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Transport Across Chloroplast Membranes: Optimizing Photosynthesis for Adverse Environmental Conditions

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ABSTRACT

Chloroplasts are central to solar light harvesting and photosynthesis. Optimal chloroplast functioning is vitally dependent on a very intensive traffic of metabolites and ions between the cytosol and stroma, and should be attuned for adverse environmental conditions. This is achieved by an orchestrated regulation of a variety of transport systems located at chloroplast membranes such as porines, solute channels, ion-specific cation and anion channels, and various primary and secondary active transport systems. In this review we describe the molecular nature and functional properties of the inner and outer envelope and thylakoid membrane channels and transporters. We then discuss how their orchestrated regulation affects thylakoid structure, electron transport and excitation energy transfer, proton-motive force partition, ion homeostasis, stromal pH regulation, and volume regulation. We link the activity of key cation and anion transport systems with stress-specific signaling processes in chloroplasts, and discuss how these signals interact with the signals generated in other organelles to optimize the cell performance, with a special emphasis on Ca^{2+} and reactive oxygen species signaling.

Key words: stroma, thylakoid, p.m.f., envelope, reactive oxygen species, programmed cell death

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INTRODUCTION

In 2009, the United Nations Food and Agriculture Organization stated the need for a 70% increase in food production to feed a predicted population of 9 billion by 2050. This represents a 38% increase in crop production over the historical trends observed for the last 50 years (Tester and Langridge, 2010). Meeting this target is an extremely challenging task, given the decreased size of the arable land and current and predicted resource limitations (Shabala, 2013). The key to success, therefore, is to identify and implement the best agronomy and breeding practices to ensure the maximum crop yield under hostile soil and environmental conditions. Such implementation requires a comprehensive understanding of the operation and optimization of plant photosynthesis.

Optimizing plant photosynthesis may be achieved at various levels of plant structural organization, from the whole plant (e.g. optimizing the light interception by leaf orientation or its color/albedo) to tissue (stomata density and anatomy; water use efficiency) and molecular (primarily photosynthetic processes in chloroplasts) levels. This review focuses on the last component and, specifically, the role of chloroplast envelope and thylakoid ion channels and transporters in optimizing photosynthesis for adverse environmental conditions.

Chloroplasts are primarily sites of photosynthesis in plant cells that convert light energy into the energy of ATP and sugars, and ultimately determine crop yield. Any adverse environmental factors (drought, flooding, salinity, temperature extremes, nutritional disorders, pathogens, viruses) that affect plant photosynthetic performance will lead to very substantial yield penalties. Chloroplasts harbor three types of membranes: a double (inner and outer) envelope membrane and a thylakoid membrane. Each of these membranes is equipped with a unique set of ion channels and transporters that enable transport of nutrients, solutes, and metabolites in and out of the chloroplast. In the following sections we reveal the molecular nature and operating modes of these transporters, and discuss how their orchestrated

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Name[a]	γ/pS[b]	P_{K+}:P_{Cl-}	Voltage dependence[c]	Transporting function	References
OEP16	330	13	Bell-shaped P_O ~0.8 (0 mV) P_O ~0.3 (+80 mV) P_O ~0.03 (−80 mV)	Export of amines and amino acids (excludes sugars)	Pohlmeyer et al., 1997; Steinkamp et al., 2000
OEP21	350	0.3 (−ATP) 4 (+ATP)	Bell-shaped P_O ~1 (+20–+30 mV) P_O ~0.1 (> +60 mV; < −10 mV)	Export of phosphorylated carbohydrates, ATP (excludes NADH) phosphate import	Bolter et al., 1999; Hemmler et al., 2006
OEP23	350	15	None P_O ~1 (±100 mV)	ND	Goetze et al., 2015
OEP24	500	3.7	Bell-shaped P_O ~0.8 (0 mV) P_O ~0.2 (+130 mV) P_O ~0.1 (−130 mV)	Triose and hexose phosphates, dicarboxylates, ATP, phosphate, charged amino acids, sugars	Bolter et al., 1999; Bräutigam et al., 2008
OEP37	500	14	Bell-shaped P_O ~1 (±60 mV) P_O ~0.5 (±120 mV)	Peptides import (?) Cations (?)	Goetze et al., 2006

Table 1. Basic Functional Properties of the Envelope Porin-like Channels.

The question marks (?) stands for sugegsted but not confirmed function.

[a]The numbers in nomenclature reflect protein molecular weights (in kDa). OEP is an outer envelope protein.

[b]A single-channel conductance was measured directly at the symmetrical 250 mM KCl solution, or the value was extrapolated to this concentration. Most channels displayed multiple substates, but only the fully open state was considered for the selectivity ratio and conductance.

[c]P_O stands for open probability at a given electric potential (potential range).

regulation affects critical metabolic processes via modulation of thylakoid structure, electron transport and excitation energy transfer, proton-motive force partition, ion homeostasis, stromal pH regulation, and volume regulation. We then link the activity of key cation and anion transport systems with the stress-specific signaling processes in chloroplasts, and discuss how these signals interact with signals generated in other organelles to optimize the performance of the cell as the entity, with a particular emphasis on emerging information on the chloroplast-related redox and Ca^{2+} signaling.

METABOLITE EXCHANGE

Chloroplasts are also unique sites for the biosynthesis of fatty acids, and nine out of 20 amino acids are exclusively produced by chloroplasts (Breuers et al., 2011). Chloroplasts also produce NADPH, ATP, and purines, and synthesize a variety of carbohydrates and triose phosphates. These and other, more specific substances (e.g. sulfolipids) produced by chloroplasts need to be exported to the rest of the cell. Metabolites have to cross two envelope membranes. For a long time, only the inner envelope (IE) was considered as being osmotically active, whereas the outer envelope (OE) was considered as a large pore sieve incapable of forming a barrier for the transport of the low molecular weight compounds. This view has been then challenged (Flügge, 2000). Indeed, if the OE is merely a "sieve," why then is it equipped with a substrate-specific amine and amine acid transporting channel outer envelope porin 16 (OEP16), and also with highly cation-selective OEP23 and OEP37 channels (Table 1)? Proteomic analysis has shown that, in contrast to IE that harbors a large variety of transporters, OE is enriched in five proteins with transport functions, namely an ABC transporter with unknown function and OEPs 16, 21, 24, and 37 (Gutierrez-Carbonell et al., 2014). Recently, OEP23 was added to this list (Goetze et al., 2015).

Unlike other porin-like channels in the chloroplast envelope and porins of the outer membrane of plastid ancestors such as Gram-negative bacteria that are β barrels, OEP16 is made from the transmembrane α helices only (Linke et al., 2004). Thus, it is plausible that this porin originates from the plasma membrane of the endosymbiontic bacteria or a host eukaryote. Similar to the mitochondrial inner membrane translocon proteins (TIM) and bacterial amino acid permease, it belongs to a PRAT family of pre-protein and amino acid transporters. Some reports claimed that OEP16 could mediate a precursor protein transport (Reinbothe et al., 2004; Samol et al., 2011). This view, however, was strongly opposed by the experimental evidence presented by Pudelski and co-workers (2010, 2012). Based on the properties of the reconstituted OEP16 (Table 1), it becomes evident that this protein operates physiologically like an amino acid permease. Interestingly, the OEP16.1 transcript is increased in pea embryos upon overexpression of the plasma membrane amino acid permease on the background of a high amino acid concentration in the medium (Weigelt et al., 2008). Of the three existing OEP16 isoforms, OEP16.1 and OEP16.2 are the most abundant. Of these, OEP16.1 is localized in the OE of leaf chloroplasts, whereas OEP16.2 is found in the OE of plastids from seeds, cotyledons, and pollen (Pudelski et al., 2010). OEP16.1 is strongly upregulated by cold stress, implying its important role in the amino acid shuttling upon cold acclimation. However, salicylic acid, drought, or abscisic acid (ABA) had little if any effect on the OEP16.1 expression (Pudelski et al., 2010). Contrary to this, expression of OEP16.2 in the late stages of seed development is under strict ABA control, and *oep16* knockout mutant showed ABA hypersensitivity (Pudelski et al., 2012).

In the absence of phosphorylated carbohydrates or ATP in the intermembrane space, OEP21 possessed slightly anionic selectivity. In the presence of the physiologically relevant ATP

concentrations (1 mM) it changes its selectivity to cationic, showing also a voltage-dependent block (Table 1). Both effects were caused by the voltage-dependent binding of ATP^{4-} within the channel's pore. Phosphorylated carbohydrates, the principal photo-assimilates exported from chloroplasts, had a similar effect on OEP21 selectivity and gating, yet with a lower potency compared with ATP. Consequently, their presence in the intermembrane space tended to moderate the effect of ATP in a competitive manner (Bolter et al., 1999; Hemmler et al., 2006). This blockage by ATP does not imply that the channel does not transport ATP. On the contrary, experimental evidence presented in aforementioned studies implied that ATP is transported by OEP21, but in a "tight fit" manner. This situation is reminiscent of the highly specific transport of ATP by VDAC, a major mitochondrial outer membrane solute channel (Colombini, 2012). Both OEP21 and VDAC displayed a relatively steep voltage dependence, which could be further modulated by some metabolites. In the case of OEP21 the peak of the open probability was ~+25 mV (cytosol positive). At the zero voltage, when the activity of other OEPs was close to maximal (Table 1), activity of OEP21 was reduced by an order of magnitude (Bolter et al., 1999). The question arises of whether there is any electric potential difference between the intermembrane space and the cytosol, and if so how large. Also, does the intermembrane space represent a metabolically separate compartment, different from the cytosol, as it stands for the periplasmic space of the ancestor Gram-negative bacteria? In the case of mitochondria, there is evidence that the intermembrane space has a different pH and is more negative than the cytosol by about 30 mV, reflecting the Donnan equilibrium caused by the accumulation of large impermeable anions in the intermembrane space (Colombini, 2012). The concentration dependence of the OEP21 regulation by phosphorylated compounds suggested their accumulation in the space between OE and IE, and it is conceivable that there is a small but significant voltage difference (cytosol more positive) across the chloroplast OE. The increase in ATP to triose phosphates plus phosphoglycerate and phosphate ratio, observed upon a transition from light to dark conditions, is expected to change the barrier properties of the OE, due to the modulation of the OEP21 ionic selectivity and current rectification (Bolter et al., 1999).

The functions of the three remaining OEPs (OEP23, OEP37 and OEP24) are less clear. OEP37 represents a relatively highly-selective peptide-sensitive cation channel, expressed ubiquitously but at a low level, and controlled by the redox status upon cysteine oxidation (Goetze et al., 2006; Breuers et al., 2011). OEP24 is the only low-selective OEP with a very broad spectrum of transported compounds (Table 1). Its role was attributed to the induction of programmed cell death (PCD) and operation at high metabolic flux rates, when the transport capability of more selective OEPs becomes limiting (Breuers et al., 2011). Some clues for the possible roles of OEP37 and OEP24 came from the proteomics studies of C3 plant (pea) and C4 plant (maize). Comparison of their OE proteomes displayed major differences in the abundance of solute channels. Here, OEP24 and OEP37 were more abundant and OEP21 less abundant in a C4 plant, with no difference in the abundance for OEP16. The protein import channels Toc75 and Tic110 were expressed at a high level and not different between C3 and C4 species (Bräutigam et al., 2008). A higher abundance of the OEP24 is consistent with a higher metabolic flux across the envelope of C4 plants, whereas high and similar abundance of the protein translocon elements is consistent with their housekeeping function.

ION TRANSPORT ACROSS PHOTOSYNTHETIC MEMBRANES AND REGULATION OF LIGHT-DEPENDENT PHOTOSYNTHESIS

Effect of Ions on the Thylakoid Structure and Regulation of Electron and Excitation Energy Transfers

As elegantly shown by Schönknecht et al. (1990) and then confirmed by electron tomography (Shimoni et al., 2005), the thylakoid network represents a single electric unit or a sole large vesicle, packed to occupy lesser volume with a larger surface. Thylakoid membranes also possess the highest known protein to lipid ratio. Thus, protein complexes are in a tight contact with each other, both laterally and vertically, providing a functional connection between neighboring disks in grana stacks. The lateral distribution of different protein complexes is not random: whereas appressed regions are rich with photosystem II (PSII) reaction centers and respective light-harvesting complex (LHCII), non-appressed and stroma-exposed membranes contain primarily photosystem I (PSI) and elements of the cyclic electron transfer system, as well as the F-type H^+-ATPase (Pribil et al., 2014). This is explained by a fact that as a distance between neighboring grana disks membranes is just few nanometers, the protruded parts of the PSI and F-type ATPase are simply excluded from the appressed regions (Kirchhoff, 2013). The appressed regions are also stabilized by non-specific interactions between positively and negatively charged groups of the LHCII in neighboring membranes. Thus, light-induced phosphorylation of the LHCII (introduction of a negative charge) causes weakening of these interactions and migration of the LHCII toward PSI (Pesaresi et al., 2011). A consequence of this grana transition should be a regulation of the spillover of the excitation energy between two photosystems and control of the linear to non-linear (cyclic) electron flows, i.e. NADPH to ATP production (Nevo et al., 2012). The mismatch between NADPH and ATP production and consumption, which may occur upon metabolic status changes, has to be avoided, otherwise a "back pressure" will be exerted on light reactions, leading to the increased photoinhibition and reactive oxygen species (ROS) formation.

Under strong light, adaptive structural rearrangements such as swelling of thylakoids and increase of partitioning gaps in the grana stacks are observed, facilitating access to the photo-damaged D1 protein of PSII and its removal (Yoshioka-Nishimura et al., 2014). Light-induced thylakoid swelling also facilitates the diffusion of the plastocyanin between bf6 complex and a PSI reaction center, enhancing the overall electron transfer rate (Kirchhoff et al., 2011). Computer simulations also predicted that in tightly stacked thylakoids the proton movement in the gaps between the neighboring disks is strongly restricted, resulting in localized pH values >9.5. This explains the arrest of the plastoquinol reduction and decrease of the linear electron transport rate, reversed upon thylakoid swelling (Tikhonov, 2015).

This swelling could only occur when there is a net influx of ions from the stromal side, which drags the water into the thylakoid lumen. Based on the polarity of the transmembrane electric potential difference ($\Delta\Psi$) generated by a light-driven pump, there should be an efflux of Cl^- (or other anion) to the thylakoid lumen, to allow thylakoid swelling but prevent (balance) membrane polarization at the same time (Spetea and Schoefs, 2010). Conversely, influx of the luminal K^+ to the stroma would promote thylakoid *shrinkage* (Cruz et al., 2001). Increase of the ionic strength inside chloroplasts will also result in a weakening of the stacking. Such an increase could be conceived upon salinity stress. However, in barley chloroplasts, no significant change in total ionic strength was observed upon strong salt stress, although reported chloroplast Na^+ and Cl^- concentrations increased several-fold. Osmotic balance in this case was achieved by means of organic solute accumulation (Schröppel-Meier and Kaiser, 1988). In broad beans, salt stress-induced damage in chloroplasts was primarily due to the accumulation of Na^+ and not of Cl^- or K^+ (Slabu et al., 2009). By contrast, salt toxicity and inhibition of the photosynthesis in soybean were more dependent on the Cl^- but not Na^+ accumulation in chloroplasts (Chen and Yu, 2007). A shift in anion balance shifted the solar energy distribution from PSII to PSI, affecting the function of the water-splitting complex and electron transport to and from the plastocyanin (Jajoo et al., 2001; Singh-Rawal et al., 2011). This is also true for cations, which affected light photosynthesis via modification of the membrane surface charge (Barber et al., 1977). NaCl stress-induced decrease in the photosynthetic performance was correlated with thylakoid swelling (Barhoumi et al., 2007). In rice, this swelling was correlated with the enhanced light-induced H_2O_2 production; both phenomena were abolished by the ascorbate addition (Yamane et al., 2012). Thus, H_2O_2 or other ROS appear to control ion fluxes across the thylakoid membrane. It may be hypothesized that ROS could favor anion uptake over cation leak from thylakoids (e.g. by inhibiting constitutive cation fluxes). However, we are not aware of a single study addressing ROS regulation of ion channels/transporters in the chloroplast membranes.

The Role of Thylakoid Ion Channels and Transporters in Proton-Motive Force Partitioning

Photo-phosphorylation, the most fundamental bioenergetic process on Earth, is carried out by H^+-ATP synthase (F-type ATPase) of the thylakoid membrane, energized by the sunlight-generated H^+-motive force (p.m.f.) (Mitchell, 1966) made of two components, $\Delta\Psi$ and ΔpH. From the thermodynamic point of view, only the size of the p.m.f. matters. From the kinetic point of view, however, the composition of the p.m.f. is very important. The $\Delta\Psi$ portion is of primary importance for the turnover rate of F-ATPase. In *Escherichia coli* and mitochondria, the ATP synthesis rate slowed down more than 50 times at zero $\Delta\Psi$ and could not be compensated by the ΔpH increase in the thylakoids, however, the F-ATPase displayed about one-third of its maximal velocity at zero $\Delta\Psi$ (Fischer and Gräber, 1999; Cruz et al., 2001). While the energy-coupling membrane in the mitochondria separates cytosol and stroma, two highly metabolically active compartments, the thylakoid lumen does not display a substantial metabolic activity and thus allows its large acidification upon energization. Therefore, it was widely accepted for some time that ΔpH is a highly dominant contributor to the thylakoid p.m.f. with gradients of up to 3 pH units reported. However, such views are now rejected. Using a non-invasive technique based on the measurements of the electrochromic shift (ECS) of membrane-embedded pigments, it was demonstrated that under typical light and atmospheric conditions, the steady state $\Delta\Psi$ *in vivo* could reach up to 50% of the p.m.f. (e.g. −60 mV; Cruz et al., 2001; Kramer et al., 2003, 2004). Above saturating light intensities, the ΔpH contribution toward p.m.f. grew again and could reach 70%–95% (Takizawa et al., 2007; Klughammer et al., 2013; Johnson and Ruban, 2014).

Why is the existence of a significant contribution of $\Delta\Psi$ at the expense of ΔpH at steady state so important? Assuming that the stromal pH at a steady state under light is kept constant (8.0), the value of ΔpH defines the degree of the lumen acidification. At most extreme conditions (high light intensity, low CO_2), luminal pH drops to 5.5–6.2, as evaluated by both ECS (Takizawa et al., 2007) and indirect methods (Tikhonov et al., 1981; Schönknecht et al., 1995). On the one hand, the luminal acidification is necessary to activate the non-photochemical quenching (NPQ), which alters the antenna (LHCII) and allows part of the excitation energy to be dissipated as a heat, to prevent the PSII photo-damage and ROS generation (Figure 1). On the other hand, lowering pH below 5.5–6 could produce a loss of Ca^{2+} (by the water-splitting complex) and Cu (by plastocyanin). Even more moderate acidification (pH $\leq$6.5) would slow down the electron transport from b6f complex to the PSI. Thus, the luminal pH should be kept between 6.5 and 5.8 under normal conditions in the light (Kramer et al., 2003) and at about 7.7 in the dark (Takizawa et al., 2007). Transmembrane transport of electrons also depends on $\Delta\Psi$. High $\Delta\Psi$ favored a charge recombination in the PSII reaction center and formation of the P680 triplet state, and increased photo-damage by sensitizing cells for singlet oxygen (Bennoun, 1994). The redox potentials of carriers attached to thylakoid surface such as plastocyanin are also modulated by the surface potential (Itoh and Nishimura, 1986). Thus, a fine balance between $\Delta\Psi$ and ΔpH across thylakoid membrane is required to optimize light-dependent photosynthetic reactions.

A massive accumulation of H^+ in the thylakoid lumen at the expense of a $\Delta\Psi$ is due to the significant ionic conductance of the thylakoid membrane, which tends to dissipate the electric potential difference. For a total shift from of $\Delta\Psi$ to ΔpH in p.m.f. storage it is sufficient to have high (non-limiting) ionic permeability of the thylakoid membrane and an abundance of permeable ions. When this is not the case, and $\Delta\Psi$ contribution is substantial at a steady state, the thylakoid ion conductance has to be limited by gating, e.g. by voltage, pH, Ca^{2+}, ligands, and/or by channel expression. On the other hand, an increase in the luminal pH buffering capacity will tend to diminish ΔpH contribution, and cause a compensatory increase of $\Delta\Psi$. Such an effect was reported upon uptake of weak bases such as putrescine, which is synthesized and naturally occurs in stroma (Ioannidis et al., 2012). Another factor that will tend to dissipate ΔpH and decrease ΔpH/$\Delta\Psi$ is an uncoupling activity of the electroneutral K^+ (Na^+)/H^+ exchangers, present in the thylakoid membrane (Tsunekawa et al., 2009; Kunz et al., 2014). In this context, a thylakoid Na^+/H^+ antiporter seems to play a dual role under saline stress conditions: (1) promoting Na^+ accumulation in the thylakoid lumen and thus detoxifying the cytosol and

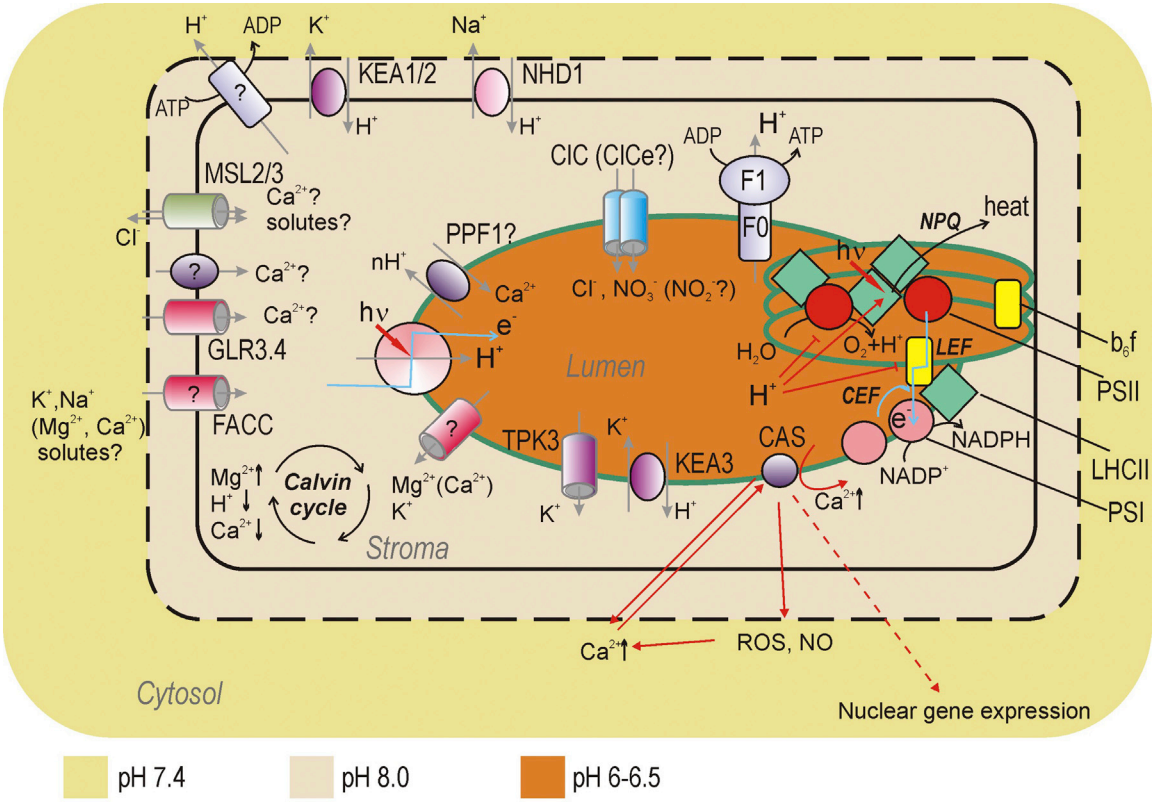

Figure 1. Transport of Major Ions Across Chloroplast Membranes, Regulation of pH and Photosynthesis, and Chloroplast-Related Ca^{2+} Signaling.

MSL, mechanosensitive MscS-like channel; GLR, glutamate receptor channel; FACC, fast activated cation channel; TPK, tandem-pore K^+-selective channel; ClC, anion channel of ClC family; KEA, cation/proton antiporter; NHD, sodium/proton antiporter; F1F0, thylakoid ATP synthase; CAS, thylakoid Ca^{2+} sensor; PPF1, product of the gibberellin-induced and vegetative growth-specific gene; LEF, linear electron flow; CEF, cyclic electron flow; NPQ, non-photosynthetic quenching; LHC, light-harvesting complex, b_6f, cytochrome b_6f complex; PSI and PSII, reaction centers of photosystems I and II.

(2) acting as uncoupler by accelerating the electron transport, thus reducing the leak of electrons and ROS formation (Tsunekawa et al., 2009).

It was demonstrated that Cl^- efflux from and Mg^{2+} and K^+ influx to the stroma are the major counterion fluxes balancing the light-driven H^+ efflux to thylakoid lumen (Figure 1). At low K^+ (<5 mM) Mg^{2+} flux will dominate (Hind et al., 1974); the opposite is true for K^+ >5 mM. Thus, at physiological ionic conditions K^+ influx appears to be more important for the charge balance, whereas Mg^{2+} efflux likely plays a regulatory role, connecting stromal metabolism to light reactions (see the next section). In *Arabidopsis* a member of a tandem-pore K^+ channel family, TPK3, was localized to the thylakoids. Importantly, plants silenced for the TPK3 gene showed lower CO_2 fixation and altered NPQ; this was correlated with a two-fold higher $\Delta\Psi$/ΔpH ratio (Carrarretto et al., 2013). Although displaying a 2-TM (two transmembrane helices with a pore region in between) topology, TPK3 channel has been shown to share the same homology with a 6-TM voltage-dependent K^+ -selective channel, SynK, present in the thylakoids and a plasma membrane of a photosynthetic prokaryote (Zanetti et al., 2010). A preliminary functional characterization of the recombinant TPK3 revealed a K^+-permeable Ba^{2+}-sensitive channel, with a high K^+/Cl^- selectivity (Carrarretto et al., 2013). Modulation of this channel by natural factors was unexplored, although it appeared to display a higher activity at high (>100 μM) Ca^{2+}. It should be noted that a vacuolar-expressed member of the same family, TPK1, is activated by the micromolar cytosolic Ca^{2+} (Gobert et al., 2007) and cytosol acidification (Allen et al., 1998). The function of the TPK3 as a balancing element for light-driven H^+ uptake requires the recuperation of the luminal K^+, depleted in the light. It was proposed that K^+ is taken up into the lumen via activity of the K^+/H^+ exchanger *KEA3* (Finazzi et al., 2015). Yet if this was essential for the maintenance of TPK3-mediated K^+ influx in the light, there should be a decrease of steady state ΔpH in the *kea3* loss-of-function mutant. Instead, *kea3* showed an *increase* in ΔpH (Kunz et al., 2014), because electroneutral K^+/H^+ antiporter activity tends to dissipate ΔpH selectively, with a compensatory increase of $\Delta\Psi$. From our point of view, both the exodus of K^+ from thylakoids in the light and its return to the lumen in the dark could be channel mediated. The driving force for the restoration of the luminal K^+ will be $\Delta\Psi$ that will change its polarity upon light–dark transition (Kramer et al., 2003) combined with a lumen-directed gradient in K^+ concentration, generated in the light. The same logic is applicable to the channel-mediated Cl^- flux (see below).

Light-induced anion (Cl^-, NO_3^-) efflux from the stroma to thylakoids is likely to be mediated by the voltage-dependent anion

channel, whose functional properties (unitary conductance, voltage dependence) are conserved in higher plants (*Peperomia metallica*, spinach; Schönknecht et al., 1988; Pottosin and Schönknecht, 1995b) and Charophyte algae (Pottosin and Schönknecht, 1995a). Anion channel activity displayed a bell-shaped voltage dependence, with a maximum at zero voltage and a very low open probability at large (>30 mV) voltages of either sign at the steady state (Pottosin and Schönknecht, 1995a, 1995b). However, transient changes of $\Delta\Psi$, mimicking those for the light–dark transitions (Kramer et al., 2003) evoked transient anion channel activation, which slowly (over seconds) relaxed to a steady state (Schönknecht et al., 1988; Pottosin and Schönknecht, 1995a). Thus, the voltage-dependent kinetics of the channel was designed in a way to produce anion permeability "on demand," synchronized with the dark–light transitions. The single-channel kinetics of the anion channel revealed that while the close–open transitions of a pair of anion channels were independent from each other, there was also a slow common gate, which opened and closed both channels in a highly synchronous manner (Pottosin and Schönknecht, 1995a). Such a tandem-gating behavior is a hallmark property of the ClC channels in animal cells (Miller, 2006). Most members of this family encode Cl^-/H^+ antiporters, but few of them, which form channels, conserve the activation by external H^+, although probably losing the ability to mediate actual H^+ influx.

It remains, therefore, to be elucidated whether thylakoid anion channels are activated by the low luminal pH. If this turns out to be true, the lumen acidification in the light could be both the *cause* for and a *consequence* of the higher anion permeability. This could explain the increase of the thylakoid membrane ion conductance in the pre-illuminated chloroplasts (Johnson and Ruban, 2014 and references therein) and also an increase in the ΔpH component at the expense of $\Delta\Psi$ at high light intensities Klughammer et al., 2013). One of seven ClC family members in *Arabidopsis*, ClCe, is targeted to the thylakoid membrane, and *clce* mutant showed altered photosynthesis, which may be associated with a reduced speed of the plastoquinol pool reduction, possibly caused by the thylakoid structural rearrangements due to distorted anion homoeostasis (Marmagne et al., 2007). Curiously, ClCe exerted a strong control on a global nitrate homoeostasis *in planta* and was suggested to mediate the uptake of nitrite (NO_2^-) by the thylakoids (Monachello et al., 2009). Clearly there is a need for the direct patch-clamp study of *Arabidopsis* thylakoids, to reveal the identity of the thylakoid Cl^- (ClC) channel, its detailed properties (selectivity, pharmacology, gating, pH regulation) and, in a combination with the reverse genetic approach and non-invasive ECS measurements, the roles executed by this channel *in planta.*

TRANSPORT AND HOMEOSTASIS OF DIVALENT CATIONS AND THEIR IMPACT ON CHLOROPLAST FUNCTION UNDER ADVERSE ENVIRONMENTAL CONDITIONS

Metalloproteins are core elements of the photosynthetic electron transport chain: Mg (central ion in chlorophyll molecule), Cu (in prosthetic group of plastocyanin), Fe (cytochrome hemes, FeS proteins), and Mn (water-splitting complex). Metal ions also participate in nutrient assimilation (Fe), amino acid biosynthesis (Zn), scavenging of ROS (Cu/Zn-superoxide dismutase [SOD]), among other functions. Respective ions have to be imported from the cytosol, and for all of them except Mn, specific transporters (for Fe and Mg) and pumps (for Cu and Zn) were characterized in the IE membrane (for a recent review see Finazzi et al., 2015). In this section we focus on Ca^{2+} and Mg^{2+}, the two most physiologically abundant divalent metal ions, which could be potentially transported also by channels.

Magnesium Transport and Function

Concentrations of free Mg^{2+} in the stroma are estimated to be between 0.5 and 1 mM in the dark and 2–3 mM in the light (Portis and Heldt, 1976; Ishijima et al., 2003). Several key enzymes including ATPases, ribulose-1,5-bisphosphate carboxylase (Rubisco), RNA polymerase, and protein kinases are known to be activated by Mg (Marschner, 1995; Shaul, 2002). Light-induced increase of stromal Mg^{2+} is essential for the activation of the two key enzymes of Calvin cycle, fructose-1,6-bisphosphatase (FBPase) and sedoheptulose-1,7-bisphosphatase (SBPase) (Hertig and Wolosiuk, 1980; Wolosiuk et al., 1982). Thus, influx of Mg^{2+} to stroma in the light stimulates the Calvin cycle and thus CO_2 fixation (and hence, the overall photosynthesis). Impairment in the photosynthetic CO_2 fixation in Mg-deficient leaves causes an over-reduction in the photosynthetic electron transport chain (Laing et al., 2000) that potentiates the generation of highly reactive ROS species (Cakmak and Kirkby, 2008). This implies a higher physiological requirement for Mg in plants grown under high-light conditions.

Light-induced Mg^{2+} influx from thylakoids to the stroma was insensitive to La^{3+} but inhibited by ruthenium red. It was also stimulated by NH_4Cl (Ishijima et al., 2003), most likely as a result of the increase in the driving force for Mg^{2+} due to increase in the $\Delta\Psi$. Thus, an ion channel (or uniporter) for Mg^{2+} has to operate in the thylakoid membrane. Initial evidence for the existence of a Ca^{2+}- and Mg^{2+}-permeable channel in the thylakoid membranes was provided by the reconstitution study by Enz et al. (1993). Later, similar channels were detected and studied in more details by patching intact swollen thylakoids from freshly broken chloroplasts (Pottosin and Schönknecht, 1996). The reported cation channel was permeable to Mg^{2+} ($\sim$20 pS conductance) as well as to K^+ and Ca^{2+}, and showed a moderate voltage dependence. The channel's open probability increased four-fold (from 0.1 to 0.4) upon a voltage shift from $-$50 mV (stroma negative) to +50 mV. In the light, therefore, a voltage-dependent decrease in the open probability compensated the decrease in a driving force for Mg^{2+} influx, so that time-averaged current through a single Mg^{2+} channel was constant (around $-$0.3 pA) at voltages between $-$60 and $-$20 mV (Pottosin and Schönknecht, 1996). Quantitative estimates suggested that Mg^{2+} flux, mediated by the whole thylakoid population of Mg^{2+}-permeable channels, will be sufficient to increase stromal Mg^{2+} by 1 mM in just 1 s, if not accounting for a possible stromal Mg^{2+} buffering. The molecular identity of a higher-plant thylakoid divalent cation-permeable channel is enigmatic but, based on its physiological potential, clearly warrants further study.

Calcium Content and Transport in Chloroplasts

According to various estimates, chloroplasts of different plant species contain between 3 and 25 mM of total Ca^{2+} (Larkum, 1968; Nobel, 1969; Portis and Heldt, 1976; Hochmal et al., 2015). Most of it is bound to the negatively charged thylakoid membranes or to calcium-binding proteins, keeping free Ca^{2+} concentration in the stroma in the light at a level of ~150 nM (Sai and Johnson, 2002). Luminal free Ca^{2+} levels are largely unknown but are assumed to be higher than stromal ones.

Giving a negative trans-envelope potential difference, Ca^{2+} could be transported from the cytosol to stroma downhill, via a uniporter or a channel. Initially, two pumps were proposed to transport Ca^{2+} across the envelope. Later, one of them (ACA1) was shown to be expressed specifically in roots, and its subcellular location is at the tonoplast and ER membranes (Dunkley et al., 2006). Another one is a heavy metal pump (AtHMA1), which primarily transports Cu^{2+} and Zn^{2+} across the IE (Seigneurin-Berny et al., 2006). In addition, experimentally observed chloroplast Ca^{2+} influx was not accompanied by pH changes, did not require ATP, and was linear in a wide range of extra-chloroplast Ca^{2+} concentrations (Kreimer et al., 1985; Roh et al., 1998). This is consistent with Ca^{2+} uptake by a uniporter or a channel. As discussed earlier, glutamate receptor channels and/or MscS-like channels may be likely candidates (Figure 1).

Ca^{2+} was shown to be accumulated in the light in the thylakoid lumen via Ca^{2+}/H^+ antiport (Ettinger et al., 1999). The product of PPF1, a gibberellin-induced gene controlling (accelerating) flower development, is associated with chloroplasts and thought to encode a putative Ca^{2+} transporter. PPF1-antisense plants have shown a decreased Ca^{2+} storage (Wang et al., 2003). Moreover, overexpressors displayed a higher meristemic cell death due to increased Ca^{2+} mobilization, which promoted the DNA fragmentation (Li et al., 2004). Thus, it was speculated that PPF1 could support Ca^{2+} storage in thylakoids, possibly by mediating Ca^{2+}/H^+ exchange across the thylakoid membrane (Rocha and Vothknecht, 2012; Nomura and Shiina, 2014). The subchloroplast location of PPF1 and its ion transporting function *in planta* remain to be elucidated, however. Light–dark transition at the end of the day was accompanied by a massive and long-standing (20–25 min) increase in the stromal Ca^{2+}, which by some estimates reached 5–10 μM (Johnson et al., 1995; Sai and Johnson, 2002). Such a massive increase in Ca^{2+} is thought to represent a hibernation signal for the CO_2 fixation machinery (see below). Unexpectedly, the source of Ca^{2+} release was not a thylakoid lumen, which was charged with Ca^{2+} during daytime, but an as yet unknown alternative chloroplast Ca^{2+} store (Sai and Johnson, 2002).

Calcium and Its Downstream Targets

At a biochemical level, Ca^{2+} is believed to control two key enzymes of the Calvin cycle, FBPase and SBPase. At low resting free stromal Ca^{2+} concentration the activity of these enzymes is high; an increase in the stromal Ca^{2+} following the light to dark transition leads to their inhibition (Kreimer et al., 1988). It is conceivable, therefore, that nocturnal Ca^{2+} release into stroma assists to put the CO_2 fixation cycle "into bed" (Sai and Johnson, 2002). However, both enzymes also require Ca^{2+}, directly or via the thioredoxin/ferroredoxin system, for their activity, and are potentiated by the high stromal Mg^{2+} and pH (Charles and Halliwell, 1980; Hertig and Wolosiuk, 1980; Wolosiuk et al., 1982; Rocha and Vothknecht, 2012). Thus, rather than the effect of stromal Ca^{2+} alone, a concerted effect of Ca^{2+}, pH, Mg^{2+}, and redox state on the Calvin cycle enzymes should be considered.

PSBO, a weak Ca^{2+} sensor, is one of the extrinsic proteins tightly bound close to the mouth of the H^+-releasing channel of the water-splitting complex (Kruk et al., 2003). Interestingly, it displays a pH-dependent conformational change, and exposes its Ca^{2+}-binding groups to the lumen only at slightly acidic (light) conditions (Shutova et al., 2005). In addition to controlling the activity of the water-splitting complex, PSBO and Ca^{2+} stabilized the high redox potential form of cytochrome *b*-559 (McNamara and Gounaris, 1995). Several other Ca^{2+} -dependent components control structure, recycling, and electron transfer function of PSI and PSII (Rocha and Vothknecht, 2012; Hochmal et al., 2015), by either direct Ca^{2+} binding or via Ca^{2+}-dependent phosphorylation. Supplemental Ca^{2+} also increases activities of SOD, catalase, and ascorbate peroxidase (Sairam et al., 2011), and Ca^{2+} binding to the 8 kDa subunit III of the CFo H^+ channel was shown to be essential to regulate the extent of the luminal acidification and thus activity of the xanthophyll cycle (Pan and Dilley, 2000), contributing to the chloroplast photoprotection under stress conditions.

Some caution should be exercised while discussing the effects of Ca^{2+} regulation of stromal enzymes. For example, while (p)ppGpp synthetase possesses specific Ca^{2+}-binding domain (EF-hand), it could only be activated by free Ca^{2+} of 100 μM and higher (Tozawa et al., 2007). Even more caution should be exercised while talking about "dramatic" Ca^{2+} effects on channels' activity (e.g. Tic110 channel; Balsera et al., 2009), when the tested Ca^{2+} concentration was non-physiologically high (20 mM). It also remains unproved as to whether chloroplast enzymes *in vivo* could be sensibilized to Ca^{2+} via calmodulin (CaM). NAD kinase that converts NAD to NADP is activated *in vitro* by Ca^{2+}/CaM (Chai et al., 2005). Similarly, protein import into chloroplasts is Ca^{2+} dependent, which is supposed to be due to the CaM binding to the stromal side of Tic32, an important protein of the IE protein translocon (Chigri et al., 2006). Calmodulin affinity chromatography and mass spectrometry studies revealed another 200 chloroplast proteins as potential targets for CaM (Dell'Aglio et al., 2013). Yet, despite the presence of the transit sequence for chloroplast targeting in several *Arabidopsis* CaMs and CaM-like proteins, none of these proteins has been found in chloroplasts so far (Nomura and Shiina, 2014).

Stress-Specific Ca^{2+} Signaling in Chloroplasts

Transient cytosolic calcium $[Ca^{2+}]_{cyt}$ elevations are reported in response to literally every known abiotic and biotic stimulus, and are therefore considered key components of plant signaling networks (Sanders et al., 1999; Bose et al., 2011). Over the last few years evidence has accumulated suggesting that stress-induced free Ca^{2+} transients occur also in the chloroplast stroma (Nomura and Shiina, 2014). Similar to the cytosolic Ca^{2+} signals, stromal Ca^{2+} transients are strictly stress dependent. Cold and salt stresses elicited rapid stromal Ca^{2+} spikes within a minute,

whereas hyperosmotic stress induced a biphasic long-lasting Ca^{2+} transient in the stroma (Nomura et al., 2012). Thus, it is very tempting to suggest that, similar to cytosolic Ca^{2+} transients, stress-specific Ca^{2+} signatures in the stroma may be involved in the regulation of photosynthesis and metabolic processes in this organelle. Moreover, most of the stromal Ca^{2+} signals evoked by stress stimuli were accompanied by cytosolic Ca^{2+} increases. The latter may occur after stromal Ca^{2+} increase (day–night transition), before (pathogen elicitors), synchronous with (cold, salt) or in between two stromal transients (Sai and Johnson, 2002; Nomura et al., 2012). This led to a concept that the chloroplast represents an element of the global cellular Ca^{2+} network and contributes to $[Ca^{2+}]_{cyt}$ signaling (Hochmal et al., 2015) that mediates plant-adaptive responses to the environment. In combination with ROS transients, these Ca^{2+} spikes may provide a link between stress-dependent adaptive responses in various cellular compartments, enabling integrated cellular response to the environment. Communication between Ca^{2+} signals in cytosol and stroma is not trivial. For instance, although light-induced Ca^{2+} uptake from cytosol to stroma was reported (Muto et al., 1982), this apparently was not transformed into any measurable stromal free Ca^{2+} increases. Thus, increase in the Ca^{2+} buffering capacity upon light-induced stromal alkalinization and/or efficient light ($\Delta\mu H^+$)-driven Ca^{2+} uptake by thylakoids efficiently damped stromal Ca^{2+} increase in this case.

The specificity of Ca^{2+} signaling in plant cells is further determined by the large number of potential Ca^{2+} sensors and downstream effectors. Hochmal et al. (2015) mentioned the presence of ~80 polypeptides, ~400 sensors, and ~200 target proteins that can potentially decode stress-induced Ca^{2+} signatures in plant cells. Some of these are located in chloroplasts. Of specific interest are CAS (calcium-sensing receptor) proteins.

CAS Protein

High-capacity, low-affinity Ca^{2+}-binding CAS protein was identified in the thylakoid membranes of *Arabidopsis* (Nomura et al., 2008). CAS protein has its Ca^{2+}-binding domain exposed to the stroma and represents the main target of Ca^{2+}-induced phosphorylation in chloroplasts (Stael et al., 2012). It is involved in the external Ca^{2+}-induced cytosolic Ca^{2+} increase (Weinl et al., 2008) and played a crucial role in the generation of $[Ca^{2+}]_{cyt}$ transients (Nomura and Shiina, 2014). When expressed in the heterologous expression systems such as human embryonic kidney cell line or in guard cells, CAS was shown to be involved in Ca^{2+} uptake across the plasma membrane (Han et al., 2003; Nomura et al., 2008; Wang et al., 2012). It was shown that CAS proteins regulate stomatal movements during calcium signal transduction (Tang et al., 2007; Nomura et al., 2008). CAS-mediated downstream events upon guard cell response to high external Ca^{2+} were sequential increases in H_2O_2 and NO generation, eventually leading to cytosolic Ca^{2+} transient and stomata closure (Wang et al., 2012). Drought tolerance was reduced in CAS antisense lines, as a result of an attenuated stomatal closure (Wang et al., 2014), and higher CAS expression prevented drought-induced inhibition of photosynthetic efficiency and degradation of chloroplasts in transgenic plants (Zhao et al., 2015). These antisense lines were also defective in the photosynthetic electron transport, had less efficient connection between the major LHCII and PSII reaction centers, and decreased NPQ rates (thus, a compromised ability to dissipate excessive energy). Chloroplast CAS were found to be crucial for photo-acclimation and the expression of LHCSR3, an ancient light-harvesting protein that mediates NPQ for effective photoprotection of PSII (Petroutsos et al., 2011), and thus required for photoprotection of PSII.

CAS proteins were also found to play a pivotal role in mediating plant hypersensitive responses to biotic stress. It was shown (Nomura et al., 2012) that pathogen-associated molecular pattern signals are quickly relayed to chloroplasts and evoke specific Ca^{2+} signatures in the stroma that are shaped by CAS. Thus, CAS proteins are involved in the transcriptional reprogramming of the cell during plant innate immunity. The suggested mechanism most likely involves the suppression of chloroplast gene expression by $^1O_2$-mediated retrograde signaling (Nomura et al., 2012).

Thus, CAS operates both as an emitter and a receiver of a Ca^{2+} signal (Figure 1), but the ways its transmitter activity is integrated with the chloroplast Ca^{2+} transport and ROS-generating machineries remain elusive.

ADJUSTING PHOTOSYNTHESIS FOR ADVERSE ENVIRONMENTAL CONDITIONS

Leaf Photochemistry and Redox Homeostasis Under Stress Conditions

Collectively, abiotic stresses such as drought, salinity, heat, cold, flooding, oxidative stress, nutrient deficiencies, and toxicities yield penalties for major crop plants of 50% (Atkinson and Urwin, 2012). Also detrimental are effects of biotic factors (pests and diseases) that cause a comparable yield loss. One of the common features of those stresses is a stress-induced accumulation of ROS. Under light conditions, chloroplasts are the major ROS-producing organelles, producing ~20-fold more ROS than mitochondria (Wrzaczek et al., 2013). The major sites of ROS generation are PSI and PSII (Asada, 2006; Bose et al., 2014).

Chloroplasts themselves are targets for the ROS-induced damage, and imbalance in the ROS production and consumption under stresses caused a photoinhibition, primarily due to the photo-damage of PSII (Nishiyama et al., 2001; Allakhverdiev et al., 2002; Allakhverdiev and Murata, 2004; Yang et al., 2007; Takahashi and Murata, 2008). Why are chloroplasts involved so heavily in the ROS production? Would it not be better to simply dampen the level of ROS production to some safe threshold? The answer is two-fold. First, for a large number of plants (e.g. those with C4 type photosynthesis), light intensity is a main factor limiting photosynthesis, so they are equipped (both anatomically and physiologically) with various means to maximize the light interception and absorption. As for C3 plants, although for them the light intensity is not a limiting factor, their photosynthetic machinery is organized to provide a maximum performance in a specific habitat under *optimal* conditions. Adverse environmental conditions such as drought, salinity, and unfavorable temperatures will impose both

stomatal- and non-stomatal limitation on photosynthesis, thus resulting in an excessive ROS production that will have to be dealt with.

Second, over the last 10–15 years the role of ROS as important second messengers and signaling agents has emerged. The ROS signaling network is highly conserved among aerobic organisms and controls a broad range of biological processes such as growth, development, and responses to biotic and abiotic stimuli (Mittler et al., 2011). It is believed that ROS that are produced in the chloroplast are acting as intermediates in the retrograde signaling from the chloroplast to the nucleus during acclimation of photosynthesis (Nott et al., 2006; Galvez-Valdivieso and Mullineaux, 2010). The H_2O_2 exodus from the chloroplast is mediated by aquaporins (Borisova et al., 2012), and the relatively long life of H_2O_2 makes it a highly efficient signaling molecule for the intracellular communication. This communication seems to occur in both directions, with apoplastic ROS signals being also transduced to chloroplasts (Sierla et al., 2013). Thus, chloroplasts may assume the dual role of both "amplifiers" and "executers" in the ROS signaling (Shapiguzov et al., 2012). This may explain a biphasic ROS production curve observed by many researchers in response to various stimuli (Sierla et al., 2013), for instance, the ROS burst observed upon pathogen infection (Mandal et al., 2011).

Could Chloroplast Channels and Transporters Be Targets for ROS Signaling?

In the plasma membrane (PM) of the higher plants, ROS inhibit or modulate a variety of constitutively expressed K^+-selective and cation channels, and induce a variety of novel conductances for K^+ and Ca^{2+} (Pei et al., 2000; Zhang et al., 2001; Demidchik et al., 2002, 2003, 2007, 2010; Garcia-Mata et al., 2010; Zepeda-Jazo et al., 2011; Laohavisit et al., 2012). In parallel with the stress-induced ROS increases, the increase in polyamines (PAs) levels is observed as a by-product of the metabolic alterations, also in a stress-specific manner (Alcazar et al., 2006, 2010). PAs can act both as a source of ROS but also as ROS scavengers and activators of key antioxidant enzymes (Kusano et al., 2008; Pottosin and Shabala, 2014), and are able to potentiate ROS-induced conductance across the PM (Zepeda-Jazo et al., 2011; Velarde-Buendia et al., 2012). PAs also are known to be able to inhibit ion channels' activity in plant PM and vacuoles (Dobrovinskaya et al., 1999; Shabala et al., 2007; Pottosin and Shabala, 2014) and to modulate ionotropic Ca^{2+} and H^+ pumps of the PM (Pottosin and Shabala, 2014).

Modulation of activities of ion channels and transporters by ROS and PAs leads to significant alterations in Ca^{2+} signaling and K^+ homoeostasis. ROS-induced Ca^{2+} permeability (with or without PAs) is involved in a polarized growth, hormonal regulation, stress responses (e.g. hypersensitive response, drought, and salt), and, generally, in a switch between survival and PCD scenarios (reviewed in Pottosin and Shabala, 2014). On the other hand, such a switch may be also controlled by the cytosolic K^+, with K^+ retention underlying abiotic stress resistance and K^+ loss being a trigger for PCD (Bortner and Cidlowski, 2007; Chen and Yu, 2007; Demidchik et al., 2010; Pottosin and Shabala, 2014; Shabala and Pottosin, 2014).

As far as we know, none of these scenarios was exploited in relation to the chloroplast ion transport machinery. Particularly, the effects of ROS and PAs on chloroplast ion channels and transporters are unknown. Yet there are some clues clearly indicating a need for such studies. Putrescine (Put) is naturally produced in chloroplasts by arginine decarboxylase, and its import in thylakoid lumen decreased the ΔpH component of the p.m.f. and stimulated photophosphorylation (Ioannidis et al., 2012). Interestingly, although at drought stress Put production was increased, ΔpH component of p.m.f. *increased*, presumably as a result of the higher ionic strength (Ioannidis et al., 2012). Regarding ROS, several chloroplast proteins including H^+-ATPase are potential targets for the reversible oxidation in chloroplasts (Wrzaczek et al., 2013). As one of the mechanisms, protein cysteines and methionine oxidation link oxidative signals with protein phosphorylation (Hardin et al., 2009; Turkeri et al., 2012). There is also evidence that proper K^+/Na^+ balance controls the chloroplast performance. In particular, Na^+ appears to be essential for O_2 evolution, H^+ uptake, and thylakoid stacking (Alia et al., 1992; Mehta et al., 2009; Goh et al., 2010). On the other hand, K^+ deficient plants are light sensitive and show greater superoxide production (Cakmak, 2005). The relative importance of Na^+ and K^+ in chloroplasts may be addressed by manipulation of relative expression of Na^+ and K^+ non-differentiating channels and/or H^+ antiporters and those selective for K^+, on the background of high salt and/or K^+ deficiency.

K^+ retention under salt stress was strongly enhanced by the exogenous application of compatible solutes such as glycine betaine or amino acids (Cuin and Shabala, 2005, 2007a), and pre-treatment of cells with these organic osmolytes were also efficient in ameliorating detrimental effects of oxidative stress on their performance (Cuin and Shabala, 2007b; Shabala et al., 2012). It was also shown that betain enhances the stability of photochemical performance of the reaction centers in the thylakoid membranes (Papageorgiou and Murata, 1995; Allakhverdiev et al., 2003). This warrants a further investigation of the mode of production and transport of organic osmolytes within the chloroplasts, and its impact on chloroplast ion homeostasis and photosynthetic performance.

ROS response upon pathogen infection involves production of the defense-related hormones such as salicylic acid (SA) (Nomura et al., 2012). The most important pathway for SA synthesis is a chloroplastic pathway (Wildermuth et al., 2001; Vlot et al., 2008). SA transport in plants occurs under strict ROS and Ca^{2+} control (Jayakannan et al., 2015a), and SA is known to modulate activity of the PM H^+-ATPase (Liu et al., 2008, 2009a, 2009b), and also affected (most likely, indirectly) the activity of voltage- and ROS-gated K^+ permeable channels at the PM (Jayakannan et al., 2013, 2015b). If the same scenario is applicable to the chloroplast membranes, this then can explain the reported effects of SA on photosynthetic electron transport (Janda et al., 2012).

Recently, an important role of chloroplasts in PCD in plant leaves has emerged (Ambastha et al., 2015). This process is mediated by the singlet oxygen ($^1O_2$) produced by the PSII and its light-harvesting antennae (Krieger-Liszkay et al., 2008; Shapiguzov et al., 2012), and is observed as microlesions in the green plant

tissues. Chloroplast-based PCD is executed by caspase-like proteases (11 families in *Arabidopsis* genome; Ambastha et al., 2015). Moreover, stress-inducible type I metacaspase AtMC1b was found in *Arabidopsis* chloroplasts (Castillo-Olamendi et al., 2007). Whether PCD is mediated by the chloroplast generated ROS directed to extra-chloroplast targets, or whether it also involves intra-chloroplast targets (e.g. Ca^{2+}-permeable channels in chloroplast membranes), remains to be elucidated.

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AUTHOR CONTRIBUTIONS

I.P. wrote the sections Metabolite Exchange and Ion Transport Across Photosynthetic Membranes and Regulation of Light-Dependent Photosynthesis, and S.S. wrote the sections Introduction and Adjusting Photosynthesis for Adverse Environmental Conditions. Both authors jointly wrote the section Transport and Homeostasis of Divalent Cations and Their Impact on Chloroplast Function under Adverse Environmental Conditions, and contributed to editing and revising other sections.

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