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Review

Regulation of photosynthesis by ion channels in cyanobacteria and higher plants

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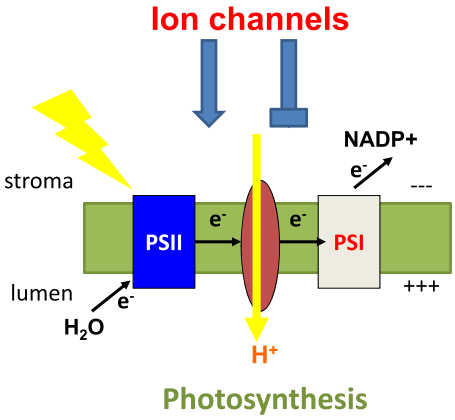
HIGHLIGHTS

- Ion channels regulate photosynthesis in cyanobacteria and higher plants.
- Some organellar ion channels are identified from molecular point of view.
- Genetic approach identifies channels important for light utilization.

GRAPHICAL ABSTRACT



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ABSTRACT

Photosynthesis converts light energy into chemical energy, and supplies ATP and NADPH for CO_2 fixation into carbohydrates and for the synthesis of several compounds which are essential for autotrophic growth. Oxygenic photosynthesis takes place in thylakoid membranes of chloroplasts and photosynthetic prokaryote cyanobacteria. An ancestral photoautotrophic prokaryote related to cyanobacteria has been proposed to give rise to chloroplasts of plants and algae through an endosymbiotic event. Indeed, photosynthetic complexes involved in the electron transport coupled to H^+ translocation and ATP synthesis are similar in higher plants and cyanobacteria. Furthermore, some of the protein and solute/ion conducting machineries also share common structure and function. Electrophysiological and biochemical evidence support the existence of ion channels in the thylakoid membrane in both types of organisms. By allowing specific ion fluxes across thylakoid membranes, ion channels have been hypothesized to either directly or indirectly regulate photosynthesis, by modulating the proton motive force. Recent molecular identification of some of the thylakoid-located channels allowed to obtain genetic proof in favor of such hypothesis. Furthermore, some ion channels of the envelope membrane in chloroplasts have also been shown to impact on this light-driven process. Here we give an overview of thylakoid/chloroplast located ion channels of higher plants and of cyanobacterium *Synechocystis* sp. PCC 6803. We focus on channels shown to be implicated in the regulation of photosynthesis and discuss the possible mechanisms of action.

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Contents

Introduction

During photosynthesis, taking place in the thylakoid membrane, photons are absorbed by the antenna pigments and the excitation energy is transferred from the site of absorption to the reaction centers. In prokaryotic photosynthetic organisms such as cyanobacteria, thylakoid membranes enclosing the lumen compartment, are not isolated from the cytosol. Instead, in eukaryotic photosynthetic organisms, including algae and higher plants, the thylakoids are surrounded by the stroma and two envelope membranes, forming together the chloroplast as bioenergetic organelle. In higher organisms but not in cyanobacteria [1], thylakoids are organized into stacked membranes called grana. The sites of photon absorption are the light-harvesting complexes of the thylakoid membrane which contain protein-bound chlorophyll and carotenoids, and the phycobilisomes containing phycobilins as pigments in higher plants and cyanobacteria, respectively.

Photosynthetic electron transport and proton motive force

In the reaction center of photosystems, excitation is converted into charge separation, which drives electron flow from photosystem II (PSII) to photosystem I (PSI) *via* the cytochrome b_6f complex [*e.g.* 2]. Most components of these macromolecular complexes are highly conserved between cyanobacteria and eukaryotic photosynthetic organisms. In addition, in cyanobacteria the thylakoid membrane is also the site of respiration [3]. In general, the net result of the light-driven electron flow is the oxidation of water molecules, molecular oxygen evolution, the reduction of $NADP^+$, and generation of a proton gradient (ΔpH) across the membrane with an acidic pH generated on the luminal side of the thylakoid membrane. The linear electron flow is strictly correlated with the evolution of molecular oxygen, the rate of which can be taken as a measure of photosynthetic efficiency (for recent review see *e.g.* [2]). However, a light driven cyclic electron transport around PSI and cytochrome b_6f is also operative, which does not evolve oxygen, nor induces NAD^+ reduction but only contributes to proton translocation.

The light-driven trans-thylakoid proton motive force (*pmf*), is composed of an osmotic component ΔpH and of an electric component ΔΨ, and serves both to drive the synthesis of ATP *via* the ATP-synthase and to regulate light capture by the photosynthetic antenna to prevent photodamage (*e.g.* [4]). Indeed, the ΔpH component of *pmf*, through significant acidification of the lumen, is crucial for initiating photoprotection of the photosynthetic apparatus through energy dependent non-photochemical quenching (qE), a process that thermally dissipates the excess absorbed light energy, thereby limiting the production of reactive oxygen species (ROS) *e.g.* [5–8]. qE, a component of non-photochemical quenching (NPQ), is also called energy-dependent excitation quenching, because thermal dissipation is stimulated by ΔpH, which builds up across the thylakoid membrane during photosynthetic electron transport. Since qE involves the de-excitation of singlet excited chlorophyll, therefore it is also called feedback de-excitation. ATP synthase mediates exit of protons from the thylakoid lumen. Its malfunction was recently shown to increase the steady-state proton motive force, resulting in strong lumen over-acidification. This was found to substantially inhibit linear electron flux, causing NPQ activation even upon exposure to low intensity light [9].

Ion fluxes across thylakoid membranes might contribute to regulation of photosynthesis (and respiration in cyanobacteria) by modulating the electric component of the transthylakoid proton motive force. Approximately 50% of the steady-state trans-thylakoid *pmf* is ascribed to the electric field both in higher plants and eukaryotic algae (*e.g.* [4,10]). The exact mechanism of how *pmf* is partitioned into ΔΨ and ΔpH is still unclear, but at least three factors seem to be important, including the capacitance of the thylakoid membrane (which determines the ΔΨ generated for the transfer of a charge across the membrane) and the proton-buffering capacity of the lumen (which sets the actual value of luminal pH following proton translocation). A recent study reported evidence that permeant buffers, such as putrescine, are able to dissipate the ΔpH component, to favor ΔΨ, and thus, to adjust the ΔpH/ΔΨ ratio. Elevated putrescine level in infiltrated leaves caused a decreased qE [11]. The third factor concerns the ionic composition on the two sides of the thylakoid membrane. Influx of protons into the lumen causes accumulation of positive charges, thus a development of inside-positive membrane potential. Ion movement determines the degree to which the ΔΨ component can be dissipated. In higher plants, it has been postulated that the efflux of cations from the lumen toward the stroma or flux of anions in the opposite direction would permit dissipation of the transmembrane electrical potential while conserving the pH gradient. According to Kramer and colleagues, in the steady state, the inward proton flux is balanced by the ATP synthase which becomes activated and a large fraction of *pmf* might be stored prevalently as ΔΨ. The partial dissipation of ΔΨ by counterion movements *via* ion channels would allow the development of significant ΔpH across the thylakoid membrane (and acidification of the lumen) and would thus lead to activation of qE at a given *pmf*. Overall *pmf* increases upon illumination. Thus, differential partitioning of the thylakoid *pmf* into ΔpH/ΔΨ could contribute to optimization of the regulation of energy transduction, and under stress conditions, to triggering of the photoprotective mechanism qE. In this scenario, ion channels would play a regulatory role even at constant total *pmf*.

The electrical compensations of light-driven H^+ uptake into thylakoids have been proposed already long time ago to be achieved by concomitant fluxes of Cl^-, K^+ and Mg^{2+} [11–14] but the molecular entities responsible for these fluxes are being determined only nowadays. Fig. 1. illustrates the ion channels identified from molecular point of view and among them the ones shown to regulate photosynthesis in cyanobacteria and chloroplasts.

Cyanobacterial ion channels

Thanks to the complete genome sequencing of various cyanobacteria species, several putative ion channels have been identified based on sequence similarity to ion channels from higher organisms and/or on

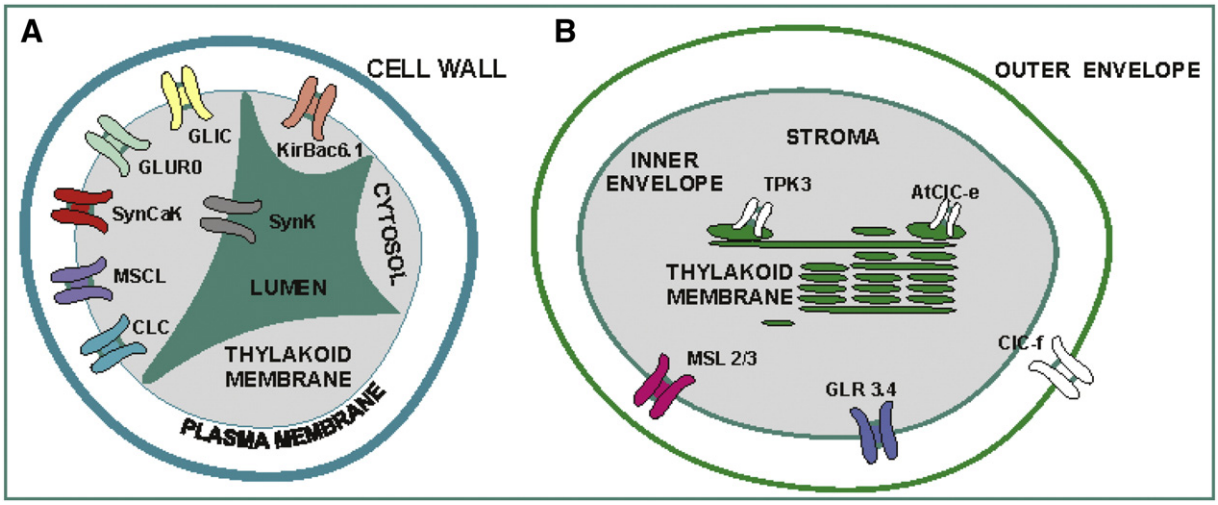

Fig. 1. Ion channels identified in cyanobacteria and higher plant chloroplasts. A) Different ion channels identified from molecular point of view in cyanobacteria are shown. In all cases, ion channel activity of the shown proteins has been proven, in most cases by electrophysiological techniques employing heterologous expression systems. The thylakoid-located SynK has been shown to regulate photosynthetic activity. B) Ion channel proteins identified in higher plant chloroplast outer, inner envelope and thylakoid membranes are shown. Electrophysiological experiments suggest the presence of an activity ascribable to a glutamate receptor in the inner envelop membrane and MSL2/3 that have been proved to work as ion channels. The other proteins (empty forms) indicate putative channels not proved to function as ion-conducting pathways yet. Genetic evidence indicates that the shown ion channel proteins affect photosynthesis (except for the case of TPK3). See text for further description.

the presence of typical amino acid sequences, such as the selectivity filter sequences [15]. Several of these putative channels have been expressed in heterologous systems in order to study their functional electrophysiological properties. Among these channels are the first prokaryotic glutamate receptor from *Synechocystis* which was found to be potassium-selective and represents an evolutionary link between two transmembrane containing potassium channels and glutamate receptors of higher organisms [16] and a proton-gated ion channel from the nicotinic acetylcholine receptor family of *Gloeobacter violaceus*, named GLIC [17]. Both channels have been successfully crystallized and gave important insight into the function of their eukaryotic counterparts [18,19]. In addition, a 6 TM potassium channel named SynK was identified [20] as well as the cyanobacterial homolog of the CLC chloride channel family, which has been recently crystallized and proved to function as a slow transport-rate chloride–proton antiporter [21].

Only a few reports deal with determination of the function of cyanobacterial ion channels in cyanobacteria themselves. These studies take advantage of the fact that homoplasmic knock-out mutants can be obtained in cyanobacteria by homologous recombination. Nazarenko and colleagues provided evidence that the *Synechocystis* sp. PCC 6803 large mechanosensitive channel of the plasmamembrane (MscL) operates as a verapamil/amiloride-sensitive outward Ca^{2+} channel that is involved in the plasma-membrane depolarization-induced Ca^{2+} release from the cells under stress conditions [22]. In a recent study, a K^+-dependent slight growth defect was observed when the cyanobacterial homolog of KirBac6.1 (inward rectifying K^+ channel) was deleted from *Synechocystis*, suggesting that KirBac might contribute to low affinity uptake of K^+ [23]. Our group has recently identified a calcium-dependent potassium channel as well, whose deletion results in a photosynthesis-unrelated phenotype and confers increased resistance to zinc [24].

Cyanobacterial channels/transporters and photosynthesis

As mentioned above, we have recently identified a putative, 6 transmembrane-domain K^+ channel, SynK in the genome of *Synechocystis* whose amino acid sequence contains the typical selectivity filter of potassium channels (amino acid sequence TMTTVGYGD). SynK mediated K^+ transport when it was expressed in *Escherichia coli* (*E. coli*) mutant strain LB2003 lacking endogenous K^+ channels and gave rise to an outwardly rectifying voltage-dependent potassium selective activity when expressed in Chinese hamster ovary cells (CHO). Using membrane fractionation and Western blot as well as immunogold electron microscopy, SynK was located to *Synechocystis* thylakoid and to plasma membrane [20]. To our knowledge, SynK is the only by far identified *bona fide* channel in cyanobacteria thylakoid. In order to understand its physiological role, a SynK-less mutant was obtained, which displayed a clear photosynthetic phenotype: the SynK-less mutant showed clear photosensitivity already at moderate light intensity and was unable to efficiently build up a proton gradient upon illumination [25]. SynK was suggested to modulate the balance between the osmotic (ΔpH) and electric ($\Delta\Psi$) components of the transthylakoid proton gradient. It has been proposed that proton pumping into the lumen results in the building of a large $\Delta\Psi$ component, which activates proton efflux through the ATP synthase [26], preventing the accumulation of high proton concentrations in the lumen. Movements of thylakoid membrane-permeable counter ions, such as efflux of cations or influx of anions through ion channels would partially dissipate the $\Delta\Psi$, thereby allowing establishment of significant ΔpH (Fig. 2). Thus, the ionic composition, as well as the activity of ion-flux pathways, in thylakoids is expected to determine the degree to which the movement of ions can dissipate the $\Delta\Psi$ [4]. In accordance with this proposal, in ΔSynK, we found an increased $\Delta\Psi$ and a decreased ΔpH as evaluated by measuring the turnover of the cytochrome b_6f complex under single-turnover flashes regime [26] and acridine orange fluorescence for pH changes [3]. It is to note that in cyanobacteria the photoprotective mechanism NPQ does not seem to take place, therefore the change in the ΔpH/$\Delta\Psi$ ratio leads to photosensitive phenotype most probably by NPQ-independent mechanisms.

While this review is focused on ion channels, it has to be briefly mentioned that several transporters have been shown to affect efficiency of photosynthesis, including PM-located aquaporin [27], thylakoid-located Na^+/H^+ antiporter NhaS3transporter [28], and the copper-transporting ATPase [29].

Chloroplast ion channels

Several different solute transporters have been identified in chloroplasts of higher plants. Ion channels have been recorded directly in chloroplast membranes using the patch clamp technique or after reconstitution of purified envelope membrane, or thylakoid vesicles into the planar lipid bilayer (for reviews see [30–35]). Several chloride,

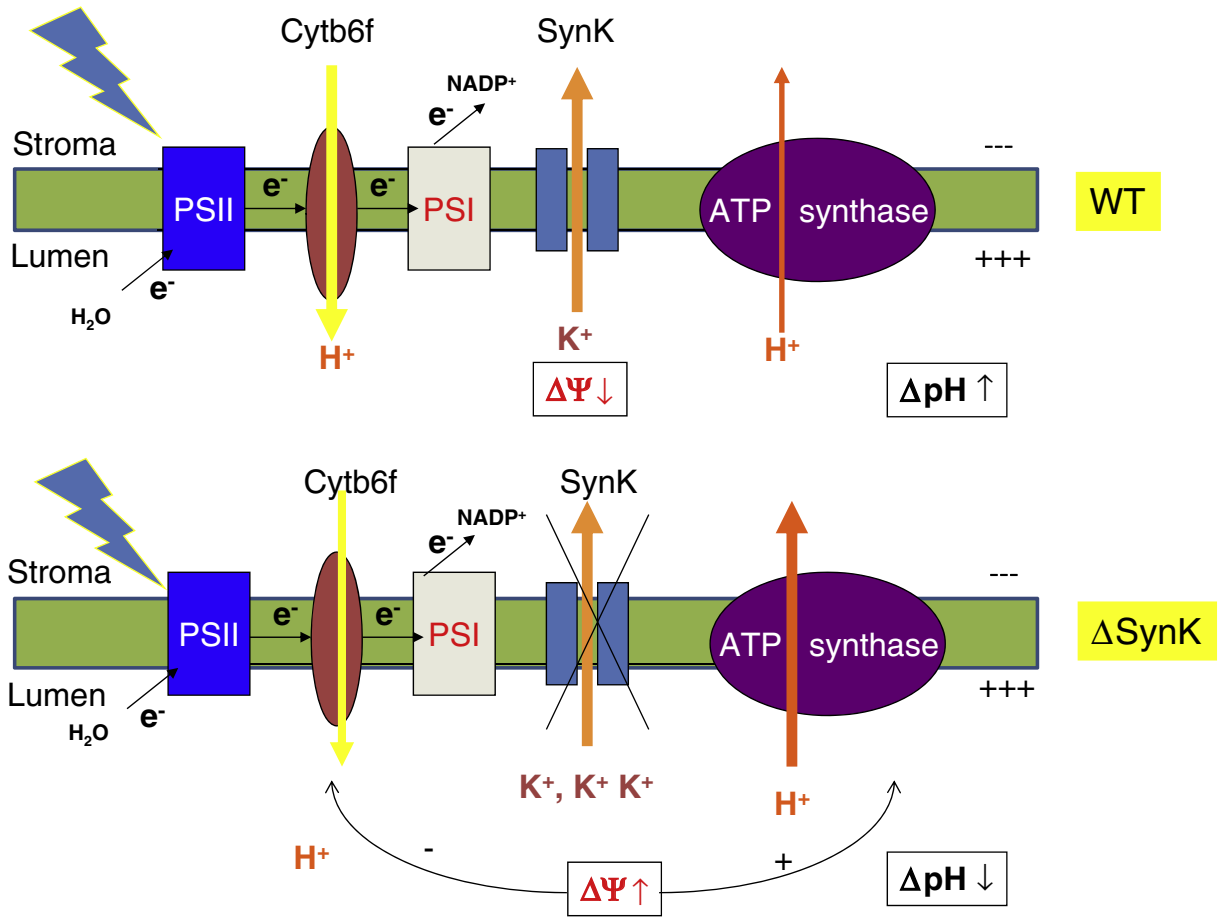

Fig. 2. Hypothetical model for the mechanism of regulation of photosynthesis by a thylakoid potassium channel in cyanobacteria. Upper part: light-driven proton pumping into the lumen results in the building of a large ΔΨ component (positive-inside), which drives proton efflux through the ATP synthase, preventing thus the accumulation of high proton concentrations in the lumen. Movements of thylakoid membrane-permeable counter ions, such as efflux of potassium *via* SynK would partially dissipate the ΔΨ, thereby allowing establishment of significant ΔpH. The ionic composition, as well as the activity of ion-flux pathways, in thylakoids is expected to determine the degree to which movement of ions can dissipate the ΔΨ. Higher ΔΨ might have also additional effects, *e.g.* restraining electron transport at the level of the cytochrome b_6f complex. In the case counterion movement does not occur *via* SynK, a decreased ΔpH can be established across the thylakoid membrane (lower panel).

potassium, and divalent cation selective ion channels [36–50] and light-induced currents [51–54] have been recorded from all three chloroplast membranes (outer, inner envelope and thylakoid). All ion channels and transporters of chloroplasts are encoded by the nuclear genome, and the protein products are imported into the chloroplast during its biogenesis after their synthesis in the cytoplasm. Even though bioinformatics algorithms exist for prediction of chloroplast localization of nucleus-encoded proteins, the validity of the prediction for localization has to be proved by biochemical evidence, or alternatively, by studying targeting *in vivo*, using proteins in fusion with fluorescent proteins. Mass spectrometry (MS) might also be a valid alternative for protein localization, although this option is mostly limited in the case of ion channels because of their very low abundance and high hydrophobicity. Indeed, while numerous transporters of chloroplasts have been identified by MS (*e.g.* [55,56]), only very few *bona fide* channels were revealed by this technique (see *e.g.* [57]). Thus, since a high-throughput approach is not available for the molecular identification of the channels residing in chloroplast membranes, this task remains challenging. In the few cases in which molecular identification was successfully achieved (see also next paragraph), precious information could be obtained on the physiological roles of these channels by using knock-out *Arabidopsis* plants. For example, MscS (small mechanosensitive channel)-like *Arabidopis* homolog AtMSL3 was shown to rescue the osmotic-shock sensitivity of a bacterial mutant lacking MS-ion-channel activity, suggesting that it functions as a mechanosensitive ion channel [58] and was later studied by electrophysiology [59]. Interestingly, two homologs, MSL2 and MSL3, located in the envelope, have been shown to control plastid size and shape [58], to protect plastids from hypoosmotic stress [60] and were identified as components of the chloroplast division machinery [61]. Expression of a truncated version of a *Chlamydomonas* homolog of MscS (MSC1) in *E. coli* gave rise to mechanosensitive currents and knock-down of the full-length protein caused abnormal localization of chlorophyll [62]. Unfortunately, in none of these works were the photosynthetic parameters determined. It is to note, that paradoxically, knowledge about the physiological role is available on some channel proteins whose ion-conducting properties in native membranes or in heterologous systems are still not proved (no electrophysiological studies on *Arabidopsis* chloroplast membranes are available to our knowledge).

Chloroplast ion channels and photosynthesis

As mentioned above, chloride, magnesium [63] and potassium [41] have been proposed to act as dominant counterions in order to counterbalance proton entry into the thylakoid lumen. The relative contribution of these three ions is still unclear. In higher plants, a K^+ flux out of the thylakoid was measured upon illumination [64,65] and tetraethylammonium$^+$ (TEA^+), a potassium channel inhibitor, was shown to reduce photosynthetic electron transport rate (ETR) [66]. The authors proposed that restriction of K^+ efflux in the light would lead to an increased membrane potential (the lumen becoming more positive)

across the thylakoid concomitantly with light-induced proton pumping. The buildup of positive charge within the lumen would increase the electrical gradient against which proton pumping must occur, thus imposing an increased restraint on electron transport. However, 10 mM TEACl has also been observed to increase ETR in other experiments because of its action as uncoupler [67,68]. Instead, another general potassium channel blocker, Cesium, significantly decreased ETR (100 mM) [67]. A channel activity, inhibited by 500 mM TEA^+, was recorded with thylakoid vesicles incorporated into artificial membrane [41]. The conductance of all cation-permeable channels recorded in thylakoids is large enough to account for an efficient counterbalance through these channels as elegantly calculated by Pottosin [46]. At least one potassium channel protein is indeed present in thylakoids, given that a thylakoid protein of 33 kDa, recognized by an antibody specific for the pore region in K^+ channels, was found in spinach [66]. We have recently reported detection of the putative two-pore potassium channel 3 from WT *Arabidopsis thaliana* (AtTPK3) in thylakoid membrane using a specific monoclonal antibody which specifically recognizes the 51 kDa protein [20]. This localization was further confirmed by other methods (Carraretto et al, unpublished result) but the channel activity of TPK3 remains to be established. Work is under way in our laboratory to determine the role, if any, of TPK3 in the regulation of photosynthesis, in light of the observation that in cyanobacteria a potassium channel regulates photosynthesis as described above.

Chloride channel activities in the thylakoid membrane have been reported by Schönknecht et al. [36] in higher plant, and in an alga [44]. The chloride channel (CLC) family comprises seven members in *Arabidopsis*, present in various membrane compartments [69]. Interestingly, AtCLCe sequence is highly similar to cyanobacterial CLCs [70] and prokaryotic ClCs were shown to function as H^+/Cl^- antiporters [71,72]. AtClCe was proposed to function in maintaining the H^+ gradient across the thylakoid membrane [73], however no proof in favor of such hypothesis has been obtained. The *clce* mutant displayed slight alterations in the kinetics of fluorescence changes upon transfer of dark-adapted leaves to light. Based on increased nitrite content in the cytosol of *clce* mutant plants, it has been proposed that CLCe transports NO_2^- to compensate for excess positive charge in the thylakoid lumen [74]. It is to note, that the closest homolog of ClCe in *Synechocystis* works as a chloride/proton antiporter [71]. If this was the case also for AtClCe, influx of chloride (or nitrite) as counterion would be accompanied by efflux of protons, and would lead to partial uncoupling. Besides ion channels, various transporters have been identified in the thylakoid membrane, including the ADP/ATP translocator, a sodium-dependent phosphate carrier and a copper-transporting P-type ATP-ase. All these transporters exert and impact on photosynthetic efficiency (for recent review see [75]). Recently, the presence of aquaporins in the thylakoid membrane has been postulated. These proteins could be beneficial for the light-induced rapid volume changes of the lumen [76].

In addition to thylakoid-located ion channels, some channels present in the envelope membrane were shown to affect photosynthesis. Ion fluxes across the envelop membrane are expected to impact on stromal concentration of ions which might be crucial for efficient photosynthesis: for example, chloride and calcium ions are known to be required for correct functioning of the oxygen evolving complex [77]. Our group has localized a member of the plant glutamate receptor family, GLR3.4 to the chloroplast envelope membrane, by using specific antibody and GRL3.4-GFP fusion protein [78]. Knock-out plants lacking GLR3.4 were characterized by a slightly decreased photosynthetic activity as assessed by measurement of chlorophyll fluorescence parameters. In addition, an inhibitor of the mammalian glutamate receptors, CNQX (6-cyano-7-nitro-quinoxaline-2,3-dione), decreased oxygen evolution (strictly related to the efficiency of photosynthesis) in intact spinach chloroplasts but not when it was added to broken chloroplasts (thylakoids). An analogous inhibitor, DNQX (6,7-dinitroquinoxaline-2,3-dione) was instead shown to abolish the 35 pS channel activity recorded in a medium containing only calcium (100 mM/300 mM gradient) using purified inner envelope membranes from spinach [79]. These results indicated that a functional, calcium-permeable glutamate receptor is expressed in the inner envelope membrane and its pharmacological inhibition or its deletion affects photosynthetic activity by a still ill-defined mechanism. In addition to GLR, a ClC family member, ClC-f, which was identified in the chloroplast envelop by mass spectrometry as well as by Western blot, also seems to impact on photosynthetic efficiency according to experiments using putative ClC inhibitors [70].

Indirect regulation of photosynthesis by non-chloroplast channels in higher plants

Interestingly, photosynthesis is affected in an indirect way also by channels that reside outside the chloroplasts as proved by genetic means as well. For example, knock-out plants lacking the mitochondrial uncoupling protein UCP1 are characterized by restriction in photorespiration, leading to an associated reduced photosynthetic carbon assimilation rate. UCP1 was proposed to contribute to maintaining the redox poise of the mitochondrial electron transport chain to facilitate photosynthetic metabolism [80]. Accordingly, transgenic lines overexpressing UCP1 germinated faster, and adult plants showed better responses to drought and salt stress than wild-type (WT) plants [81].

Plasmamembrane ion channels impact on photosynthesis also through affecting intracellular salt concentration. At medium or high salinity, leaf photosynthesis is severely inhibited (*e.g.* [82]), possibly due to a reduction in chlorophyll content (usually associated with leaf chlorosis). Salt-induced decrease in the total amount of chlorophyll in affected leaves, as well as changes in the chlorophyll a/b ratio have been reported [83]. Salinity may also affect plant photosynthetic performance indirectly by reducing stomatal conductance *via* the osmotic component of salt stress [84] and thus inducing an overall reduction in net CO_2 assimilation in affected plants [85]. Indeed, SLAC1, a slow anion channel in the membrane of stomatal guard cell has been shown to allow CO_2 passage and thus to provide CO_2 for photosynthesis in the guard cells [86]. *Arabidopsis* aquaporins PIP1 and PIP 2 also contribute to CO_2 passage [87].

Conclusion

The recent molecular identification of some ion channels in photosynthetic organisms has allowed to prove by genetic means an important contribution of ion channel activities to the regulation of photosynthesis, either directly or indirectly. For mammalian mitochondria a compendium of proteins with proven mitochondrial localization (either mass spectrometry or targeting of proteins fused to GFP), named MitoCarta is available [88]. Excellent mass spectrometry studies became available on chloroplast sub-membranes as well [89,90] and hopefully will help the molecular identification of further channels and understanding of their physiological roles.

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