100%) were higher than those found in healthy animals (33.3-40.6%). Thus, these data suggest that AEEC strains with the eae[beta] gene are associated with neonatal diarrhoea in ruminants. The eae[gamma]1, eae[zeta] and eae[iota] genes were found in low percentages in the strains studied (4.5, 2.8 and 7.3%, respectively). All the types of the eae gene, except the type [iota], showed a close correlation with the types of the espB gene: the eae[beta] and eae[epsiv] genes with the espB[beta] gene, the eae[gamma]2 and eae[zeta] genes with the espB[alpha] gene and the eae[gamma]1 gene with the espB[gamma] gene.

Payne, C. J., T. M. Ellis, et al. (1999). "Sequence data suggests big liver and spleen disease virus (BLSV) is genetically related to hepatitis E virus." Veterinary Microbiology **68**(1-2): 119.

http://www.sciencedirect.com/science/article/B6TD6-3XMPK3F-F/2/67949212e2a1fd3dedbb8b6f7c748932

A monoclonal antibody (mAb) that reacted specifically with a 16 kDa big liver and spleen disease virus (BLSV) protein was used to identify the protein in western immunoblots of infected liver extracts and enable partial amino acid sequence analysis of the protein. Based on this sequence, a degenerate primer was designed that was used in conjunction with random hexamers in a reverse transcriptase-POR (RT PCR), to amplify a 523 bp product from RNA extracted from homogenates of BLSV-infected livers. There was 62% nucleotide sequence identity between this sequence and the sequence of the helicase gene of human hepatitis E virus (HEV). POR primers designed from this 523 bp fragment were able to amplify a 490 bp product from livers of virus-infected chickens but not chickens from virus-free flocks.

Prager, R., R. Bauerfeind, et al. (2004). "Prevalence and deletion types of the pathogenicity island ETT2 among Escherichia coli strains from oedema disease and colibacillosis in pigs." Veterinary Microbiology **99**(3-4): 287.

http://www.sciencedirect.com/science/article/B6TD6-4BVRTCV-1/2/54b87ac9c8e61ac0785562d55836d4e4

Piglet pathogenic Escherichia coli encoding Shigatoxin 2e and F18 adhesins are the etiological agents of oedema disease as well as of non-oedema disease colibacillosis. In order to reveal virulence differences among this pathogen, the presence of the pathogenicity island (PAI) E. coli type three secretion system 2 (ETT2) was examined. Using PCR and Southern blot techniques for the identification of the right, the middle, and the left region of this 29.9 kb large genetic element, the entire ETT2 was found among E. coli O138:H-, O139:H1, and O147:H6 strains originated from cases of oedema disease in Germany between 1995 and 2001 and belonging to various clonal types. In contrast, non-oedema disease E. coli isolates (e.g. O8:H19, 101:H-, O141:H4) contain deleted subtypes of ETT2. These deletions cover the translocon part of the putative ETT2-encoded type III secretion apparatus. It is suggested that the entire ETT2 is associated with a particular virulence trait of piglet oedema disease E. coli (EDEC).

Pratelli, A., A. Tinelli, et al. (2004). "Safety and efficacy of a modified-live canine coronavirus vaccine in dogs." Veterinary Microbiology **99**(1): 43.

http://www.sciencedirect.com/science/article/B6TD6-4BG3TDW-B/2/72f81e715a6ebf78c56621590d89cdb7

The safety and the efficacy of a modified-live (ML) canine coronavirus (CCoV) vaccine strain 257/98-3c was evaluated in 14 dogs seronegative and virus negative for CCoV. For the safety test, four dogs were inoculated, two by intramuscular and two by oronasal route, with 10 times the vaccinal dose. During the observation period (28 days) all dogs did not display any local or systemic reaction. For the efficacy test, eight dogs were vaccinated by intramuscular (four dogs--group A) or by oronasal route (four dogs--group B). Two dogs were maintained as non-vaccinated controls. In the dogs of group A, vaccinal virus was not detected in faecal samples by virus isolation (VI) and by PCR assay, while in the dogs of group B, the virus was revealed for six median days only by PCR. Twenty-eight days later, the vaccinated and control dogs were challenged with a field CCoV strain. After the challenge, the vaccinated dogs did not display clinical signs and the dogs of group A shed virus for 5.5 median days, evaluated by VI, and for 10 median days evaluated by PCR. Virus shedding was not observed, both by VI and PCR assay, in the dogs of group B. The two control dogs displayed moderate clinical signs and the virus was detected by VI for 14.5 median days starting from day 3 post-challenge (dpc 3) and by PCR assay for 23 median days starting from dpc 1.

Ramasoota, P., N. Chansiripornchai, et al. (2001). "Comparison of Mycobacterium avium complex (MAC) strains from pigs and humans in Sweden by random amplified polymorphic DNA (RAPD) using standardized reagents." Veterinary Microbiology **78**(3): 251.

http://www.sciencedirect.com/science/article/B6TD6-427JW5Y-6/2/cde6d22a3651ea7a004e61ba0e1b5812

Infections with atypical mycobacteria belonging to the Mycobacterium avium/intracellulare complex (MAC) can cause infection in both animals and humans. Using a standardized reagents commercial kit for random amplified polymorphic DNA (RAPD) analysis, 49 MAC strains isolated from 32 slaughter pigs and 17 humans in Sweden were identified and sorted out, yielding 6 RAPD types. By combining the results of RAPD primers 4 and 5 and the primer IS1245A, we found that pigs and humans may be infected with the same types of MAC strains, since 14 strains from humans and 8 strains from pigs were essentially identical and together, comprised RAPD type 2, the largest group of strains (44.8% of strains). With respect to grouping of strains, serotype and RAPD type were uncorrelated, except for serotype 20 and RAPD type 6. Using standardized beads, RAPD analysis is a reproducible technique for typing MAC strains, as the indistinguishable banding patterns obtained with repeated analyses of two isolates from each strain in this study demonstrate. However, primer selection and DNA purity were crucial for differentiating closely related strains.

Ritelli, M., M. Amadori, et al. (2003). "Use of a macrophage cell line for rapid detection of Mycobacterium bovis in diagnostic samples." Veterinary Microbiology **94**(2): 105.

http://www.sciencedirect.com/science/article/B6TD6-48J45KC-1/2/30abed3acd07e3b1980ee24483d038c9

Mycobacterium bovis isolation on bacteriological media from suspected cases of bovine tuberculosis (TB) demands laborious and time-consuming procedures. Even polymerase chain reaction (PCR) and radiometric analyses are secondary procedures and not alternatives to bacteriological procedures. Therefore, there is a need to develop new techniques aimed at rapid M. bovis detection in diagnostic samples. The human macrophage cell line THP-1 was thus

investigated in experiments of M. bovis propagation and isolation from reference lymph node suspensions. THP-1 cells were shown to support a high-titered propagation within 48 h of minute amounts of both M. bovis BCG and fully pathogenic M. bovis strain 503. A semi-nested PCR for TB-complex-specific insertion sequence IS6110 revealed M. bovis infection in THP-1 cells. The same was true of a flow cytometry (FC) assay for expression of M. bovis chaperonin 10 in infected cells. The reduced time for isolation and identification of M. bovis (48-72 h) and the consistency of the test results make the use of macrophage cell cultures attractive and cost-effective for veterinary laboratories involved in TB surveillance.

Segales, J., M. Calsamiglia, et al. (2002). "Porcine reproductive and respiratory syndrome virus (PRRSV) infection status in pigs naturally affected with post-weaning multisystemic wasting syndrome (PMWS) in Spain." Veterinary Microbiology **85**(1): 23.

http://www.sciencedirect.com/science/article/B6TD6-44W2JX4-1/2/97839f97d540c6ca602f8aff52a0d952

The purpose of the study reported here was to determine the prevalence of porcine reproductive and respiratory syndrome virus (PRRSV) in pigs affected with post-weaning multisystemic wasting syndrome (PMWS), a disease believed to be caused by porcine circovirus type 2 (PCV2). From May 1997 to February 2000, PMWS was diagnosed in 277 pigs (from 120 farms) submitted to the Veterinary Pathology Diagnostic Service, Veterinary School of Barcelona, Spain. In each case, the PMWS diagnosis was based on clinical history and the detection, by in situ hybridization, of nucleic acid of PCV2 in characteristic histologic lesions. Antigens for PRRSV were detected by immunohistochemistry in tissues of 66 (23.8%) of the same 277 pigs. Sera, which were available for 93 of the 277 pigs, were tested for PRRSV by a multiplex reverse transcription-polymerase chain reaction (RT-PCR). A total of 33 of these sera were RT-PCR positive, three for a North American strain(s) of PRRSV. In addition, 76 of the 93 sera were tested for antibodies to PCV2 (indirect immunoperoxidase) and PRRSV (enzyme-linked immunoassay). Antibodies for PCV2 and PRRSV were detected, respectively, in 56 (73.9%) and 43 (56.6%) of the 76 sera. Collectively, these results suggest that while infection with PRRSV may be common, it is not an essential component of PMWS.

Sharma, V. K. and E. A. Dean-Nystrom (2003). "Detection of enterohemorrhagic Escherichia coli O157:H7 by using a multiplex real-time PCR assay for genes encoding intimin and Shiga toxins." Veterinary Microbiology **93**(3): 247.

http://www.sciencedirect.com/science/article/B6TD6-485PC6R-1/2/b7c6991fe8063e491d13b93dce0b042b

A multiplex real-time PCR (R-PCR) assay was designed and evaluated on the ABI 7700 sequence detection system (TaqMan) to detect enterohemorrhagic Escherichia coli (EHEC) O157:H7 in pure cultures, feces, and tissues. Three sets of primers and fluorogenic probes were used for amplification and real-time detection of a 106-bp region of the eae gene encoding EHEC O157:H7-specific intimin, and 150-bp and 200-bp segments of genes stx1 and stx2 encoding Shiga toxins 1 and 2, respectively. Analysis of 67 bacterial strains demonstrated that the R-PCR assay successfully distinguished EHEC O157:H7 serotype from non-O157 serotypes and provided accurate profiling of genes encoding intimin and Shiga toxins. Bacterial strains lacking these genes were not detected with this assay. The detection range of the R-PCR assay for the three genes was linear over DNA concentrations corresponding from 103 to 108 CFU/ml of EHEC O157:H7. The R-PCR allowed construction of standard curves that facilitated quantification of EHEC O157:H7 in feces and intestinal tissues. Detection sensitivity of the R-PCR assay ranged from 104 to 108 CFU/g of feces or tissues without enrichment. Enrichment of feces in a non-

selective broth for 4 and 16 h resulted in the detection of levels (from 100 to 103 CFU/g of feces) considered sufficient for infection in humans. The R-PCR assay for eaeO157:H7, stx1, and stx2 proved to be a rapid test for detection of EHEC O157:H7 in complex biological matrices and could also potentially be used for quantification of EHEC O157:H7 in foods or fecal samples.

Staats, J. J., M. M. Chengappa, et al. (2003). "Detection of Escherichia coli Shiga toxin (stx) and enterotoxin (estA and elt) genes in fecal samples from non-diarrheic and diarrheic greyhounds." Veterinary Microbiology **94**(4): 303.

http://www.sciencedirect.com/science/article/B6TD6-48TKH2G-1/2/c66a82233654c95c189120045619d877

Virulence factors responsible for acute diarrhea in greyhounds have not been well established. The objective of this study was to determine if a correlation exists between disease and the presence of the Escherichia coli toxin genes in non-diarrheic and diarrheic greyhound feces. DNA extracted from broth cultures was evaluated for the presence of Shiga toxin and enterotoxin genes and broth samples were evaluated for Shiga toxin and heat-labile enterotoxin. Shiga toxin (stx1 and stx2) and enterotoxin (et and estA) genes were identified in both non-diarrheic and diarrheic samples after in vitro cultured of swabs at 37 [deg]C for 16-24 h. The stx1 gene was present in 3% of non-diarrheic and 15% diarrheic samples and the stx2 gene was identified in 36 and 23%, non-diarrheic and diarrheic samples, respectively. Shiga toxin was present in 48% diarrheic and 25% of the non-diarrheic in vitro cultured samples. The elt gene was detected in vitro cultured swabs in 12% of the non-diarrheic and 7% of the diarrheic samples. Labile toxin was present in the feces of small numbers of both groups of dogs. A significant correlation existed between the presence of both stx1 genes and Shiga toxin in feces, and lack of disease in non-diarrheic (P=0.01) and presence of disease in diarrheic (P=0.024) greyhounds. Correlation between production of Shiga toxin and detection of stx1 or stx2 was significant in both the diarrheic and non-diarrheic feces (P=0.03); however, only the presence of stx1 correlated with diarrhea in both groups of samples (PE. coli in both non-diarrheic and diarrheic greyhounds indicates a zoonotic potential from dogs to humans and requires further study.

Stine, L. C., D. K. Hoppe, et al. (1997). "Sequence conservation in the attachement glycoprotein and antigenic diversity among bovine respiratory syncytial virus isolates." Veterinary Microbiology **54**(3-4): 201.

http://www.sciencedirect.com/science/article/B6TD6-3RGSY4F-1/2/171e29f230c3b33d7cc801f66082ffa4

Partial nucleotide sequences were determined from the coding regions of the attachment glycoprotein (G) mRNAS of eight isolates of bovine respiratory syncytial virus (BRSV). The antigenic characteristics of 18 field and reference isolates were analyzed using the reactivity patterns of monoclonal antibodies (MAbs) directed against the human respiratory syncytial virus (HRSV) and BRSV G, fusion protein (F), nucleoprotein (N), and phosphoprotein (P), by radioimmunoprecipitation and immunofluorescence assays. The MAb reaction patterns demonstrated some random antigenic differences among the isolates, but for the most part were cross-reactive to the viral protein epitopes, expecially on the F protein. Structural differences in the F and P proteins were observed among BRSV isolates; the P protein migrated at three different apparent molecular weights on PAGE gels. Antigenic and structural variation occurs among isolates, however, the structural differences in the P protein did not correlate with the antigenic differences among the F, N and P proteins. The G mRNA nucleotide sequence identities were high, ranging from 94.1 to 99.9%, and the predicted amino acid sequence identities ranged from 89.9 to 99.6%. Variance was due to substitution point mutations. The G

protein ectodomains contained areas of sequence divergence flanking a highly conserved region, with four cysteine residues, which is analogous to the putative HRSV receptor binding domain. The high sequence and amino acid identities and random antigenic diversity among the isolates indicates that the BRSV isolates analyzed belong in a monophyletic group.

Subramaniam, S., J. Frey, et al. (2000). "Immunoblot assays using recombinant antigens for the detection of Mycoplasma hyopneumoniae antibodies." Veterinary Microbiology **75**(1): 99.

http://www.sciencedirect.com/science/article/B6TD6-40J1DVM-B/2/54bea9f42f11c1c7712a0cc07cde4ad8

The 36 kDa -lactate dehydrogenase (LDH) and a 29 kDa partial fragment of an ABC transporter ATP-binding protein analogue/multidrug resistance protein homologue (PR2) of Mycoplasma hyopneumoniae were tested for their potential as diagnostic antigens. Recombinant LDH was genetically engineered to contain six histidine residues at its C-terminal end, expressed in Escherichia coli and purified to a high degree using Ni2+-chelate affinity chromatography. A partial 262 amino acid segment representing the C-terminal end of the PR2 protein was cloned as a glutathione S-transferase (GST) fusion protein, expressed in E. coli and purified by urea extraction. Purified recombinant LDH-6 x His and PR2-GST were then reacted with pig sera in immunoblot assays. Our immunoblots showed that both proteins detected anti-M. hyopneumoniae antibodies in field and experimentally infected pig sera but not in any of the SPF control sera. The two proteins were specific for M. hyopneumoniae as they did not react with sera of pigs infected with the closely related Mycoplasma flocculare and Mycoplasma hyorhinis which are frequently isolated in pigs but are not of particular concern.

Tola, S., G. Idini, et al. (2001). "A physical map of the Mycoplasma agalactiae strain PG2 genome." Veterinary Microbiology **80**(2): 121.

http://www.sciencedirect.com/science/article/B6TD6-42R0S3M-2/2/69e372662526a1ee4cb5b6cdf957e2f4

We have constructed a physical map of the Mycoplasma agalactiae strain PG2 chromosome analyzing it by pulsed field gel electrophoresis in a contour-clamped homogeneous electric-field system. We mapped 33 cleavage sites generated with SmaI, XhoI, SalI, EclXI and BsiWI restriction endonucleases using double digestions, one- and two-dimensional pulsed electrophoresis, cross-hybridization and linking clones. We have also mapped the loci of some genes by Southern hybridization.

Vandekerchove, D., F. Vandemaele, et al. "Virulence-associated traits in avian Escherichia coli: Comparison between isolates from colibacillosis-affected and clinically healthy layer flocks." Veterinary Microbiology **In Press, Corrected Proof**

http://www.sciencedirect.com/science/article/B6TD6-4FR8PMD-2/2/7988fac2bb6a2e7db0b5feafad9ad850

Colibacillosis appears to be of increasing importance in layer flocks. The aim of this study was to determine characteristics of avian pathogenic Escherichia coli associated with the occurrence of colibacillosis outbreaks at flock level. Forty E. coli strains originating from layers from healthy flocks ('control isolates'), consisting of 25 caecal and 15 extra-intestinal isolates, were compared with 40 strains isolated from layers originating from colibacillosis-affected flocks ('outbreak

isolates'), consisting of 20 caecal and 20 extra-intestinal isolates. The examined characteristics were adhesins, invasivity in T84 cell culture, serum resistance, iron uptake, colicin production, and toxinogenicity. The following traits were significantly more often detected in the outbreak isolates than in the control isolates: tsh, iss, iucA, iutA, irp2, fyuA, iroC, cvaC, colicin and colicin V production. A comparison of the extra-intestinal outbreak isolates and the caecal control isolates yielded the same results as when the caecal isolates, extra-intestinal isolates and total number of isolates of the outbreak and the control group were compared. When comparing the caecal and extra-intestinal isolates within the control and within the outbreak group, no significant differences were detected. The O78 and O2 groups showed significant differences with other O-types and NT strains for prevalence of most of the same characteristics. The combination of type 1 fimbriae, tsh, serum resistance, iss, traT, iucA, fyuA, iroC and colicin or colicin V production was significantly more often present in extra-intestinal outbreak isolates than in extra-intestinal control isolates. Only the combination of serum resistance, fyuA and colicin production was present in all outbreak isolates, with a significantly lower prevalence in the control isolates. None of the characteristics or combinations examined were exclusive to the outbreak isolates.

Vicca, J., T. Stakenborg, et al. (2003). "Evaluation of virulence of Mycoplasma hyopneumoniae field isolates." Veterinary Microbiology **97**(3-4): 177.

http://www.sciencedirect.com/science/article/B6TD6-4B2CNMM-2/2/4d8422faaba1012e6545456d5280053c

The course of enzootic pneumonia, caused by Mycoplasma hyopneumoniae, is strongly influenced by management and housing conditions. Other factors, including differences in virulence between M. hyopneumoniae strains, may also be involved. The aim of this study was to evaluate the virulence of six M. hyopneumoniae field isolates and link it to genetic differences as determined by randomly amplified polymorphic DNA (RAPD) analysis.Ninety, conventional M. hyopneumoniae-free piglets were inoculated intratracheally with the field isolates, a virulent reference strain or sterile culture medium. Animals were examined daily for the presence of disease signs and a respiratory disease score (RDS) was assessed per pig. Twenty-eight days post infection, pigs were euthanized, blood sampled and a lung lesion score was given. Lung samples were processed for histopathology, immunofluorescence testing for M. hyopneumoniae and isolation of M. hyopneumoniae. RAPD analysis was performed on all M. hyopneumoniae strains.Significant differences between isolates were found for the RDS, lung lesion score, histopathology, immunofluorescence and serology. Based on the results of the different parameters, isolates were divided into three "virulence" groups: low, moderately and highly virulent strains. Typically, a 5000 bp RAPD fragment was associated with the highly and moderately virulent strains whereas it was absent in low virulent strains. It was concluded that high variation in virulence exists between M. hyopneumoniae strains isolated from different swine herds. Further studies are required to determine whether the 5000 bp fragment obtained in the RAPD analysis can be used as a virulence marker.

Walker, J. K., J. H. Morgan, et al. (1994). "Listeria innocua isolated from a case of ovine meningoencephalitis." Veterinary Microbiology **42**(2-3): 245.

http://www.sciencedirect.com/science/article/B6TD6-476TTW8-H/2/b2ef78ab562c02155fdd7c170ee9a1c7

This paper reports a naturally occurring case of meningoencephalitis associated with Listeria innocua in a Polled-Dorset ewe. The ewe was one of a housed group of twenty-five, fed ad lib. on wrapped baled silage. L. innocua was isolated after one week from cold enrichment culture of brain and pituitary tissue. Its identity was confirmed by conventional biochemical tests, API

Listeria (BioMerieux UK Ltd), the absence of hly and prfA genes using PCR assay and sequencing two variable regions of 16S rDNA. Histological examination demonstrated lesions of vasculitis and perivascular cuffing in the midbrain which were consistent with listeriosis although limited in distribution and severity.

Wards, B. J., D. M. Collins, et al. (1995). "Detection of Mycobacterium bovis in tissues by polymerase chain reaction." Veterinary Microbiology **43**(2-3): 227.

http://www.sciencedirect.com/science/article/B6TD6-3YS8YC7-P/2/ca1c465321b4f8086049395c9f229328

A polymerase chain reaction (PCR) test was developed to detect Mycobacterium bovis in tissues. The test was based on amplification of a 248 bp segment of the insertion sequence, IS1081, present in six copies in strains of M. bovis and other members of the tuberculosis complex. The procedure involved digestion with proteinase K, lysis with sodium dodecyl sulphate, and extraction with hexadecyl tetramethyl ammonium bromide and phenol:chloroform:iso-amyl alcohol. When agarose gel electrophoresis was used for detection, the method was able to detect 1 fg of pure DNA, or 0.2 genome equivalents. It could also detect as few as 10 organisms from pure cultures and between 200-500 organisms from tissues spiked with cultured organisms. Detection by hybridization was only marginally more sensitive. The method was tested on 110 selected tissues recovered post mortem from a variety of animals. Fifty three of 58 samples diagnosed as M. bovis culture positive, including all samples containing microscopically visible acid-fast bacilli, were positive on duplicate testing by PCR. Five of 52 culture negative samples were also positive by PCR including three which contained large numbers of acid-fast organisms. Ten of the culture negative samples came from animals in a herd known to be free of bovine tuberculosis and all these were negative by PCR.

Wisselink, H. J., F. H. Reek, et al. (1999). "Detection of virulent strains of Streptococcus suis type 2 and highly virulent strains of Streptococcus suis type 1 in tonsillar specimens of pigs by PCR." Veterinary Microbiology **67**(2): 143.

http://www.sciencedirect.com/science/article/B6TD6-47YY90F-6/2/41c91c121115fde0d05fc7e440c9fb13

We developed a PCR assay for the rapid and sensitive detection of virulent Streptococcus suis type 2 and highly virulent S. suis type 1 in tonsillar specimens from pigs. The PCR primers were based on the sequence of the gene encoding the EF-protein of virulent S. suis type 2 strains (MRP+EF+) and highly virulent S. suis type 1 strains (MRPsEF+) and of the EF* protein of weakly virulent S. suis type 2 strains (MRP+EF*). The latter strains give rise to larger PCR products than the virulent strains of S. suis type 1 and 2. A positive control template was included in the assay to identify false negative results. The PCR was evaluated using tonsillar specimens from herds known (or suspected) to be infected and herds without an S. suis history. The results obtained with the PCR assay were compared with the results obtained with a newly developed bacteriological examination. In this bacteriological examination we were able to identify the EF-positive strains directly in the tonsillar specimens. From the 99 tonsils examined, 48 were positive in the PCR and 51 negative. All specimens from which EF-positive S. suis strains were isolated were also positive in the PCR assay. Three samples were positive in the PCR, but negative by bacteriological examination. The results demonstrated that the PCR is a highly specific and sensitive diagnostic tool for the detection of pigs carrying virulent strains of S. suis type 2 and highly virulent strains of type 1. Application of the assay may contribute to the control of S. suis infections.

Woodward, M. J., P. J. Carroll, et al. (1992). "Detection of entero- and verocyto-toxin genes in Escherichia coli from diarrhoeal disease in animals using the polymerase chain reaction." Veterinary Microbiology **31**(2-3): 251.

http://www.sciencedirect.com/science/article/B6TD6-476VJ18-DG/2/b7e7bef6d8ddbd6fd5deab8e0a747e7f

Oligonucleotide primers were designed for the specific polymerase chain reaction (PCR) amplification of the enterotoxins STIa and LTI and of the verocytotoxins VT1 and VT2. All of 184 E. Coli isolates from cases of diarrhoea from pigs, cattle and sheep gave identical toxin profiles by PCR and gene probe. Differentiation between VT2 and VT2v was achieved using two oligonucleotide primers pairs in PCR and showed that all of 34 VT2+ porcine isolates, of which 23 were 0138:K1, harboured VT2v whereas 20 VT2+ bovine and ovine isolates harboured VT2. No isolate harboured both VT2 polymorphs. Simplified methods for sample preparation for PCR were examined and showed that PCR was not inhibited by direct addition of broth culture to the reaction mixture.

Woodward, M. J. and J. S. Redstone (1994). "Deoxynucleotide sequence conservation of the endoflagellin subunit protein gene, flaB, within the genus leptospira." Veterinary Microbiology **40**(3-4): 239.

http://www.sciencedirect.com/science/article/B6TD6-476VKWD-143/2/941debcc05853dccffa835c75ace8ee5

A Polymerase chain reaction was developed to amplify the entire open reading frame of flaB, the gene encoding the endoflagellin subunit protein. The 852 bp amplified products from 23 serovars of the genus Leptospira were subjected to restriction endonuclease analysis and the profiles correlated well with phylogenetic relationships between these serovars. The flaB deoxynucleotide sequences of L. hardjo-bovis, L. hardjo-prajitno and L. grippotyphosa were determined. The deduced primary amino acid sequences of each were highly conserved with only three amino acid residue differences observed. The deoxynucleotide sequences showed genetic drift with alternative bases in the third position of codons. The PCR product derived by amplification of flaB from L. grippotyphosa was cloned into the expression vector pGEX-2T and a recombinant FlaB fusion protein made. As predicted from the deduced amino acid sequences, the recombinant FlaB cross-reacted with heterologous antiserum derived from a rabbit infected with L. hardjo-bovis.

Zhou, N. N., D. A. Senne, et al. (2000). "Emergence of H3N2 reassortant influenza A viruses in North American pigs." Veterinary Microbiology **74**(1-2): 47.

http://www.sciencedirect.com/science/article/B6TD6-405KDD3-5/2/b5873a86dd4cebeefa2aea80d2d84f87

In late summer through early winter of 1998, there were several outbreaks of respiratory disease in the swine herds of North Carolina, Texas, Minnesota and Iowa. Four viral isolates from outbreaks in different states were analyzed, both antigenically and genetically. All of the isolates were identified as H3N2 influenza viruses with antigenic profiles similar to those of recent human H3 strains. Genotyping and phylogenetic analysis demonstrated that the four swine viruses had emerged through two different pathways. The North Carolina isolate is the product of genetic

reassortment between human and swine influenza viruses, while the others arose from reassortment of human, swine and avian viral genes. The hemagglutinin genes of the four isolates were all derived from the human H3N2 virus circulating in 1995. It remains to be determined if either of these recently emerged viruses will become established in the pigs in North America and whether they will become an economic burden.

Zhou, W., R. F. Cook, et al. (2002). "Multiple RNA splicing and the presence of cryptic RNA splice donor and acceptor sites may contribute to low expression levels and poor immunogenicity of potential DNA vaccines containing the env gene of equine infectious anemia virus (EIAV)." Veterinary Microbiology **88**(2): 127.

http://www.sciencedirect.com/science/article/B6TD6-46BMTXT-2/2/d62052c6b5bfc208576930e9a6413dfa

The env gene is an excellent candidate for inclusion in any DNA-based vaccine approach against equine infectious anemia virus (EIAV). Unfortunately, this gene is subjected to mutational pressure in E. coli resulting in the introduction of stop codons at the 5' terminus unless it is molecularly cloned using very-low-copy-number plasmid vectors. To overcome this problem, a mammalian expression vector was constructed based on the low-copy-number pLG338-30 plasmid. This permitted the production of full-length EIAV env gene clones (plcnCMVenv) from which low-level expression of the viral surface unit glycoprotein (gp90) was detected following transfection into COS-1 cells. Although this suggested the nuclear export of complete env mRNA moieties at least two additional polypeptides of 29 and 20 kDa (probably Rev) were produced by alternative splicing events as demonstrated by the fact that their synthesis was prevented by mutational inactivation of EIAV env splice donor 3 (SD3) site. The plcnCMVenv did not stimulate immune responses in mice or in horses, whereas an env construct containing an inactivated SD3 site (plcnCMV[Delta]SD3) did induce weak humoral responses against gp90 in mice. This poor immunogenicty in vivo was probably not related to the inherent antigenicity of the proteins encoded by these constructs but to some fundamental properties of EIAV env gene expression. Attempts to modify one of these properties by mutational inactivation of known viral RNA splice sites resulted in activation of previously unidentified cryptic SD and slice acceptor sites.

Veterinary Parasitology (22)

Alhassan, A., W. Pumidonming, et al. "Development of a single-round and multiplex PCR method for the simultaneous detection of Babesia caballi and Babesia equi in horse blood." Veterinary Parasitology **In Press, Corrected Proof** http://www.sciencedirect.com/science/article/B6TD7-4FH0WBR-2/2/b1978c988c7289fffacddd962daa36d6

With the aim of developing more simple diagnostic alternatives, a differential single-round and multiplex polymerase chain reaction (PCR) method was designed for the simultaneous detection of Babesia caballi and Babesia equi, by targeting 18S ribosomal RNA genes. The multiplex PCR amplified DNA fragments of 540 and 392 bp from B. caballi and B. equi, respectively, in one reaction. The PCR method evaluated on 39 blood samples collected from domestic horses in Mongolia yielded similar results to those obtained from confirmative PCR methods that had been established earlier. Thus, the single-round and multiplex PCR method offers a simple tool for the differential diagnosis of B. caballi and B. equi infections in routine diagnostic laboratory settings

as well as in epidemiological studies.

Barlough, J. E., J. E. Madigan, et al. (1996). "Nested polymerase chain reaction for detection of Ehrlichia equi genomic DNA in horses and ticks (Ixodes pacificus)." Veterinary Parasitology **63**(3-4): 319.

http://www.sciencedirect.com/science/article/B6TD7-3VYTHT9-13/2/b9c82c78e5b5fd06b54361ad5e028b69

A nested polymerase chain reaction for detecting Ehrlichia equi in horses and ticks (Ixodes pacificus) was developed. A major second-round PCR product of 928 bp could be readily visualized in ethidium bromide-stained agarose minigels. An internal probe was used to verify the identity of the amplified product by non-radioactive (digoxigen-based) Southern blotting: additional confirmation was provided by DNA sequence analysis. A dilution study testing the sensitivity of the PCR indicated that DNA derived from 3 infected neurophils was sufficient to generate a PCR signal. The specificity of the PCR was examined using a panel of rickettsiae, of which only E. equi and the closely-related human granulocytotropic ehrlichia produced PCR bands. In an in vivo infection study, E. equi DNA was detected in blood buffy-coat cells of an experimentally-infected horse on days three through 14 post-inoculation. In a separate study, three of six adult I. pacificus that as nymphs had been fed on an experimentally infected horse were found to be PCR-positive for E. equi.

Barlough, J. E., Y. Rikihisa, et al. (1997). "Nested polymerase chain reaction for detection of Ehrlichia risticii genomic DNA in infected horses." Veterinary Parasitology **68**(4): 367.

http://www.sciencedirect.com/science/article/B6TD7-3RGSY66-9/2/288701958c8b315399b62700ef18bd3b

A nested polymerase chain reaction was developed for amplifying a 529-bp segment of the 16S ribosomal RNA gene of Ehrlichia risticii from equine buffy coat cells. Confirmation of identity of the amplified bands was accomplished by Southern hybridization and DNA sequencing. The study indicated a detection limit of > 10 copies of the target gene, and specificity for E. risticii as based on a panel of test rickettsiae. Ticks (Ixodes pacificus) collected in an area of northern California enzootic for equine monocytic ehrlichiosis were found to be negative for E. risticii DNA.

Bautista, J. L. R., H. Ikadai, et al. (2001). "Molecular evidence of Babesia caballi (Nuttall and Strickland, 1910) parasite transmission from experimentally-infected SCID mice to the ixodid tick, Haemaphysalis longicornis (Neuman, 1901)." Veterinary Parasitology **102**(3): 185.

http://www.sciencedirect.com/science/article/B6TD7-44SHF4R-7/2/122727509d9e86cd16cd8f5297f313d1

Molecular evidence that suggests the possible role of the ixodid tick, Haemaphysalis longicornis and its eggs in the transmission of equine Babesia caballi parasites is presented herein. Using polymerase chain reaction (PCR) to assay for DNA in parasites, presumably acquired by ticks that were allowed to feed on splenectomized-SCID mice, experimentally exposed to in vitro-cultivated B. caballi, we have obtained positive bands that corresponded to the expected B. caballi-specific 430 bp gene fragment in 50% of female ticks used, and in 75 and 25% of eggs and larval progeny, respectively. Also, parasite DNA was detected in ticks, eggs and larvae as late as the 16th to the 20th day post-host infestation. Present findings support to the potential role

of H. longicornis in the transmission of B. caballi parasites. Its capability, however, to successfully transmit the infection to horses under natural conditions in the field needs to be further ascertained. To our knowledge, this is the first documented study incriminating H. longicornis as a most and likely biological vector of equine babesias.

Cobo, E. R., C. M. Campero, et al. (2003). "Ultrastructural study of a tetratrichomonad species isolated from prepucial smegma of virgin bulls." Veterinary Parasitology **117**(3): 195.

http://www.sciencedirect.com/science/article/B6TD7-49W1WSF-3/2/623875c7344c2d21fcc8131b1cd5965f

We present observations on an unusual tetratrichomonad species isolated from preputial smegma of virgin bulls. Ultrastructural studies were performed using scanning and electron microscopy techniques. This protozoan presents four anterior flagella of unequal length and a recurrent one forming the undulating membrane. It shows one anterior nucleus, a Golgi complex, an axostyle, and a costa. The hydrogenosomes are rather elongated, seen in groups, and presenting different electron densities. Vacuoles of different sizes containing bacteria and material in process of digestion were frequently found. PCR was also used in order to compare the species herein described with other trichomonad species. The amplification products were seen only with primers TFR1 and TFR2 (specific to trichomonads), but not with TFR3 and TFR4 (specific to Tritrichomonas foetus), suggesting that although collected from the genital tract of the bull, this protist was not T. foetus. We propose that the appearance of these tetratrichomonads were probably due to the sodomy practiced among bulls. Concomitant contamination of preputial cavity with feces could explain the presence of the opportunistic organism. The observations presented here show the importance of the correct diagnostic when investigating samples obtained from the urogenital tract of cattle. We also suggest that this flagellate belongs to the species Tetratrichomonas buttreyi.

Criado-Fornelio, A., L. Gutierrez-Garcia, et al. (2000). "A parasitological survey of wild red foxes (Vulpes vulpes) from the province of Guadalajara, Spain." Veterinary Parasitology **92**(4): 245.

http://www.sciencedirect.com/science/article/B6TD7-4177K34-1/2/c6ca5d9c0f344c054d6dcac60bd443de

An epizootiological survey of leishmaniosis, coccidiosis and parasitic helminths in 67 foxes (Vulpes vulpes) was conducted in Guadalajara (central Spain). Examination for parasitic protozoa revealed prevalences of 74% Leishmania (determined by molecular methods) and 2.9% coccidia oocysts (fecal flotation). Survey of parasitic helminths (fecal flotation/necropsy) demonstrated the presence of nine species, including six nematodes, two cestodes and one trematode. Nematodes were the most common parasites of foxes, followed by cestodes and trematodes. Greater levels of nematodes like Uncinaria, with a free-living stage in its life-cycle, were found in foxes in areas where moist soils were likely to exist, in contrast to areas of semiarid characteristics, where Toxascaris leonina or Trichuris vulpis were predominant. With regard to helminths of importance as human pathogens, trichinoscopy revealed the presence of a relatively high number of foxes (8.9%) infected with Trichinella spiralis. Finally, Toxocara canis infection was less frequent (4.4%) than trichinellosis.

Criado-Fornelio, A., A. Martinez-Marcos, et al. (2003). "Molecular studies on Babesia, Theileria and Hepatozoon in southern Europe: Part I. Epizootiological aspects." Veterinary Parasitology **113**(3-

4): 189.

http://www.sciencedirect.com/science/article/B6TD7-48407K0-2/2/231d335adecab18886eab357f361bf03

Molecular epizootiology of piroplasmids (Babesia spp., Theileria spp.) and Hepatozoon canis was studied in mammals from southern Europe (mainly from Spain, but also from Portugal and France). Partial amplification and sequencing of the 18s rRNA gene was used for molecular diagnosis. In some particular cases (B. ovis and B. bovis) the complete 18s rRNA gene was sequenced.Blood samples were taken from domestic animals showing clinical symptoms: 10 dogs, 10 horses, 10 cows, 9 sheep and 1 goat. In addition, DNA samples were isolated from blood of 12 healthy dogs and from spleen of 10 wild red foxes (Vulpes vulpes). The results of the survey were the following:- Piroplasmid infections: Approximately from 50 to 70% of wild or domestic mammals (symptomatic) were infected.- Piroplasmids detected in ruminants were:Cow: B. bovis, T. annulata and Theileria sp. (type C).Sheep and goat: B. ovis.- Piroplasmids present in canids were: Babesia canis vogeli, Babesia canis canis, Theileria annae and B. equi. The only piroplasmid found in asymptomatic dogs was B. equi.- Piroplasmids found in horse were: B. equi and B. canis canis.- H. canis infections in canids: H. canis was absent of domestic dog samples, whereas all foxes studied were infected by this protozoa.Genetic analysis showed that most of piroplasmid and Hepatozoon isolates from southern Europe matched unambigously with previously described species, as demonstrated by the high level sequence identity between them, usually between 99 and 100%. Minor differences, usually detected in hypervariable regions of 18s rRNA gene are probably due to strain variations or rare genetic polymorphisms. A possible exception was B. bovis, which shows a relatively lower degree of homology (94%) with regard to other B. bovis isolates from several countries. The same is true for B. ovis, that showed a 94% identity with regard to Babesia sp. from South African cow and a 92% with rapport to B. bovis from Portugal.

Ewing, S. A., J. E. Dawson, et al. (1997). "Attempted transmission of human granulocytotropic Ehrlichia (HGE) by Amblyomma americanum and Amblyomma maculatum." Veterinary Parasitology **70**(1-3): 183.

http://www.sciencedirect.com/science/article/B6TD7-3RJG29C-N/2/e230cbf8bca7ef06fd0d89a868d0db1c

Transstadial transmission of human granulocytotrophic Ehrlichia (HGE) was attempted in dogs using Amblyomma americanum (L.) and A. maculatum Koch, two species that, as adults, feed readily on human beings. Larvae and nymphs were acquisition-fed on a dog that was parasitemic with HGE. Two months later, following digestion of the blood meal and subsequent molting to nymphal or adult stage, these ticks were fed to repletion on HGE-naive dogs. None of the dogs developed clinical evidence of ehrlichiosis. Parasites were not observed in blood smears by light microscopy, HGE DNA was not detected by polymerase chain reaction, and none of the dogs seroconverted. Based on this trial, we conclude that, unlike E. chaffeensis, HGE is probably not transmitted from dog to dog by either A. americanum or A. maculatum.

Foil, L. D., F. Guerrero, et al. "Association of the kdr and superkdr sodium channel mutations with resistance to pyrethroids in Louisiana populations of the horn fly, Haematobia irritans irritans (L.)." Veterinary Parasitology **In Press, Corrected Proof**

http://www.sciencedirect.com/science/article/B6TD7-4FM01M6-1/2/302fefd500692e3cb6e5ae9606b392e2

Pyrethroid resistance in three horn fly populations in Louisiana was monitored by weekly fly counts, filter paper bioassays, and diagnostic PCR assays for the presence of pyrethroid resistance-associated mutations in the sodium channel gene coding region. The PCR assay for the knockdown resistance (kdr) and superkdr sodium channel mutations was used to determine the frequency of the target site insensitivity mechanism in the populations of horn flies, which possessed varying degrees of insecticide resistance. The bioassays and frequency of homozygous-resistant (RR) kdr genotypes were relative predictors of the fly control subsequently observed. Flies exposed to filter paper impregnated with a discriminating concentration of one of four different insecticides were collected when 50% mortality was estimated. Genotypes for the dead flies and the survivors were determined by the PCR assay. The results of the PCR assays indicated that the genotype at the kdr locus of the flies exposed to the two pyrethroids had an effect upon whether the flies were considered to be alive or dead at the time of collection. The kdr genotype of flies exposed to either diazinon or doramectin was unrelated to whether the flies were considered to be alive or dead, except for a single comparison of flies exposed to diazinon. When possible interactions of the kdr and superkdr mutations were compared, we found that there were no associations with the response to diazinon and doramectin. For one location, there were no survivors of the 75 flies with the SS-SS (superkdr-kdr) homozygous susceptible wild type genotype exposed to pyrethroids, while there were survivors in all of the other five genotypes. The SS-RR genotype flies were more susceptible to the pyrethroids than the SR-RR flies, but that was not the case for exposure to diazinon or doramectin. In the St. Joseph population, there were an adequate number of flies to demonstrate that the SS-SR genotype was more susceptible to pyrethroids than the SS-RR and that flies with the SR-SR genotype were more susceptible to pyrethroids than the flies with the SR-RR genotype.

Grahn, R. A., R. H. BonDurant, et al. (2005). "An improved molecular assay for Tritrichomonas foetus." Veterinary Parasitology **127**(1): 39.

http://www.sciencedirect.com/science/article/B6TD7-4DS8063-2/2/630e437aa5629ff4653eee862beb8c91

Tritrichomonas foetus (T. foetus) is the causative agent of bovine trichomonosis, a sexually transmitted disease leading to abortion (from 1 to 8 months gestation), infertility, and occasional pyometra. The annual losses to the U.S. beef industry are estimated to be in the hundreds of millions of dollars. Currently, the "gold standard" diagnostic test for trichomonosis in most countries is the cultivation of live organisms from reproductive secretions. The cultured organisms can then be followed by PCR assays with primers that amplify T. foetus to the exclusion of all other trichomonad species. Thus, negative results present as null data, indistinguishable from failed PCR amplification during T. foetus specific amplification. Our newly developed assay improves previously developed PCR based techniques by using diagnostic size variants from within the internal transcribed spacer 1 (ITS1) region that is between the 18S rRNA and 5.8S rRNA subunits. This new PCR assay amplifies trichomonad DNA from a variety of genera and positively identifies the causative agent in the bovine trichomonad infection. This approach eliminates false negatives found in some current assays as well as identifying the causative agent of trichomonad infection. Additionally, our assay incorporates a fluorescently labeled primer enabling high sensitivity and rapid assessment of the specific trichomonad species. Moreover, electrophoretic separation of amplified samples can be outsourced, thus eliminating the need for diagnostic laboratories to purchase expensive analysis equipment.

Hurtado, A., G. Aduriz, et al. (2001). "Single tube nested PCR for the detection of Toxoplasma gondii in fetal tissues from naturally aborted ewes." Veterinary Parasitology **102**(1-2): 17.

http://www.sciencedirect.com/science/article/B6TD7-44CHMK7-

2/2/13254e59381fefe7adad5290cdd7203a

A single tube nested polymerase chain reaction (PCR) assay targeting the multicopy 18S-5.8S rRNA internal transcribed spacer (ITS1) region has been developed for the diagnosis of Toxoplasma gondii-induced abortion in ovine fetal tissues. In all, 145 ovine fetal samples including brain, spleen, lung, liver, kidney, placenta and fetal fluids from 53 fetuses and stillborns of 32 farms in Northern Spain were analyzed. Thirty-six samples belonging to nine fetuses and one stillborn lamb were T. gondii PCR-positive. Although T. gondii DNA was amplified from different types of tissues, brain was the tissue with the highest detection rate. All animals that had histopathological lesions associated to T. gondii infection were positive by PCR. In addition, four fetuses whose histological examination was hindered by autolysis were PCR-positive. Results obtained by PCR and indirect fluorescent antibody test (IFAT) showed good correspondence, demonstrating the diagnostic value of the two techniques. However, PCR has the advantage over serology in its ability to diagnose T. gondii infection at earlier stages of gestation when the fetus is not yet immunocompetent and in lambs that have taken colostrum. Once other abortifacient agents are ruled out, PCR detection of the ITS1 region in fetal tissues is a valuable and relatively rapid technique for the diagnosis of ovine abortion caused by T. gondii.

Johnson, D. R., J. Sales, et al. (2005). "Local cytokine responses in Dictyocaulus viviparus infection." Veterinary Parasitology **128**(3-4): 309.

http://www.sciencedirect.com/science/article/B6TD7-4F9FR21-1/2/88922f52245a2fa31e751aa5b5c3f5ee

The high degree of immunity induced by the bovine lungworm, Dictyocaulus viviparus, makes it an ideal model in which to study nematode-induced protective immune responses. Here, cytokine responses were measured over the course of an experimental infection of D. viviparus. Local cytokine messenger RNA (mRNA) transcripts were measured in lung parenchyma, tracheal rings and draining lymph nodes using semi-quantitative reverse transcriptase-polymerase chain reaction. Responses were measured in animals necropsied at 15, 22 and 43 days post-infection (DPI). The responses elicited at these time points were compared with cytokine levels observed in uninfected animals. Interleukin (IL)-4, IL-5, IL-10, IL-12p35, IL-13 and interferon gamma (IFN[gamma]) mRNA levels were measured in duplicate at each site. By 42 DPI, very few parasites were recovered, either from faeces or lungs. Transcripts of all cytokines increased in the lung parenchyma, tracheal rings and caudal mesenteric lymph nodes by 15 DPI. The response was rapid and peaked during the time of larval migration through the lungs. By 42 DPI, expression levels of most cytokines were reduced to levels similar to, or below, base line values measured in uninfected animals. Highest levels of IL-10, IL-12p35, IL-13 and IFN[gamma] transcript were measured in the bronchial lymph nodes of uninfected animals. IgG1 levels were negatively correlated with expression levels of all cytokines. The results demonstrate that a mixed cytokine response occurs over the course of a primary infection during which the parasites were eliminated by day 43 DPI. These results agree with those obtained for other helminths in cattle and challenge the hypothesis that polarised Th2 responses are essential for protection against nematodes in this species. These observations are important in the development of recombinant vaccines, particularly when considering adjuvant choice.

Lee, A. J., J. Huntley, et al. (2002). "Expression and characterisation of a Psoroptes ovis glutathione S-transferase." Veterinary Parasitology **105**(1): 49.

http://www.sciencedirect.com/science/article/B6TD7-458P8Y8-1/2/768fdd7fcdc10fe3c142671360e33b98

The astigmatid mite Psoroptes ovis is the causative agent of sheep scab, a highly contagious parasitic disease of sheep. Infection causes severe allergic dermatitis, resulting in damage to the fleece and hide, loss of condition and occasional mortality. Interest in the P. ovis allergens led us to characterise a glutathione S-transferase (GST) which displays homology to GST allergens isolated from the house dust mite, Dermatophagoides pteronyssinus and the cockroach, Blatella germanica. A cDNA encoding a mu-class GST from P. ovis was expressed in Escherichia coli and the recombinant protein purified for biochemical analysis. SDS-PAGE analysis indicated that the purified product was homogeneous and had an apparent molecular weight of 30 kDa. The recombinant GST (rGST) is active towards the substrate 1-chloro-2,4-dinitrobenzene (CDNB), whereas 1,2-dichloro-4-nitrobenzene (DCNB) is a poor substrate. The recombinant protein was also tested for recognition by IgE and IgG antibodies in serum from P. ovis naive and P. ovis infested sheep. Neither IgE nor IgG antibodies were detected to the rGST. Prausnitz-Kustner testing with rGST did not provoke a characteristic weal and flare response. Biopsies collected at the PK test sites were stained for eosinophils, neutrophils, mast cells and basophils. Neutrophil, mast cell and basophil counts were not significantly different to the controls. Eosinophil numbers were significantly higher than controls, but were not due to an IgE response.

Lee, A. J., R. E. Isaac, et al. (1999). "The construction of a cDNA expression library for the sheep scab mite Psoroptes ovis." Veterinary Parasitology **83**(3-4): 241.

http://www.sciencedirect.com/science/article/B6TD7-3WV9GC7-7/2/d9817b446c71ef9325bf884814adb340

The need for alternative control strategies for sheep scab is critical. One approach is to develop vaccines based on 'concealed' antigens derived from Psoroptes ovis. This strategy requires the identification and characterisation of potential target antigens, which has been hampered by the problem of limited biological material for isolation of protein antigens. To aid the discovery of P. ovis antigens and to provide a resource for generating recombinant protein, we constructed a P. ovis cDNA expression library, using total RNA isolated from 250 mg of mixed-stage P. ovis and the Clontech SMART(TM) cDNA synthesis kit. The presence of P. ovis-specific sequences was confirmed using PCR amplification and sequencing of actin. The sequences of cDNA inserts from six random clones included one with high homology to the Dermatophagoides pteronyssinus (house dust mite) antigen p Dp15. This is a glutathione S-transferase known to be an important house dust mite antigen. We conclude that this library will be a useful tool for the identification of potential target antigens for the immunological control of P. ovis and to further our understanding of the pathology of sheep scab.

Manna, L., F. Vitale, et al. (2004). "Comparison of different tissue sampling for PCR-based diagnosis and follow-up of canine visceral leishmaniosis." Veterinary Parasitology **125**(3-4): 251.

http://www.sciencedirect.com/science/article/B6TD7-4DDR7TJ-1/2/6f60dca5406d6d17757f82d22f8df39f

In this study, different types of tissue sampling for PCR-based diagnosis and follow-up of canine visceral leishmaniosis were compared. Skin, whole blood and lymph node samples were collected from 95 naturally infected dogs living in South Italy, where the disease is endemic. Twenty-nine of these 95 dogs, treated with meglumine administered concurrently with allopurinol for 30 days, and then with allopurinol alone, were monitored during a period of 2 years. The DNA extracted from the clinical specimens was amplified by PCR using as target DNA a 116-bp fragment in the constant region of the kinetoplast DNA minicircle. PCR analysis was more sensitive than indirect immunofluorescence antibody test in detecting Leishmania infection in symptomatic dogs: 99% of lymph node samples resulted positive, whereas 94% of blood samples

and 95% of skin samples gave a positive result. PCR analysis of samples from dogs followed up 2 years showed that: (1) all subjects resulted positive in at least one of the three types of samples; (2) all time the dogs had a relapse, PCR resulted positive in all three types of samples; (3) when dogs were apparently healthy, PCR analysis was positive on skin and lymph node samples, but not always on blood samples. Since lymph node sampling is invasive and sometimes difficult in healthy asymptomatic dogs, our results suggest that, independently from the presence or not of cutaneous lesions, skin biopsy represents a good substratum for PCR-based diagnosis and follow-up of canine visceral leishmaniosis.

Muller, N., V. Zimmermann, et al. (2003). "PCR-based detection of canine Leishmania infections in formalin-fixed and paraffin-embedded skin biopsies: elaboration of a protocol for quality assessment of the diagnostic amplification reaction." Veterinary Parasitology **114**(3): 223.

http://www.sciencedirect.com/science/article/B6TD7-48HXRKR-3/2/93dc5b374016a6c0de7a41e73033c2e5

Diagnosis of the cutaneous form of canine leishmaniosis is mostly performed by histological or immunohistological examination of skin biopsies. In modern histology, the polymerase chain reaction (PCR) has gained increasing importance as a complementary tool to directly demonstrate the presence of parasite DNA in the tissue sections. For the present study, a previously described Leishmania-PCR has been further developed and optimised in view of its practicability for routine histological application. Since formalin-fixation of histological specimens causes partial DNA-destruction, which may hamper diagnostic PCR analysis, primers specific for the highly conserved [alpha]-actin gene sequences were used to pre-diagnostically assess the isolated sample-DNA for its functionality in a PCR-reaction. This [alpha]-actin-specific PCR detects DNA from a large variety of mammalian species and thus exhibits relevance for both human and veterinary medical application. A recombinant internal positive control was introduced to monitor possible sample-related inhibitory effects during the amplification reaction. We performed a retrospective evaluative study with 18 formalin-fixed samples from dogs with suspected or proven leishmaniosis. Six samples were PCR-incompatible. In turn, 9 of the other 12 samples were PCR-positive, and immunohistochemical results matched these findings. Based on these technical achievements, the Leishmania-PCR proved to be a valuable tool to complement conventional histological and immunohistological methods for diagnosis of cutaneous leishmaniosis in formalin-fixed, paraffin-embedded skin biopsies.

Murphy, G. L., S. A. Ewing, et al. (1998). "A molecular and serologic survey of Ehrlichia canis, E. chaffeensis, and E. ewingii in dogs and ticks from Oklahoma." Veterinary Parasitology **79**(4): 325.

http://www.sciencedirect.com/science/article/B6TD7-3V51CF7-6/2/fba7015f04108e60c55c1eb7b14f443c

Polymerase chain reaction and Southern hybridization were used to survey for the presence of Ehrlichia canis, Ehrlichia chaffeensis, and Ehrlichia ewingii in blood samples of 65 dogs that harbored ticks from northcentral and northeastern Oklahoma. Dog blood samples were also examined for antibodies against E. canis and E. chaffeensis, using an immunofluorescent antibody test. Ten of 65 dogs (15.4%) examined were positive for Ehrlichia spp. by PCR. Four (6.2%) were positive for E. ewingii, 2 (3.1%) for E. canis, and 4 (6.2%) for E. chaffeensis. Seven dogs (10.8%) were seropositive for E. canis or E. chaffeensis. Ticks collected from PCR-positive dogs were examined by PCR for the presence of Ehrlichia DNA. Several groups of ticks were PCR-positive for E. ewingii or E. canis. E. canis was detected in Rhipicephalus sanguineus, which is considered the major vector for that organism. E. ewingii was detected in a larger variety of ticks, including the only known vector Amblyomma americanum, as well as in Dermacentor

variabilis and R. sanguineus. Results suggest that Ehrlichia spp. which are canine and human pathogens circulate in dogs in Oklahoma and in several tick species that feed on dogs.

Pinitkiatisakul, S., J. G. Mattsson, et al. "Immunisation of mice against neosporosis with recombinant NcSRS2 iscoms." Veterinary Parasitology **In Press, Corrected Proof** http://www.sciencedirect.com/science/article/B6TD7-4FG2XCN-1/2/18295f1c544fb39c3228280e75da9997

The coccidian parasite Neospora caninum is an intracellular protozoan, causing abortion in cattle in many countries around the world. In this study, the protective potential of the major N. caninum surface antigen NcSRS2, expressed in Escherichia coli and formulated into immunostimulating complexes (iscoms), was investigated in an experimental mouse model. The recombinant protein was specially designed for binding to iscoms via biotin-streptavidin interaction. Two groups of 10 BALB/c mice were immunised twice, on days 0 and 28 with iscoms containing either the recombinant NcSRS2 (NcSRS2 iscoms) or similar iscoms with NcSRS2 substituted by an unrelated recombinant malaria peptide (M5) as a control (M5 iscoms). A third group of 10 age-matched BALB/c mice served as an uninfected control group. Immunisation with recombinant NcSRS2 iscoms resulted in production of substantial antibody titres against N. caninum antigen, while the mice immunised with M5 iscoms produced only very low levels of antibodies reacting with N. caninum antigen. After challenge infection with N. caninum tachyzoites on day 69, mice immunised with NcSRS2 iscoms showed only mild and transient symptoms, whereas the group immunised with M5 iscoms showed clinical symptoms until the end of the experiment at 31 days post inoculation. A competitive PCR assay detecting Nc5-repeats was applied to evaluate the level of parasite DNA in the brain. The amount of Nc5-repeats in the group vaccinated with NcSRS2 iscoms was significantly lower than in the control group given M5 iscoms. In conclusion, it was found that the recombinant NcSRS2 iscoms induced specific antibodies to native NcSRS2 and immunity sufficient to reduce the proliferation of N. caninum in the brains of immunised mice.

Pusterla, N., C. M. Leutenegger, et al. (2000). "Detection and quantitation of Ehrlichia risticii genomic DNA in infected horses and snails by real-time PCR." Veterinary Parasitology **90**(1-2): 129.

http://www.sciencedirect.com/science/article/B6TD7-40BG4H5-F/2/ef46450785c81558d397bc511abdf460

A real-time quantitative PCR using the TaqMan fluorogenic detection system (TaqMan PCR) was established for identification of Ehrlichia risticii, the agent of Potomac horse fever (PHF). The TaqMan PCR identified an 85 base pair section of the 16S rRNA gene by use of a specific fluorogenic probe and two primers. This technique was specific for eight tested E. risticii strains. The TaqMan system identified 10 copies of a cloned section of the 16S rRNA gene of E. risticii. The sensitivity and specificity of the TaqMan PCR were similar to those of conventional nested PCR. The TaqMan PCR was evaluated on horses with infectious colitis and on freshwater stream snails collected from regions with a history of PHF. E. risticii could be detected in 22 of 153 (14.4%) horses with infectious colitis and in 25 of 234 (10.7%) snails in the TaqMan PCR. The same results were obtained in the conventional nested PCR. The Ehrlichia-load was in the range of 10,000-9,000,000 and 35,000-680,000,000 Ehrlichia equivalents per [mu]g leukocyte DNA and snail DNA, respectively.

Shoda, L. K. M., A. C. Rice-Ficht, et al. (1999). "Bovine T cell responses to recombinant thioredoxin of Fasciola hepatica." Veterinary Parasitology **82**(1): 35.

http://www.sciencedirect.com/science/article/B6TD7-3W379CH-4/2/221d06b725db203b5af5e9dc98f9272b

Fasciolosis is an economically significant disease of ruminants, caused by infection with the digenetic trematodes, Fasciola hepatica and F. gigantica. Some vaccination trials using irradiated metacercariae or isolated proteins have been shown to afford significant protection. However, the mechanisms of specific immunity against this pathogen have not been elucidated. We have identified thioredoxin, a tegument antigen of F. hepatica, among several proteins that are common to both the juvenile and adult fluke within the mammalian host and have undertaken studies to characterize bovine T cell responses to recombinant thioredoxin protein (FH 2020). Peripheral blood mononuclear cells from immune cattle proliferated specifically to crude F. hepatica antigenic extract but not to FH 2020. However, after repeated stimulation of lymphocytes by alternating crude extract and FH 2020, FH 2020-specific proliferation by T cell lines was observed. T cell clones were subsequently generated and found to respond specifically but weakly to both crude antigen and FH 2020. Thioredoxin appears to be only weakly antigenic for bovine T cells and is, therefore, an unpromising candidate for inducing resistance to F. hepatica.

Walker, J., R. Hoekstra, et al. (2001). "Cloning and structural analysis of partial acetylcholine receptor subunit genes from the parasitic nematode Teladorsagia circumcincta." Veterinary Parasitology **97**(4): 329.

http://www.sciencedirect.com/science/article/B6TD7-435CRSD-M/2/92a50bc7dcb423e443a5531c25592732

Nematode nicotinic acetylcholine receptors (nAChRs) are the sites of action for the anthelmintic drug levamisole. Recent findings indicate that the molecular mechanism of levamisole resistance may involve changes in the number and/or functions of target nAChRs. Accordingly, we have used an RT-PCR approach to isolate and characterise partial cDNA clones (tca-1 and tca-2) encoding putative nAChR subunits from the economically important trichostrongyloid, Teladorsagia circumcincta. The predicted tca-1 gene product is a 248 aa fragment (TCA-1) which contains structural motifs typical of ligand-binding ([alpha]-) subunits, and which shows very high sequence similarities (98.8 and 97.2% amino acid identities) to the [alpha]-subunits encoded by tar-1 and hca-1 from Trichostrongylus colubriformis and Haemonchus contortus, respectively. Sequence analyses of partial tca-1 cDNAs from one levamisole-resistant and two susceptible populations of T. circumcincta revealed polymorphism at the predicted amino acid level, but there was no apparent association of any particular tca-1 allele with resistance. tca-2 encodes a 67 aa fragment (TCA-2) containing the TM4 transmembrane domain and carboxyl terminus of a putative nAChR structural (non-[alpha]) subunit. The deduced amino acid sequence of TCA-2 shows highest similarity (75% amino acid identity) to ACR-2, a structural subunit involved in forming levamisole-gated ion channels in Caenorhabditis elegans, but low similarity (43% identity) to the corresponding regions of TAR-1 and HCA-1. tca-2 is the first nAChR subunit gene of this type to be isolated from parasitic nematodes, and it provides a basis for further characterisation of structural subunits in trichostrongyloids.

Winterrowd, C. A., W. E. Pomroy, et al. (2003). "Benzimidazole-resistant [beta]-tubulin alleles in a population of parasitic nematodes (Cooperia oncophora) of cattle." Veterinary Parasitology **117**(3): 161.

http://www.sciencedirect.com/science/article/B6TD7-49WMXP6-3/2/f00a13d32c11c7463ae4409aa418b69c

Three anthelmintic classes with distinct mechanisms of action are commercially available. Selection of nematode populations resistant to all these drugs has occurred, particularly in trichostrongyloid parasites of sheep. Anthelmintic resistance in cattle parasites has only recently been recognized and appears to be less pronounced, even though very similar species infect both hosts. To understand the bases for differences in the rate of resistance development in sheep versus cattle parasites, it is important to first demonstrate that the same kinds of resistance alleles exist in both. The benzimidazoles (BZ), which have been used for more than 40 years, were chosen as an example. BZ-sensitive (BZS) and BZ-resistant (BZR) nematodes that parasitize sheep have been distinguished at the molecular level by a single nucleotide change in the codon for amino acid 200 of a [beta]-tubulin gene, a switch from TC (phenylalanine) to TC (tyrosine). PCR primers were designed to completely conserved regions of trichostrongyloid [beta]-tubulin genes and were used to amplify DNA fragments from Haemonchus contortus (cDNA from a BZS and a BZR library) as positive controls. The technique was then extended to the cattle parasites, Cooperia oncophora and Ostertagia ostertagi (from genomic DNA). Sequence analysis proved the presence of amplified BZS alleles in all three species and BZR alleles in the BZR population of H. contortus. Based on these data, nested PCR primers using the diagnostic or as the most 3' nucleotide were designed for each species. Conditions for selective PCR were determined. To demonstrate feasibility, genomic DNA was recovered from individual H. contortus L3 larvae from both BZS and BZR populations. Genomic DNA was also isolated from >70 individual adult male C. oncophora collected from a cattle farm in New Zealand with reported BZ resistance. Allele-specific PCR discriminated among heterozygotes and homozygotes in both species. This method could find utility in studying the molecular epidemiology of BZ resistance in cattle parasites and for defining the variables that limit the development and spread of anthelmintic resistance in this host.

Waste Management (1)

Watabe, M., J. R. Rao, et al. (2004). "Identification of novel eubacteria from spent mushroom compost (SMC) waste by DNA sequence typing: ecological considerations of disposal on agricultural land." Waste Management **24**(1): 81.

http://www.sciencedirect.com/science/article/B6VFR-49VC5MR-1/2/3e6ea7d36f725a2012882b224a2643d1

A small study was undertaken to examine the microbiological characteristics of spent mushroom compost (SMC), which is the major waste by-product of the mushroom industry and which is regularly disposed off by application to agricultural land. The primary aim of this study was to examine SMC for the presence of faecal bacterial pathogens, including Campylobacter spp., Salmonella spp. and Listeria monocytogenes. Secondly it was desirable to quantify bacterial and fungal populations within SMC, and also qualitatively identify the diversity of bacterial populations within SMC, through employment of rDNA PCR and direct sequencing techniques on the culturable microflora. Conventional microbiological analyses of SMC material (n=30) from six commercial operations in both Northern Ireland and the Republic of Ireland, failed to detect Salmonella spp, Listeria spp. or Campylobacter spp. in any of the SMC material examined. Total aerobic plate counts gave a mean count of log10 7.01 colony forming units (cfu) per gram SMC material (range: log10 6.53-7.52 cfu/g). Fungal counts gave a mean count of log10 4.57 cfu per gram SMC material (range: log10 3.93-4.98 cfu/g). From a total of greater than 50 colony picks, a total of 12 bacterial morphotypes were identified and were further examined by employment of partial 16S rRNA gene amplification and sequencing techniques, yielding several genera and species, including Bacillus licheniformis, Bacillus subtilis, Klebsiella/Enterobacter sp.

Microbacterium sp. Paenibacillus lentimorbus, Pseudomonas mevalonii, Sphingobacterium multivorum and Stenotrophomonas sp. This is the first preliminary report on the microbial diversity of SMC waste and demonstrates the presence of several species that have not been previously described in SMC, in addition to two potentially novel species within the genera Microbacterium and Stenotrophomonas. It is thereby important to examine the ecological microbe-microbe and plant-microbe interactions that are occurring between the native bacterial soil flora and those added annually (theoretically estimated at approximately 1018 cells) through the application of SMC. Such studies would be beneficial in helping to ascertain the ecological consequences involved in the disposal of SMC waste on agricultural land.

Water Research (9)

Eschenhagen, M., M. Schuppler, et al. (2003). "Molecular characterization of the microbial community structure in two activated sludge systems for the advanced treatment of domestic effluents." Water Research **37**(13): 3224.

http://www.sciencedirect.com/science/article/B6V73-48PV2FF-R/2/e3e5f154b3bd9fe85e475998bc2dac2b

Although activated sludge systems with enhanced biological phosphorus removal (EBPR) represent state-of-the-art technology for phosphate removal from wastewater it is still unknown which species of bacteria are responsible for the EBPR process.The aim of this study was to compare the bacterial composition of activated sludge from two laboratory plants with different modes of operation, anoxic/oxic- (EBPR, no nitrification) and Phoredox-system (EBPR, nitrification and denitrification) with particular emphasis on microorganisms responsible for EBPR process. In addition to fluorescence in situ hybridization (FISH), we applied further rRNA-based molecular techniques like terminal restriction-fragment length polymorphism analysis and comparative 16S rDNA analysis to yield additional information and to verify the results from FISH analysis, like e.g. for the identification of polyphosphate accumulating organisms (PAO).Despite the different modes of operation only minor differences in the bacterial composition were detected by FISH analysis based on the probes used in this study. In contrast T-RFLP analysis yielded characteristic community fingerprints for each of the investigated plants and comparative 16S rDNA analysis indicated highly diverse microbial communities in both plants suggesting substantial differences in the microbial structure.The results obtained by FISH analysis with specific probes for PAOs support the presumption that not only one specific organism is responsible for the EBPR. In our case Tetrasphaera spp. dominated the PAO community, but other possible PAOs, like Microlunatus spp. and members of the Rhodocyclus group, were also detected.

Haugland, R. A., S. C. Siefring, et al. (2005). "Comparison of Enterococcus measurements in freshwater at two recreational beaches by quantitative polymerase chain reaction and membrane filter culture analysis." Water Research **39**(4): 559.

http://www.sciencedirect.com/science/article/B6V73-4F3FSB2-4/2/08d994854cce2a834dbb6da18d69379d

Cell densities of the fecal pollution indicator genus, Enterococcus, were determined by a rapid (3

h or less) quantitative polymerase chain reaction (QPCR) analysis method in 100 ml water samples collected from recreational beaches on Lake Michigan and Lake Erie during the summer of 2003. Measurements by this method were compared with counts of Enterococcus colony-forming units (CFU) determined by Method 1600 membrane filter (MF) analysis using mEI agar. The QPCR method had an estimated 95% confidence, minimum detection limit of 27 Enterococcus cells per sample in analyses of undiluted DNA extracts and quantitative analyses of multiple lake water samples, spiked with known numbers of these organisms, gave geometric mean results that were highly consistent with the spike levels. At both beaches, the geometric means of ambient Enterococcus concentrations in water samples, determined from multiple collection points during each sampling visit, showed approximately lognormal distributions over the study period using both QPCR and MF analyses. These geometric means ranged from 10 to 8548 cells by QPCR analysis and 1-2499 CFU by MF culture analysis in Lake Michigan (N=56) and from 8 to 8695 cells by QPCR and 3-1941 CFU by MF culture in Lake Erie (N=47). Regression analysis of these results showed a significant positive correlation between the two methods with an overall correlation coefficient (r) of 0.68.

Kurisu, F., H. Satoh, et al. (2002). "Microbial community analysis of thermophilic contact oxidation process by using ribosomal RNA approaches and the quinone profile method." Water Research **36**(2): 429.

http://www.sciencedirect.com/science/article/B6V73-44HTG7C-7/2/917252f0590000e2f10cdf5df86ec190

Microbial community structure of a lab scale thermophilic aerobic wastewater treatment reactor was analyzed by a combination of culture-independent methods. Quinone profile method provides for chemical analysis of respiratory quinone molecular species, which corresponds to bacterial groups. Denaturing gradient gel electrophoresis (DGGE) of PCR-amplified 16S rDNA partial sequences (PCR-DGGE) clarifies community changes at species level, as DGGE can separate DNA fragments of different sequences. Certain phylogenetic groups of bacterial cells can be labeled by fluorescence in situ hybridization (FISH). Quinone profile showed a predominant presence of MK-7. PCR-DGGE revealed that constituents of the community were unchanged during the stable phase. FISH demonstrated the existence of the relatives of Bacillus lentus and B. thermocloacae in considerable proportions. The community was mainly composed of Bacillaceae, and obligate thermophilic and mesophilic Bacillus appeared in spite of the temperature fluctuation from 35[deg]C to 60[deg]C. The combination of these culture-independent methods revealed the community precisely enough to evaluate the reactor performance.

Lauderdale, C. V., H. C. Aldrich, et al. (2004). "Isolation and characterization of a bacterium capable of removing taste- and odor-causing 2-methylisoborneol from water." Water Research **38**(19): 4135.

http://www.sciencedirect.com/science/article/B6V73-4DFK5TH-2/2/8b173bfd80775c3c461e39f608a55749

2-Methylisoborneol (MIB), a metabolite of blue-green algae, has been implicated in causing unpalatable drinking water throughout the world. Current non-biological water treatment technologies are ineffective in removing MIB from potable water or are cost-prohibitive, and biological applications may address these problems. We have isolated and characterized a bacterium derived from lake water and capable of aerobically degrading MIB. Light microscopy and transmission electron microscopy revealed that this strain is a spore-forming, flagellated bacterium that is bacilloid in shape, and 16S rRNA phylogenetic analysis determined that it is most closely related to Bacillus fusiformis and Bacillus sphaericus, both members of the Bacillus sphaericus senso lato taxon. While the growth and oxidation potential of this strain was shown to

be affected beyond certain MIB concentrations in the mg/l range, it was capable of depleting MIB at mg/l and ng/l concentrations and of removing MIB to concentrations yielding no observed odor.

Monis, P. T. and C. P. Saint (2001). "Development of a nested-PCR assay for the detection of cryptosporidium parvum in finished water." Water Research **35**(7): 1641.

http://www.sciencedirect.com/science/article/B6V73-42K5F1M-3/2/4151e10063c06eae601851601a1eb124

A nested-PCR assay, incorporating an internal positive control, was developed for Cryptosporidium monitoring in finished water. This assay was capable of reproducibly detecting 8 oocysts in spiked-filtered water samples collected from 5 South Australian water treatment plants. The RT-PCR assay of Kaucner and Stinear (Appl. Environ. Microbiol. 64(5) (1998) 1743) was also evaluated for the detection of Cryptosporidium parvum. Initially, under our experimental conditions, a detection level of 27 oocysts was achieved for spiked reagent water samples. This level was improved to 5 oocysts by modification of the method. Untreated South Australian source waters concentrated by calcium carbonate flocculation were found to be highly inhibitory to the RT-PCR assay. Concentration of similar samples using Envirochek filters appeared to eliminate PCR inhibition. While both methods possessed similar sensitivities the nested-PCR assay was more reproducible, more cost effective, simpler to perform and could detect both viable and non-viable intact Cryptosporidium parvum oocysts, which is an important consideration for plant operators. These factors make the nested-PCR assay the method of choice for screening large numbers of potable water samples, where a reliable low level of detection is essential.

Muscillo, M., G. La Rosa, et al. (1995). "Comparison of cDNA probe hybridizations and RT-PCR detection methods for the identification and differentiation of enteroviruses isolated from sea water samples." Water Research **29**(5): 1309.

http://www.sciencedirect.com/science/article/B6V73-3YKKH8C-8B/2/28d09f93272fc5d2461720807bcc92fc

Fifteen enteroviruses (EVs) previously isolated from Tyrrhenian sea water samples were used. They were first identified by traditional dot-blot and Northern-blot hybridizations with a group of cDNA probes from cloned Poliovirus 1 and Coxsackievirus B4 and oligodeoxynucleotides complementary to echovirus 6 and 9 sequences. Using both wild viruses and known enteroviruses a reverse-PCR protocol was then set up followed by cDNA sequencing of the fragments generated. The sequences of primers were selected from a consensus of several 5' non-coding ends of enterovirus genomes, representing highly conserved regions. The downstream (region 577-603) and the upstream (region 436-465) oligonucleotide primers carried an extra sequence in order to generate BamHI and a HindIII restriction sites at the 5' and 3' end respectively of the amplified cDNA fragments for directional cloning in a plasmid. The downstream 5'NC primer was 5'-biotinylated in order to allow direct sequencing of the amplicon, when possible, after strand separations on streptavidin coated magnetic beads. The PCR of reverse transcribed viral RNAs resulted in a 167-170 b.p. cDNA product on ethidium bromide-stained 2% agarose gels in all the samples and reference viruses. The test is negative on reoviruses, hepatitis A and uninfected BGM cells and detects 50 viral particles. Sequences of cloned fragments were compared with sequences of cloned enteroviruses stored in commercial data banks. The 5'NC region of a reference echovirus 5 was also cloned and sequenced to improve the comparison. On the basis of deduced genetic distances, three poliovirus 1, eight coxsakievirus B5, four coxsakievirus B1 were diagnosed. One poliovirus Sabin 2 was isolated together with a coxsackievirus-related strain in the same lysate sample. The reliability and

sensitivity of this RT-PCR method makes it an attractive approach to virus detection in environmental samples.

Muscillo, M., G. La Rosa, et al. (1999). "Molecular and biological characterization of poliovirus 3 strains isolated in adriatic seawater samples." Water Research **33**(14): 3204.

http://www.sciencedirect.com/science/article/B6V73-3X70S5S-G/2/d5aa42adb731dfdc0062135f1284c323

In a previous study [Muscillo, M., Carducci, A., La Rosa, G., Cantiani, L., Marianelli, C. (1997a) Enteric virus detection in adriatic seawater by cell culture, polymerase chain reaction and polyacrylamide gel electrophoresis. Water Res. 31, 1980-1984] enterovirus strains were isolated from Adriatic seawater and estuarine water from the Foglia River, by infecting susceptible cells with ultrafiltrated water samples. In the present work we have studied three of those samples, in which routine reverse transcriptase-polymerase chain reaction (RT-PCR) and sequencing analysis had identified the presence of poliovirus type 3 (P3). In order to better estimate the risk to human health of such occurrence in bathing water (having bacteriological standards in line with the EEC directive 76/160), we set up a protocol to distinguish wild from Sabin P3 strains. Three sets of RT-PCR primers were engineered and their predicted products were: 593 nucleotides (nt) in the 5' noncoding (5'NC) region (11-603), 350 nt at the Vp3-Vp1 junction (2438-2787) of the capside protein genes, and 420 nt in the 2C (4209-4628) region, which is regarded as the hotspot of recombinant polioviruses. Eight reference ATCC strains, whose sequences were known, were also tested under the same experimental conditions in order to verify the accuracy of the RT-PCR reactions. The amplicons were directly sequenced by Big-dye(TM) terminator sequencing using a capillary automatic sequencer. The latter two regions found the same viral species Polio 3 in all the sample strains, with no meaningful distinction between P3/Leon/37 and P3/Leon/12a1b, the vaccine strain. The analyses in the 5'NC region were more useful, where genetic relationships and the predicted secondary structure suggested that the viruses were of vaccinal sources. Molecular data were confirmed by in vitro phenotypic marker tests rct/40, where all the examined samples displayed a temperature sensitive phenotype rct/40(-). Our results suggest that the 472U->C transition alone, is not a predictive marker of reversion to neurovirulence. Finally, we conclude that the 220U constantly found in the consensus sequences of the samples can serve as a good predictor of rct/40(-) phenotype.

Muscillo, M., G. La Rosa, et al. (2001). "A new RT-PCR method for the identification of reoviruses in seawater samples." Water Research **35**(2): 548.

http://www.sciencedirect.com/science/article/B6V73-41S4TCG-X/2/0d87a7d0ddaf1c88bf0a2a298246f5ec

The frequent occurrence of reoviruses in environmental samples could be a potential source of interference with enterovirus detection, especially when enterovirus isolation on cell culture is required. In order to evaluate new virus-based criteria for enforcing recreational water quality standards, a new method based on a broad reverse transcribed polymerase chain reaction (RT-PCR) was set up to detect reoviruses. Two primers were engineered to amplify a 538 base pair fragment of the Sigma 2 gene. Reovirus strains obtained from ATCC (Jones, Lang, Dearing, Abney, NC-TEV, SV59 and SV12) were used as references. Twenty-four samples of 10 l were collected from two beaches of the Adriatic sea and 12 from the neighbourhood of Fano Harbour Channel. The presence of environmental reoviruses was tested on both concentrated seawater samples and lysates of BGM cells infected with the concentrated seawater samples. The new method was used in parallel with the detection of a 3: 3: 4 electrophoretic pattern of reovirus RNA in polyacrylamide gel electrophoresis (PAGE). Enterovirus and bacteria were also screened in

compliance with EEC directives. No enteroviruses were isolated, and it was not attributable to reovirus interference. All the reovirus found by PAGE (8/72) were confirmed by RT-PCR, while several genomes (14/72) were detected only by RT-PCR. Presumptive methods of virus identification, that is CPE on BGM cells and haemagglutination test, were not able to detect them. The specificity of RT-PCR products was checked by direct nucleotide sequence analyses of the amplicons. The phylogenetic analyses showed heterogeneous taxa including human and animal reoviruses, with strong evidence that they were spreading consistently from the Harbour-Channel. This novel approach for reovirus detection will be very useful as a trace route of faecal pollution; more importantly, it could be very useful in contributing to the creation of a databank of circulating enteric viruses.

Tang, Y., T. Shigematsu, et al. (2004). "The effects of micro-aeration on the phylogenetic diversity of microorganisms in a thermophilic anaerobic municipal solid-waste digester." Water Research **38**(10): 2537.

http://www.sciencedirect.com/science/article/B6V73-4CG04X4-6/2/60bb66a8ade01599e668a5a6d89a25b5

We demonstrated previously that micro-aeration allows construction of an effective thermophilic methane-fermentation system for treatment of municipal solid waste (MSW) without production of H2S. In the present study, we compared the microbial communities in a thermophilic MSW digester without aeration and with micro-aeration by fluorescence in situ hybridization (FISH), denaturing gradient gel electrophoresis (DGGE), phylogenetic analysis of libraries of 16S rRNA gene clones and quantitative real-time PCR. Moreover, we studied the activity of sulfate-reducing bacteria (SRB) by analysis of the transcription of the gene for dissimilatory sulfite reductase (dsr). Experiments using FISH revealed that microorganisms belonging to the domain Bacteria dominated in the digester both without aeration and with micro-aeration. Phylogenetic analysis based on 16S rRNA gene and analysis of bacteria by DGGE did not reveal any obvious difference within the microbial communities under the two aeration conditions, and bacteria affiliated with the phylum Firmicutes were dominant. In Archaea, the population of Methanosarcina decreased while the population of Methanoculleus increased as a result of micro-aerations as revealed by the analysis of 16S rRNA gene clones and quantitative real-time PCR. Reverse transcription and PCR (RT-PCR) demonstrated the transcription of dsrA not only in the absence of aeration but also in the presence of micro-aeration, even under conditions where no H2S was detected in the biogas. In conclusion, micro-aeration has no obvious effects on the phylogenetic diversity of microorganisms. Furthermore, the activity of SRBs in the digester was not repressed even though the concentration of H2S in the biogas was very low under the micro-aeration conditions.