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Lemeille, Sylvain; Rochaix, Jean-David

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REVIEW

State transitions at the crossroad of thylakoid signalling pathways

Sylvain Lemeille · Jean-David Rochaix

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Abstract In order to maintain optimal photosynthetic activity under a changing light environment, plants and algae need to balance the absorbed light excitation energy between photosystem I and photosystem II through processes called state transitions. Variable light conditions lead to changes in the redox state of the plastoquinone pool which are sensed by a protein kinase closely associated with the cytochrome b_6f complex. Preferential excitation of photosystem II leads to the activation of the kinase which phosphorylates the light-harvesting system (LHCII), a process which is subsequently followed by the release of LHCII from photosystem II and its migration to photosystem I. The process is reversible as dephosphorylation of LHCII on preferential excitation of photosystem I is followed by the return of LHCII to photosystem II. State transitions involve a considerable remodelling of the thylakoid membranes, and in the case of Chlamydomonas, they allow the cells to switch between linear and cyclic electron flow. In this alga, a major function of state transitions is to adjust the ATP level to cellular demands. Recent studies have identified the thylakoid protein kinase Stt7/STN7 as a key component of the signalling pathways of state transitions and long-term acclimation of the photosynthetic apparatus. In this article, we present a review on recent developments in the area of state transitions.

Keywords Photosynthesis · State transitions · LHCII kinase · Arabidopsis · Chlamydomonas

Introduction

The primary reactions of photosynthesis occur in the thylakoid membranes and are catalyzed by the photosystem II (PSII) and photosystem I (PSI) complexes, and their associated antennae. These complexes act in series and are linked together through the plastoquinone pool, the cytochrome b_6f complex and plastocyanin within the photosynthetic electron transport chain. The collected light energy is channelled from the antennae to the reaction centres of PSII and PSI and used to induce stable charge separations across the thylakoid membrane with subsequent oxidation of water by PSII, followed by multiple electron transfer reactions, and photoreduction of ferredoxin by PSI. Ultimately, this process creates reducing power and a proton gradient across the membrane which is used by ATP synthase to produce ATP. The differentiation of thylakoids into grana and stroma lamellar regions is believed to reflect the uneven distribution of the photosynthetic complexes. Whereas PSI and the ATP synthase with their bulky stromal domains are localized in the non-appressed regions consisting of the stroma lamellae and the grana end membranes and margins, PSII and its light-harvesting system (LHCII) are embedded in the appressed regions of the grana.

The photosynthetic machinery is remarkably dynamic and has developed several mechanisms to adapt to both high- and low-light conditions. In nature, photosynthetic organisms are constantly subjected to changes in their light environment, and they need to adjust their photosynthetic activity accordingly. Under high light when the absorbed

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S. Lemeille · J.-D. Rochaix (✉)
Departments of Molecular Biology and Plant Biology,
University of Geneva, 30, Quai Ernest Ansermet,
1211 Geneva, Switzerland
e-mail: Jean-David.Rochaix@unige.ch

light energy exceeds the capacity of the photosynthetic apparatus, the excess absorbed energy is dissipated through heat, a process called non-photochemical quenching (for review, see Holt et al. 2004). At the same time, the photosynthetic system is susceptible to light-induced damage leading to photoinhibition and repair at the level of PSII.

Because the antenna systems of PSII and PSI have a different composition and hence different light absorption properties, their excitation can be unbalanced under changing light conditions. Thus, when PSII is preferentially excited by light absorbed mostly by PSII, the plastoquinone (PQ) pool is reduced, and on docking of plastoquinol (PQH_2) to the Q_o site of the cytochrome b_6f complex, a protein kinase is activated that phosphorylates LHCII (Fig. 1A). This event triggers the release of LHCII from PSII and its migration to PSI thereby rebalancing the light excitation energy between the two photosystems (state 2). The process is reversible as preferential excitation of PSI by light enriched in the far-red region induces the dephosphorylation of LHCII and its return to PSII (state 1). This rebalancing processes called state transitions also involve a remodelling of the thylakoid membranes and lead to an optimal utilization of the absorbed light energy.

State transitions have been studied in several photosynthetic organisms including algae, land plants and cyanobacteria. In this article, we review recent studies on state transitions performed mainly in the unicellular green alga *Chlamydomonas reinhardtii* and the model plant *Arabidopsis thaliana*. These two organisms have emerged as very powerful and complementary model systems in which genetic, biochemical, physiological and biophysical approaches can be coupled efficiently. It is of particular interest to compare the acclimation of a mobile unicellular alga with that of a sessile multicellular plant, and this also provides interesting insights into evolutionary aspects of light acclimation. Earlier studies on state transitions have been extensively discussed in several reviews on state transitions (Allen 1992; Wollman 2001; Rochaix 2007).

STN7/Stt7 protein kinase is the key player for state transitions

State transitions were discovered independently by Murata (1969) and Bonaventura and Myers (1969) while they were studying the fluorescence properties of two unicellular organisms, the red alga *Porphyridium cruentum* in one case, and the green alga *Chlorella pyrenoidosa*, in the other. These investigators subjected these algae to light absorbed preferentially by PSII and found that, within a few minutes, PSI absorbed more light excitation energy. In contrast, illumination with light absorbed preferentially by PSI was followed by an increased light absorption by PSII. The increase in the light absorption capacity of PSI and PSII following these changes in light conditions were referred to as state 2 and state 1, respectively. It was later found that regulation of excitation energy transfer from PSII to PSI occurred through phosphorylation of the light-harvesting complex of PSII (LHCII) and that this process was associated with fluorescence changes (Bennett 1977, 1979). The kinase involved in this phosphorylation was shown to be activated through reduction of the plastoquinone pool after preferential excitation of PSII (Allen et al. 1981; Horton and Black 1980). The finding, that the two photosystems are not equally distributed in the thylakoid membranes with PSII and PSI localized mainly in the grana and stromal lamellae, respectively (Andersson and Andersson 1980), implied that the light excitation energy needs to be redistributed between these thylakoid regions and involves the migration of LHCII between PSII and PSI (Delosme et al. 1996).

A key finding was that the cytochrome b_6f complex plays an important role in state transitions. Mutants of *C. reinhardtii* lacking this complex are unable to phosphorylate LHCII and are locked in state 1 (Wollman and Lemaire 1988). Further analysis revealed that it is not the redox state of the plastoquinone pool per se which is critical for the activation of the kinase, but the docking of plastoquinol to the Q_o site on the luminal side of the cytochrome b_6f complex (Vener et al. 1997; Zito et al. 1999).

In spite of the fact that it was possible to identify several kinase activities associated with thylakoids, attempts to isolate the LHCII protein kinase by biochemical means failed (Coughlan and Hind 1986, 1987; Sokolenko et al. 1995). Taking advantage of the fact that the N-terminal region of LHCII contains the residues that are phosphorylated during a state 1 to state 2 transition, Kohorn and colleagues used a genetic screen for proteins that interact with this region (Snyders and Kohorn 2001). This screen led to the discovery of the TAK protein kinases which form a small family related to the human TGFβ1 receptor (Snyders and Kohorn 1999). The TAK kinase was shown to be associated with PSII and the cytochrome b_6f complex. Moreover, a decline in kinase level in antisense TAK lines led to a decrease in LHCII phosphorylation and to a partial deficiency in state transitions, and to light sensitivity (Snyders and Kohorn 2001). The fact that no TAK ortholog could be identified in Chlamydomonas suggests that the TAK kinases perform a role which is specific to land plants. Alternatively, these kinases may have diverged considerably between algae and plants. Surprisingly, no further studies have been performed on the TAK kinases since the early studies of Kohorn.

Chlamydomonas reinhardtii has proven to be especially attractive for a genetic approach for studying state transitions because transition from state 1 to state 2 is associated

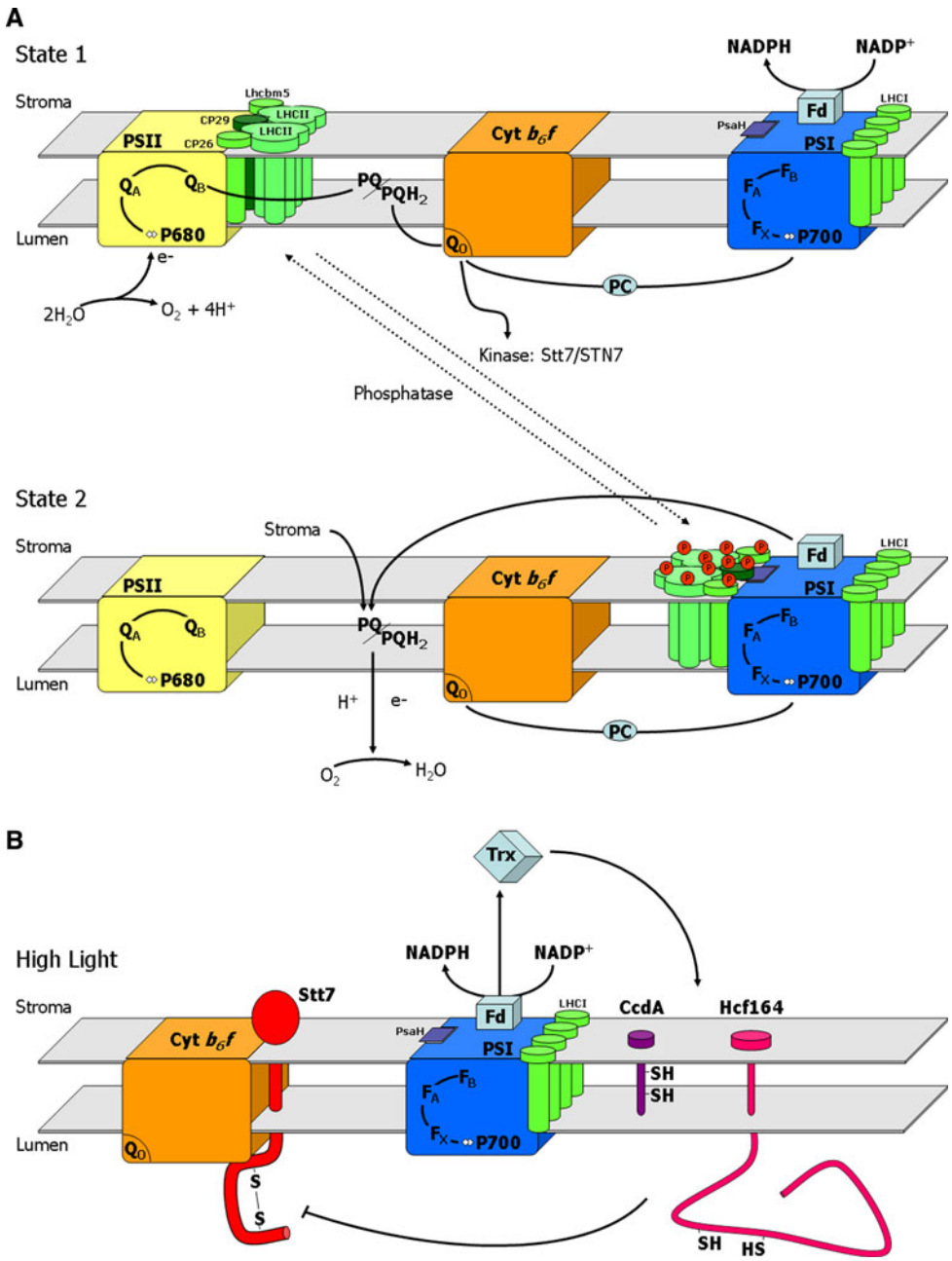

Fig. 1 Model for state transitions in the thylakoid membrane. **A** The redox state of the PQ pool determines state transitions. When PSI is preferentially excited, the PQ pool is oxidized. In this state, called state 1, LHCIIs are bound to PSII. Monomeric LHCIIs (CP29, CP26 and Lhcbm5) act as connectors between PSII core and the trimeric LHCII, and the photosynthetic electron flow proceeds in a linear mode generating both a proton gradient across the thylakoid membrane which is used for ATP production, and NADPH. In contrast, when PSII is preferentially excited, the PQ pool is reduced. Docking of plastoquinol to the Q_o site of the cytochrome b_6f leads to the activation of the Stt7/STN7 kinase which is required for the phosphorylation of CP29, CP26 and Lhcbm5 and the LHCII trimers, and to the displacement of LHCII from PSII to PSI (state 2). The docking of LHCII occurs on the PsaH side of PSI whereas the LHCI belt is on the other side of PSI. In state 2 in *C. reinhardtii*, PSII is disconnected from the electron transport chain which now operates in a cyclic mode between PSI and the PQ pool generating mostly ATP. State 2 can also be induced under anaerobic conditions through the chloro-respiratory electron transport chain. **B** Model for inactivation of Stt7/STN7 under high light. Reducing equivalents are shuttled from ferredoxin and thioredoxin through a trans-thylakoid thiol-reducing pathway mediated by CcdA and Hcf164 across the thylakoid membrane and could reduce the disulphide bond in the lumenal N-terminal domain of Stt7/STN7 and thereby inactivate the kinase. P680, PSII reaction centre chlorophyll dimer; Q_A, Q_B, primary and secondary electron acceptors of PSII; PQ/PQH_2, plastoquinone/plastoquinol, P700, PSI reaction centre chlorophyll dimer; F_X, F_A, F_B, 4Fe–4S centres acting as electron acceptors within PSI; *PC* plastocyanin, *Fd* ferredoxin

with a large decrease in fluorescence in this alga (Wollman and Delepelaire 1984). A screen based on this differential fluorescence signal was used for isolating mutants deficient in state transitions (Fleischmann et al. 1999; Kruse et al. 1999). In this way, the *stt7* mutant was isolated, which is blocked in state 1 and fails to phosphorylate LHCII under state 2 conditions (Fleischmann et al. 1999). The gene affected in *stt7* is a thylakoid protein kinase called Stt7 which comprises 499 amino acids (Depège et al. 2003). The kinase is localized in the chloroplast thylakoid membranes and shown to be essential for LHCII phosphorylation and state transitions (Depège et al. 2003). This protein displays significant sequence similarity (29% sequence identity, 43% sequence similarity) with another protein kinase named Stl1. Both Stt7 and Stl1 have orthologs in Arabidopsis, called STN7 (At1g68830) and STN8 (At5g01920), which are involved in state transitions and in PSII core protein phosphorylation, respectively (Bellafiore et al. 2005; Bonardi et al. 2005; Vainonen et al. 2005). Stt7/STN7-like protein kinases are also found in other land plants, trees and marine algae (Fig. 2, Supplementary Fig. 1).

The Stt7 kinase is required for LHCII phosphorylation during a transition from state 1 to state 2. Characterization of the Stt7 kinase revealed that it contains a transmembrane domain separating its stroma-exposed catalytic domain from its lumen-located N-terminal end. Two conserved cysteine residues near the N-terminus of Stt7 are critical for its activity (Lemeille et al. 2009). In addition, coimmunoprecipitation assays showed that Stt7 interacts with LHCII proteins and cytochrome b_6f complex subunits, and also with PSI (Lemeille et al. 2009). Interaction between Stt7 kinase and cytochrome b_6f complex was expected since state transitions depend critically on this complex (Wollman 2001). Moreover, kinase activity was detected in purified fractions of the cytochrome b_6f complex (Gal et al. 1990). Further analysis revealed that Stt7 interacts with the Rieske protein of the cytochrome b_6f complex (Lemeille et al. 2009). Structural studies of the mitochondrial *bc1* and chloroplast b_6f complexes showed that electron transfer between plastoquinol at the Q_o site and cytochrome *f* is mediated by the Rieske protein which moves from a proximal position when the Q_o site is occupied by PQH_2 to a distal position when the Q_o site is unoccupied (Breyton 2000; Stroebel et al. 2003; Zhang et al. 1998). One interesting possibility is that this dynamic behaviour of the Rieske protein is coupled with the activation of the Stt7 kinase (Finazzi et al. 2001). Such a dynamic model is compatible with the low abundance of the Stt7 kinase with a molar ratio of 1:20 relative to the cytochrome b_6f complex (Lemeille et al. 2009). The active kinase would need to phosphorylate several substrates before it returns to its inactive state. The low level of Stt7 was probably the reason of the failure of all the previous biochemical attempts to purify the LHCII kinase.

An intriguing component of the cytochrome b_6f complex is its single chlorophyll *a* molecule whose chlorine ring lies between helices F and G of the PetD subunit, whereas the phytol chain protrudes near the Q_o site (Stroebel et al. 2003). Interestingly, *petD* mutants affected in the binding of chlorophyll *a*, besides having reduced cytochrome b_6f turnover, also display a delay in the transition from state 1 to state 2 as well as in protein phosphorylation indicating a slower activation of the LHCII kinase by the cytochrome b_6f complex (de Lacroix de Lavalette et al. 2008). This region of PetD may thus either form a site for interaction with the N-terminal domain of Stt7 or it could act as a sensor for the presence of PQH_2 at the Q_o site and initiate a signalling pathway through the chlorophyll *a* molecule towards the catalytic domain of Stt7 on the stromal side of the thylakoid membrane. Additional evidence for the importance of this region for the activation of the LHCII kinase comes form the analysis of a Chlamydomonas strain in which the *petD* and *petL* genes are fused to each other resulting in a chimeric protein in which the C-terminus of subunit IV (PetD) is fused to the N-terminus of PetL (Zito et al. 2002). Although this strain was unimpaired in the Q-cycle, it was unable to perform state transitions. A role of PetL in the activation of the kinase seems unlikely because mutants lacking PetL are still able to undergo state transitions (Zito et al. 2002). It is thus more likely that subunit IV is involved in this process either directly or indirectly.

The PetO subunit of the *C. reinhardtii* b_6f complex is known to be phosphorylated during state transitions (Hamel et al. 2000). In the presence of tridecyl-stigmatellin, an inhibitor of electron transport which binds to the Q_o site, and which prevents phosphorylation of LHCII, the PetO subunit is still phosphorylated under state 2 conditions, indicating that it is probably the first protein to be phosphorylated upon activation of the LHCII kinase (Finazzi et al. 2001). PetO phosphorylation could be part of the signalling pathway of state transitions and may act at an early step. However, no ortholog of this protein could be found in land plants suggesting that this protein is specific to Chlamydomonas.

The N-terminal region of Stt7 contains two cysteine residues that are critical for its activity (Lemeille et al. 2009). These two cysteine residues are the only conserved cysteine residues between Stt7 and its ortholog STN7 in *Arabidopsis thaliana* (Depège et al. 2003). Moreover, these two cysteine residues are conserved in all Stt7/STN7 orthologs (Supplementary Fig. 1). It was reported that, in land plants, high-light treatment inactivates the LHCII kinase through the ferredoxin–thioredoxin system (Rintamaki et al. 1997; Hou et al. 2003). The two conserved cysteine could therefore be the targets of this redox system.

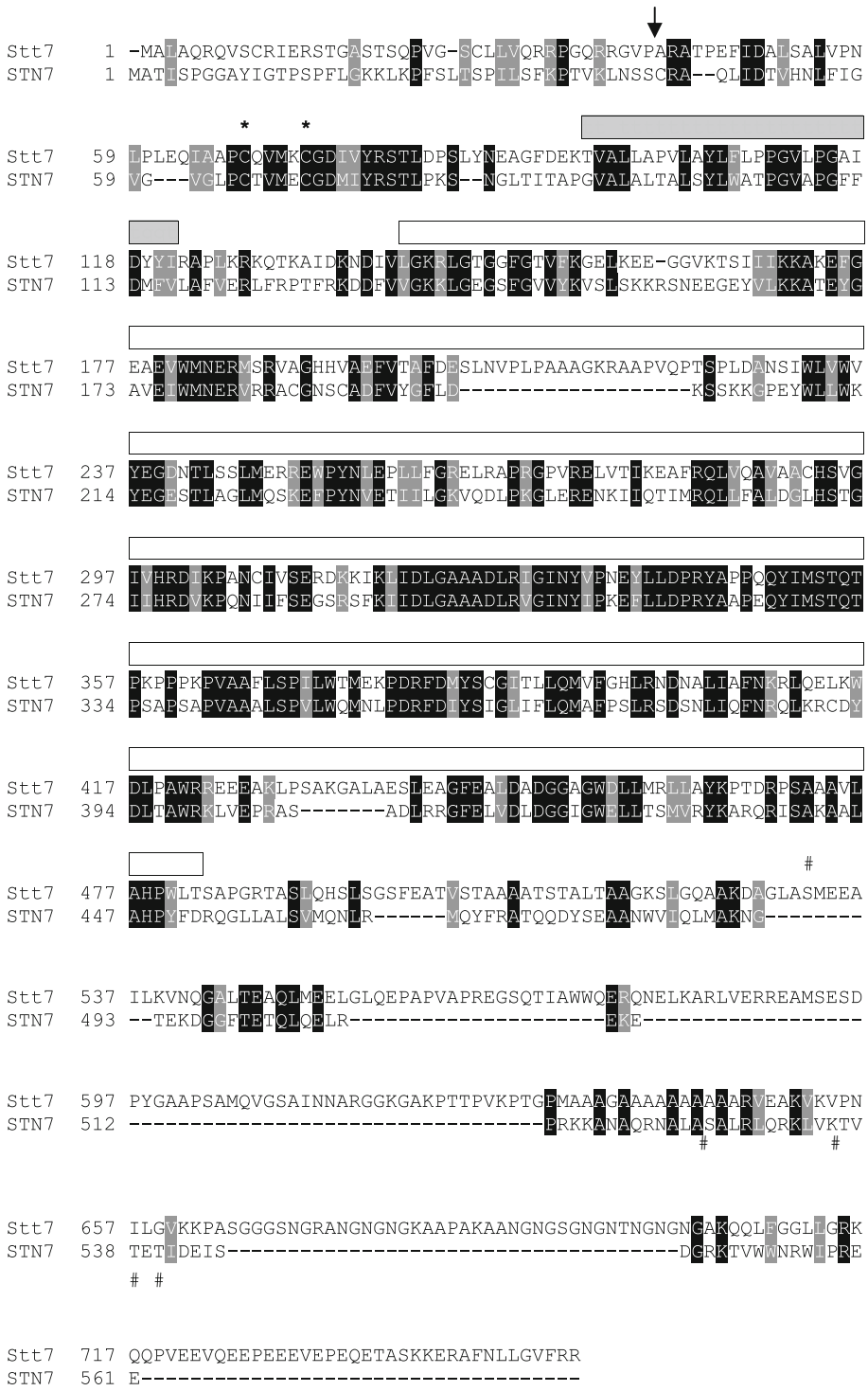

Fig. 2 Sequence comparison of the Stt7 kinase from *Chlamydomonas reinhardtii* and STN7 from *Arabidopsis thaliana*. The arrow indicates the predicted cleavage site of the chloroplast transit sequence of Stt7. The hydrophobic region is marked by a *grey box* above the amino acid sequence. The kinase catalytic domain is indicated by an *open box* above the amino acid sequence. The *asterisks* indicate the conserved cysteine residues that are potential targets for the ferredoxin–thioredoxin system. Identified phosphorylated serine and threonine in Stt7 and STN7 are marked by # above and below the sequence, respectively

It thus appears likely that the activity of the STN7/Stt7 kinase is regulated by a complex network involving cooperative redox control by PQ and the cytochrome b_6f complex as well as by the ferredoxin–thioredoxin system in the stroma. Given the fact that these two cysteine residues are located in the lumen whereas ferredoxin and thioredoxin are present in the stroma, the question arises how the activity of the kinase is regulated under these conditions. Recently, at least two components of a trans-thylakoid thiol-reducing pathway have been identified in chloroplasts. The CcdA protein which belongs to the DsbD/DipZ family of membrane polytopic proteins is involved in transmembrane transfer of thiol reducing equivalents from the stroma to the lumen (Page et al. 2004). The second

component Hcf164, a transmembrane protein containing a thioredoxin domain in the thylakoid lumen with disulphide reductase activity, is involved in cytochrome b_6f assembly (Lennartz et al. 2001; Motohashi and Hisabori 2006). Thus, it is possible that the ferredoxin–thioredoxin system regulates the redox state of the two Cys across the membrane through CcdA/Hcf164 (Fig. 1B). Another possibility based on the observation that Stt7 forms a dimer in Chlamydomonas (S. Lemeille and J.D. Rochaix, unpublished results) is that these two conserved cysteine residues of Stt7 could form two disulphide bridges between the Stt7 monomers and that dimerization of the kinase is linked to its activation.

Regulation of Stt7 also appears to occur at the level of protein accumulation. Time-course experiments showed that the level of Stt7 protein decreases under prolonged state 1 conditions and also after high-light treatment (Lemeille et al. 2009). Thus, Stt7 is less stable under conditions when the kinase is in an inactive state. The decline of the amount of Stt7 could be prevented by addition of inhibitors of cysteine proteases. It is possible that under state 2 conditions, Stt7 is protected from proteases either because of posttranslational modifications or by its association with other proteins. At this time, there is no clear evidence for the presence of cysteine proteases in the chloroplast. However, other proteases, such as Deg proteases which are known to degrade thylakoid membrane proteins (Huesgen et al. 2009; Adam and Clarke 2002), are sensitive to inhibitors of cysteine proteases (Helm et al. 2007).

State transitions are associated with the phosphorylation of LHCII (Bennett 1977), and Stt7 is essential for these phosphorylation events (Depège et al. 2003). However, it is still not yet known whether this kinase directly phosphorylates LHCII or whether it is part of a kinase cascade involved in the signalling pathways of state transitions. Mapping of in vivo protein phosphorylation sites in thylakoid membranes of wild-type *C. reinhardtii* cells under state 1 and state 2 conditions, or when exposed to high light, identified 19 in vivo phosphorylation sites corresponding to 15 polypeptides (Turkina et al. 2006). This study revealed that the major LHCII proteins Lhcbm4, Lhcbm6, Lhcbm9 and Lhcbm11 are phosphorylated at Thr_3 and Thr_7. Because of their identical phosphopeptide sequences, it is not possible to distinguish whether all or only some of these proteins are phosphorylated.

Comparison of the thylakoid phosphoproteome of wild type and *stt7* mutant under state 1 and state 2 conditions revealed that, in state 2, several Stt7-dependent phosphorylations of specific Thr residues occur in Lhcbm1/Lhcbm10, Lhcbm4/Lhcbm6/Lhcbm8/Lhcbm9, Lhcbm3, Lhcbm5, and CP29 located at the interface between PSII and its light-harvesting system (Lemeille et al. 2010). Because of its pivotal role in state transitions, the phosphorylation patterns of CP29 are particularly interesting. Of the two phosphorylation sites detected specifically in CP29 under state 2 conditions, one is Stt7 dependent. This phosphorylation may play a crucial role in the dissociation of CP29 from PSII and/or in its association to PSI where it serves as a docking site for LHCII in state 2 (Lemeille et al. 2010). Stt7 itself is phosphorylated under state 2 conditions (Lemeille et al. 2010). However, it is not known whether this is due to autophosphorylation or whether another kinase is involved.

State transitions and cyclic electron flow

A major role of state transitions in Chlamydomonas is to adjust the ATP level to cellular demands. This probably explains why the mobile LHCII represents such a large portion of the light-harvesting system of PSII (Bulté et al. 1990). When the cellular level of ATP is low, glycolysis is stimulated through the Pasteur effect, and the reducing power is channelled through the chlororespiratory chain into the plastoquinone pool, thereby activating the LHCII kinase and promoting a transition from state 1 to state 2. This transition triggers a switch from linear to cyclic electron flow as indicated by the fact that in state 2 the PSII-specific inhibitor DCMU no longer prevents the re-reduction of the cytochrome b_6f complex (Finazzi et al. 1999). In striking contrast to the wild type, under the same state 2 conditions, DCMU blocked the re-reduction of the cytochrome b_6f complex in the *stt7* mutant indicating that this process observed in the wild type is not due to increased influx of reducing power from the chlororespiratory chain, but due to cyclic electron flow (Finazzi et al. 2002).

Such a coupling between state transitions and cyclic electron flow is not apparent in Arabidopsis. Although it was assumed earlier that cyclic electron flow may not be important for steady state photosynthesis in C3 plants, this view has changed recently (reviewed in Joliot and Joliot 2006). It now appears that the principal role of cyclic electron flow is to match the requirements of the ATP/NADPH ratio of 3/2 required for driving the Calvin–Benson cycle especially during changes in light conditions or under stress. Because linear electron flow gives rise to a lower ratio, it has been estimated that there must be 20% more PSI than PSII to allow for the recycling of one electron in five through cyclic electron transfer (Allen 2003). The cyclic pathway transfers electrons back from ferredoxin to the plastoquinone pool either through the NAD(P)H dehydrogenase-dependent pathway or through the cytochrome b_6f complex using a Q-cycle-derived mechanism (reviewed in Joliot and Joliot 2006; Shikanai 2007). Alternatively, a ferredoxin-plastoquinone reductase

has been proposed for direct reduction of plastoquinone by ferredoxin (Cleland and Bendall 1992; Moss and Bendall 1984). However, this enzyme has not yet been isolated. Two novel factors involved in cyclic electron flow, PGR5 and PGRL1, have been identified in Arabidopsis through forward and reverse genetic screens (Munekage et al. 2002; DalCorso et al. 2008). Plants deficient in either of these proteins display perturbations in cyclic electron flow. Both proteins interact physically with each other and bind to PSI suggesting that the PGRL1–PGR5 complex may facilitate cyclic electron flow in close association with PSI.

Role of STN7/Stt7 kinase in other processes

In response to changes in light conditions, photosynthetic organisms induce a short-term response such as state transitions. If preferential excitation of PSII or PSI is maintained for longer periods, long-term acclimation occurs which leads to changes in the amount of the antennae proteins of PSII and PSI and to a readjustment of photosystem stoichiometry which can be measured by the chlorophyll *a/b* ratio or the fluorescence parameter F_S/F_M (ratio between steady-state fluorescence and maximum fluorescence) (Dietzel et al. 2008). This process is called long-term response (LTR) and is achieved through a signalling network involving coordinate gene expression in the nucleus and chloroplast (Pfannschmidt 2003). When wild-type plants are illuminated for several days by light preferentially absorbed by PSI relative to PSII, the excitation pressure of PSII, as measured by the chlorophyll fluorescence parameter F_S/F_M, increases as a result of its increased antenna size, and the chlorophyll *a/b* ratio decreases because the PSII antenna is enriched in chlorophyll *b*. The opposite occurs when the plants are illuminated with PSII light. Bonardi et al. (2005) and Tikkanen et al. (2006) observed that under all light conditions, the response of the *stn7* mutant is typical of plants acclimated to PSI light indicating that STN7 is also required for LTR. Moreover, it was shown that STN7 kinase acts as a common redox sensor and/or signal transducer for both short- and long-term responses in *Arabidopsis thaliana* (Pesaresi et al. 2009), indicating that the two corresponding signalling pathways diverge at, or immediately after downstream of STN7. As suggested earlier (Allen and Pfannschmidt 2000), these two pathways may be subject to regulatory coupling. It is noticeable that, in contrast to Arabidopsis, no changes in photosystem stoichiometry could be detected between the Chlamydomonas mutants *stt7* and *dum22* locked in state 1 and state 2, respectively, suggesting that the Stt7 kinase is not involved in the readjustment of photosystem stoichiometry (Cardol et al. 2009). Earlier studies indicated that the PSI/PSII ratio changes in Chlamydomonas cells grown under photoautotrophic conditions in response to changes in the quality of irradiance and that this chromatic regulation improves the quantum efficiency of photosynthesis (Melis et al. 1996). The differences observed between the roles of Stt7 and STN7 in LTR may be due to structural differences between these two proteins. While the N-terminal region and the catalytic region are well conserved, the C-terminal domains differ considerably and may have different functions.

Analysis of transcription patterns upon long-term acclimation using microarrays failed to reveal significant differences between wild type and the *stn7* mutant (Bonardi et al. 2005; Tikkanen et al. 2006). However, the amount of some thylakoid proteins was altered. In *stn7*, the level of Lhcb1 decreased whereas an increases of Lhca1 and Lhca2 and of other chloroplast proteins were observed (Tikkanen et al. 2006). In contrast, clear differences in the expression of genes involved in photosynthesis or metabolism between wild type and *stn7* were observed during a time-course experiment following a change to PSII light (Brautigam et al. 2009). A large majority, 85% of the genes responding to the light shift did no longer respond in *stn7* indicating that most of these genes are under STN7-mediated redox control under these particular conditions. Moreover, under conditions of fluctuating light intensity, the *stn7* mutant displayed retarded growth, and the expression of stress-responsive genes was highly increased as compared to the wild type (Tikkanen et al. 2006). It has, therefore, been proposed that STN7 and state transitions are part of a buffering system which dampens rapid oscillations of redox signals produced in the photosynthetic apparatus by fluctuating environmental conditions. This allows the organisms to prevent over-reduction of the electron acceptors of PSI which would otherwise generate reactive oxygen species and induce the expression of stress response genes (Tikkanen et al. 2006). It also allows for the stabilization of metabolic fluxes and coordinates photosynthesis and metabolism for adapting plant growth to the environmental conditions (Brautigam et al. 2009).

Interplay between kinases and phosphatases

Using high-accuracy mass spectrometry, the phosphoproteome of *Arabidopsis thaliana* seedlings has been characterized revealing four phosphorylation sites in the C-terminal region of STN7 (Fig. 2) (Reiland et al. 2009). Interestingly, this C-terminal region is not conserved between Chlamydomonas Stt7 and Arabidopsis STN7 (Depège et al. 2003) suggesting that the C-terminal phosphorylation of STN7 controls responses that are specific to higher plants. Furthermore, Reiland et al. (2009) proposed that one phosphorylation site of STN7 may be a substrate

site for casein kinase 2 (CK2) which is known to phosphorylate proteins involved in transcription and post-transcriptional regulation (Link 2003; Bollenbach et al. 2004). This raises the possibility that CK2 could be involved in the phosphorylation processes in LHCII through the STN7 kinase.

Stl1 is another protein kinase related to Stt7 in *Chlamydomonas reinhardtii* which is likely to be targeted to the chloroplast because of the presence of an N-terminal chloroplast presequence (Depège et al. 2003). However, the function of this kinase is not yet known. Stl1 appears to have an ortholog in *Arabidopsis thaliana* called STN8. The availability of Arabidopsis T-DNA insertion lines with disruptions in the *STN7* and *STN8* genes provided important insights into the function of the corresponding proteins. Both STN7 (Bellafiore et al. 2005) and STN8 (Bonardi et al. 2005; Vainonen et al. 2005) are activated in thylakoid membranes by light. Loss of STN8 kinase leads to a specific decrease in phosphorylation of the PSII core proteins D1, D2 and CP43 (Bonardi et al. 2005; Vainonen et al. 2005).

The existence of the conserved kinase couple STN7/STN8 and Stt7/Stl1 in Arabidopsis and Chlamydomonas raises the question of a possible functional interaction between STN7/Stt7 and STN8/Stl1. Both proteins appear to act synergistically based on the fact that the phenotype of dephosphorylation of LHCII and of the PSII core proteins in the double mutant *stn7stn8* is more pronounced than in the two single mutants together (Bonardi et al. 2005; Vainonen et al. 2005). Moreover, field tests revealed that fitness as measured by seed production is significantly decreased in the double mutant whereas it is decreased to a smaller extent in *stn7* and not significantly affected in *stn8* (Frenkel et al. 2007). Also, the phosphorylation in state 2 of Stl1 in Chlamydomonas is dependent on the presence of the Stt7 kinase (Lemeille et al. 2010). In Arabidopsis, light quality-dependent changes in core protein phosphorylation do no longer occur in the *stn7* mutant implying that STN7 affects activity or substrate accessibility of/for STN8 and suggesting that as Stt7, STN7 acts upstream of STN8. The synergistic functions of STN8 and SNT7 in Arabidopsis were the basis for the proposal of the "sensor box" model in which both kinases interact directly or indirectly to adapt photosynthetic activity to changes in light quality and quantity (Dietzel et al. 2008). The function of Stl1 in Chlamydomonas may fulfil a similar function as STN8 in Arabidopsis based on the sequence similarity. A major role of this sensor box in *C. reinhardtii* most likely would be to mediate the response to changes in metabolic state by modulating the contributions of cyclic and linear electron flow and thereby adjusting the ATP/NADPH ratio.

Recently, the chloroplast CSK kinase was identified and proposed to act as a sensor kinase based on its homology to bacterial two-component systems (Puthiyaveetil et al. 2008). It was proposed that autophosphorylation of CSK occurs on Tyr because phosphorylation is resistant to acid and alkali treatment. However, an extensive phosphoproteomic study did not reveal any chloroplast Tyr phosphorylation site (Reiland et al. 2009). CSK appears to be conserved in most photosynthetic organisms although no closely related kinase could be found in Chlamydomonas. Chloroplast transcript accumulation was changed in CSK knock-out lines indicating that CSK affects plastid gene expression either directly or indirectly (Puthiyaveetil et al. 2008). The suggested redox regulatory coupling of CSK between photosynthesis and chloroplast gene expression requires further investigation to understand the functional role of this kinase in detail.

Besides Stt7/STN7 and Stl1/STN8 which appear to be linked through a phosphorylation cascade and the possible connection between CK2 and STN7, it is not yet clear whether there are links between these different kinases and CSK which would involve other signalling chains in the chloroplast (Fig. 3).

The reversible phosphorylation of the LHCII proteins during state transitions implies the active participation of protein phosphatases. Reversible phosphorylation of LHCII proteins was observed with isolated thylakoids indicating that at least a portion of the phosphatase is membrane associated (Bennett 1980). It was further shown that the

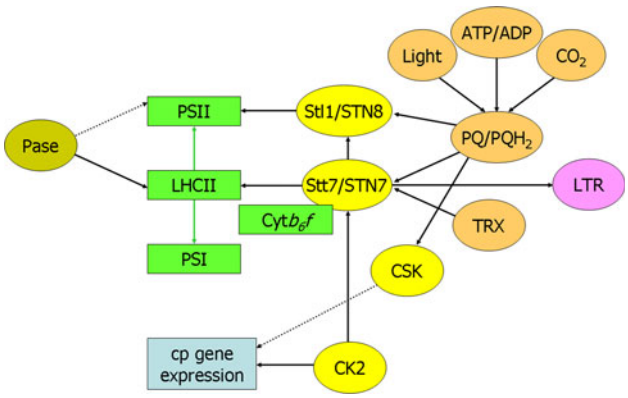

Fig. 3 Thylakoid protein kinase network. The redox state of the plastoquinone pool, which is influenced by light quality and quantity, cellular ATP level and CO_2 level, modulates the activity of the Stt7/STN7 and Stl1/STN8 kinases required for the phosphorylation of LHCII and PSII, respectively. It may also act on CSK. Activation of the Stt7/STN7 kinase depends on its close interaction with the $Cytb_6f$ complex. After it is phosphorylated, LHCII moves from PSII to PSI. A phosphatase (Pase) which specifically dephosphorylates LHCII has been identified recently (see text). Moreover, the redox state of Trx also influences the activity of Stt7/STN7. The proposed action of CK2 on Stt7/STN7 and of CSK on chloroplast gene expression is still tentative and will need further experimental support. Besides its role in short-term acclimation, STN7 is also involved in the long-term response (LTR). Protein kinases are highlighted in *yellow*

activities of thylakoid protein phosphatases are redox independent and kinetically heterogeneous (Silverstein et al. 1993). Several chloroplast phosphatase activities were identified both in thylakoid membranes and in the chloroplast stroma using as assays dephosphorylation of synthetic phosphopeptide analogues of the LHCII N-terminal phosphorylation sites or authentic phosphopeptides cleaved from thylakoid membrane proteins. In this way, a 29-kDa stromal phosphatase was purified and suggested to play a role in the dephosphorylation of LHCII (Hammer et al. 1997). However, its in vivo role remains unclear. It is not known whether the LHCII phosphorylation state is solely regulated by the LHCII kinase or whether phosphatases are also subjected to regulation.

In contrast to soluble phosphatases which associate with the thylakoid membrane and which appear to be involved in LHCII dephosphorylation (Hammer et al. 1997; Kieleczawa et al. 1992), a thylakoid membrane, PP2A protein phosphatase, was purified which was efficient in dephosphorylating PSII phosphoproteins and was regulated by the immunophilin-like TLP40 protein localized in the thylakoid lumen (Fulgosi et al. 1998; Vener et al. 2000). It was proposed that signalling from TLP40 to the protein phosphatase coordinates dephosphorylation and folding of thylakoid membrane proteins.

During the screening of Arabidopsis lines with T-DNA insertions in nuclear genes encoding putative chloroplast phosphatases, two groups have recently identified a novel phosphatase of *A. thaliana*, called TAP38/PPH1, which is specifically required for the dephosphorylation of LHCII, but not of the PSII core proteins under state 1 conditions (Pribil et al. 2010; Shapiguzov et al. 2010). This phosphatase, which belongs to the family of monomeric PP2C type phosphatases, is a chloroplast protein and is mainly associated with the stromal lamellae of the thylakoid membranes. Loss of TAP38/PPH1 leads to an increase in the antenna size of photosystem I and impairs transition from state 2 to state 1. Thus, phosphorylation and dephosphorylation of LHCII appear to be specifically mediated by the STN7 kinase–TAP38/PPH1 phosphatase pair. These two proteins emerge as key players in the adaptation of the photosynthetic apparatus to changes in light quality and quantity.

Membrane remodelling during state transitions

A remarkable feature of state transitions is that they involve considerable membrane remodelling. During a transition from state 1 to state 2, spectroscopic measurements indicate that 80% of the LHCII antenna of *C. reinhardtii* moves from PSII to PSI (Delosme et al. 1996). An immunocytochemical study revealed marked changes in the distribution of the cytochrome b_6f complex and LHCII between the appressed and unappressed thylakoid membrane regions during state transitions (Vallon et al. 1991). There was a significant enrichment of these two complexes in the unappressed regions in state 2 compared to state 1. Under similar conditions, this reorganization of LHCII and the cytochrome b_6f complex within the thylakoid membranes was suppressed in the *stt7* mutant which is locked in state 1 (Fleischmann et al. 1999).

There is a marked difference in thylakoid membrane organization between *C. reinhardtii* and land plants. Whereas clear differences are observed in Arabidopsis between grana regions and stromal lamellae, in *C. reinhardtii*, the grana regions generally consist of only a few appressed membranes. A slightly higher extent of appressed membranes was reported for *C. reinhardtii* cells in state 1 compared to state 2. Earlier studies concluded that the unappressed regions carry a higher negative surface charge than the appressed regions of thylakoid membranes (Barber 1982). It was shown that destacking of thylakoid membranes can be induced in vitro through depletion of cations presumably because of the appearance of repulsive negative charges. In contrast, readdition of cations promoted restacking through screening of the negative surface charges. The availability of mutants deficient in LHCII or PSII core protein phosphorylation has made it possible to investigate which surface charges are mainly responsible for the folding of the thylakoid membranes. Electron microscope analysis of the membranes from the *stn7stn8* double mutant of Arabidopsis deficient in the light-induced phosphorylation of LHCII and PSII core proteins revealed a more compact thylakoid organization with increased thylakoid membrane folding as compared to wild-type plants (Fristedt et al. 2009). There was a significant enhancement in the size of stacked thylakoid membranes in *stn7stn8* which was also manifested by a higher rate of gravity-driven sedimentation of isolated thylakoids. This phenotype was only seen in the absence of the STN8 kinase but not in *stn7* which lacks the STN7 kinase. Thus, PSII core protein phosphorylation plays a critical role in the folding of the thylakoid membranes. In contrast, the phosphorylation status of LHCII does not appear to have a significant effect on this process. The enhanced membrane folding in *stn7stn8* and *stn8* hindered the lateral migration of the PSII reaction centre protein D1 and of the processing FtsH protease between the appressed and the non-appressed membrane regions and slowed down D1 turnover in plants exposed to high light (Fristedt et al. 2009). These results suggest that the high level of PSII core protein phosphorylation in plants is required for adjusting the folding of the photosynthetic membranes for controlling lateral mobility of membrane proteins and photosynthetic activity.

Biochemical evidence for the displacement of LHCII from PSII to PSI was provided through the isolation of a PSI–LHCI–LHCII supercomplex from Arabidopsis plants in state 2 (Zhang and Scheller 2004). Electron microscopy revealed that this supercomplex consists of one PSI–LHCI complex and one LHCII trimer (Kouril et al. 2005). The docking site of LHCII on PSI is formed by the PsaH, PsaI and PsaO subunits as revealed by cross-linking studies which are in full agreement with the fact that mutants of Arabidopsis lacking the PsaH, PsaL and PsaO subunits are unable to perform state transitions (Lunde et al. 2000). Recent experiments with Chlamydomonas also indicate a clear association of LHCII to PSI under state 2 conditions. A PSI–LHCI–LHCII supercomplex distinctly larger than the PSI–LHCI complex was isolated which contains the three LHCII proteins CP29, CP26 and Lhcbm5 (Takahashi et al. 2006). The last-mentioned protein is a LHCII type II protein which is present in similar amounts as CP29 and CP26 but at a lower level than the other LHCII proteins. Lhcbm5 may have a similar role as CP24 in land plants. Together with CP29 and CP26, Lhcbm5 may act as a linker between the PSII core dimer and the trimeric LHCII in state 1. In state 2, these three proteins migrate to the PsaH side of PSI where they provide a binding site for the LHCII trimers on PSI. In this respect, it is interesting to note that the crystal structure of the PSI–LHCI complex reveals that four Lhca subunits form a belt on the opposite side of PsaH (Ben-Shem et al. 2003). Thus, it appears that CP29, CP26 and Lhcbm5 shuttle between PSII and PSI during state transitions and that their affinities for PSII and PSI may be modulated by phosphorylation. Phosphorylated forms of CP29 and Lhcbm5 were found associated with PSI (Takahashi et al. 2006). Of particular interest is CP29 with four sites phosphorylated in state 2 (Turkina et al. 2006). The phosphorylation of three additional sites under high light could induce its dissociation and that of LHCII from PSI and allow for thermal energy dissipation within the LHCII trimers. It thus appears that reversible phosphorylations at the interface between the PSII core and LHCII play a critical role in state transitions.

In order to examine the dissociation of LHCII from the PSII complex which occurs during a transition from state 1 to state 2, PSII-containing complexes were purified through a His-tag inserted in CP47 by nickel affinity chromatography (Iwai et al. 2008). This led to the identification of a PSII core complex, a PSII–LHCII supercomplex, and a multimer of PSII supercomplex called PSII megacomplex. The fact that the megacomplex was predominant in state 1 while the core complex was found in state 2 indicated that the LHCIIs dissociate from PSII on state transitions. Moreover, phosphorylated LHCII type I was mainly found in the supercomplex and less in the megacomplex whereas phosphorylated CP26 and CP29 were only present in the unbound form. Iwai et al. (2008) propose a model in which PSII remodelling during transition from state 1 to state 2 proceeds in two steps; first a dissociation of the megacomplex into supercomplexes mediated mainly by phosphorylation of LHCII type I and subsequent release of LHCII from the supercomplex triggered by phosphorylation of the minor LHCIIs and the PSII core subunits. In this view, the minor complexes CP29 and CP26 play a key role as their phosphorylation induces the undocking of the entire peripheral antenna during a transition to state 2. The free LHCII would then reassociate with the PSI–LHCI supercomplex. In order to further examine the role of CP29 and CP26 in state transitions in *C. reinhardtii*, RNA interference experiments were performed (Tokutsu et al. 2009). The results indicate that, in the absence of CP29, state transitions do no longer occur and although the mobile LHCII is detached from PSII, it does not bind to the PSI–LHCI complex. In contrast, the loss of CP26 did not affect state transitions in this alga. This further confirms that CP29 is essential for the docking of LHCII to PSI.

The traditional view of state transitions attributes an important role to the movement of LHCII from PSII in the grana to PSI in the stroma lamellae. A recent study has compared the phosphorylation-dependent movement of LHCII with the fluorescence changes occurring in both photosystems upon subfractionation of the thylakoid membranes by phase partitioning (Tikkanen et al. 2008). Although several LHCII proteins moved to the lamellar regions after LHCII phosphorylation, there was no increase in PSI fluorescence associated with this fraction. Such an increase in PSI fluorescence was exclusively found in the grana margin fractions indicating that besides the mobile LHCII, PSI–LHCI complexes appear to move from the stromal lamellae to the grana margins where they associate with LHCII and/or that a large fraction of PSI is located at the margins as proposed by Albertsson (2001). In this view, the dynamic nature of grana margins allows the capture of excitation energy by PSI from PSII–LHCII in a process which must be regulated in a tight and dynamic way (Tikkanen et al. 2008).

Analysis of thylakoid membranes of Arabidopsis in state 1 by electron microscope tomography revealed that the granum layers consist of repeating units that are physically connected to each other and to the adjacent stroma lamellae (Shimoni et al. 2005). According to this view, the grana consist of bifurcations of stroma lamellae into neighbouring sheets that run parallel to each other and that fuse with each other through membrane bridges that stabilize the membrane network. Reversible structural changes in thylakoid membrane organization of this type have been observed during state transitions by atomic force microscopy, scanning and transmission electron microscopy, and confocal imaging (Chuartzman et al. 2008). Based on this

analysis, a model has been proposed in which reorganization of the membranes is mediated through fusion and fission events at the interface between the granal and stromal lamellar domains of the thylakoid membranes. This membrane remodelling appears to be initiated at the curved margins of the appressed grana domains through breakage of the lateral and vertical connections within this membrane system. These localized rearrangements are proposed to undergo macroscopic cooperative changes that then propagate through the entire membrane network (Chuartzman et al. 2008). A challenging task will be to identify and characterize the intrachloroplast machinery which participates in these fission and fusion events.

Physiological significance of state transitions

It is apparent that state transitions in *C. reinhardtii* are associated with the movement of a large part of the LHCII antenna. In this organism, state transitions are influenced to a large extent by the cellular ATP level. Lack of ATP results in a transition to state 2. Moreover, a transition from state 1 to state 2 in this alga acts as a switch from linear to cyclic electron flow and, therefore, influences the ATP/NADPH ratio (Finazzi et al. 1999, 2002). It is very likely that the major role of state transitions in this alga is to adjust the cellular ATP levels in response to changes in cellular demand rather than the balancing of the light excitation energy between the two photosystems. It would be difficult to explain the migration of 80% of the LHCII antenna from PSII to PSI uniquely for reasons of antenna size readjustment.

It is surprising that no obvious growth phenotype could be detected in the *stt7* mutant of *C. reinhardtii* even under changing light conditions (Fleischmann et al. 1999). However, the situation changes drastically under conditions leading to low ATP levels. It was noticed that depletion of ATP through inhibition of mitochondrial respiration or in mitochondrial mutants deficient in respiration, in the dark, stimulates glycolysis through the Pasteur effect (Bulté et al. 1990; Cardol et al. 2003). This, in turn, leads to the production of reductants which reduce the plastoquinone pool and induce state 2. Transfer of these cells to the light restores ATP levels and state 1 partially. These findings suggested a possible link between state transitions and mitochondrial respiration. In order to further explore the interplay between state transitions and mitochondrial respiration, the growth properties of the *stt7* mutant were examined in the presence of myxothiazol, an inhibitor of respiration (Cardol et al. 2003). The growth of this mutant on minimal medium was drastically affected. Next, the double mutant *stt7 dum22* deficient both in state transitions and mitochondrial respiration was examined. Its growth rate under photoautotrophic conditions was shown to be severely affected at low light but less so under high light, indicating that the growth defect is not due to increased photosensitivity. These results indicate that Chlamydomonas cells display some energetic flexibility and are able to compensate for a lack of mitochondrial respiration and ATP synthesis through transition to state 2 and a concomitant switch to cyclic electron flow which enhances ATP production for sustained CO_2 assimilation. Indeed, an ATP to NADPH ratio of 1:5 is required to drive the Calvin–Benson cycle which cannot be achieved solely by linear electron flow. In the absence of state transitions, mitochondrial respiration can, at least, partially compensate. However, under conditions of impaired mitochondrial respiration, state transitions are essential for ensuring photoautotrophic growth under limiting light. This is because, in mitochondrial respiration mutants, increased reducing power is fed into the chloroplast plastoquinone pool together with the electrons originating from PSII. This increase must be matched by an increase in the PSI absorption capacity through a transition to state 2, thereby allowing for an efficient light utilization by both photosystems. Thus, there is a tight interplay between cyclic electron flow and respiratory capacity which is mediated, in part, through the process of state transitions.

The importance of state transitions is apparent not only in Chlamydomonas but also in Arabidopsis. The analysis of double mutants of Arabidopsis combining the *stn7* mutation with mutations affecting linear electron flow and leading to a more reduced state of the plastoquinone pool showed that the growth rate and the effective quantum yield were significantly decreased compared to the single mutants (Pesaresi et al. 2009). These results indicate that state transitions become critical when linear electron transport is impaired. Comparison of the *stn7* mutant with wild-type Arabidopsis lines in field tests revealed that the loss of state transitions leads to a significant decrease in fitness (Frenkel et al. 2007). Moreover, controlled light shift experiments with *stn7* and wild-type plants also showed marked differences in seed production (Wagner et al. 2008). Thus, when the plants were shifted every 2–3 days between PSI- and PSII-light, a period which allows for LTR, *stn7* plants produced 50% less seeds than wild-type plants. When the light shifts occurred every 20 min which correspond to the time range of state transitions, a further decrease in seed production was observed for *stn7*. It, thus, appears that state transitions and LTR provide a metabolic flexibility which allows photosynthetic organisms to acclimate both under long- and short-term light quality shifts corresponding to a wide range of light fluctuations which occur in a natural environment.

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