



## The Dynamics of the Photosynthetic Apparatus in Algae

Jean-David Rochaix\*

*Departments of Molecular Biology and Plant Biology,  
University of Geneva University, Geneva, Switzerland*

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### I. Introduction

Oxygenic photosynthetic organisms are constantly subjected to changes in light quality and quantity and have to adapt to this changing environment. On the one hand they need light energy and have to collect it efficiently especially when light is limiting, on the other, they have to be able to dissipate the excess absorbed light energy when the capacity of the photosynthetic apparatus is exceeded. The primary events of photosynthesis occur in the thylakoids, a complex network of membranes localized within chloroplasts. These primary reactions are

mediated by three major protein-pigment complexes, photosystem II (PSII), the cytochrome *b<sub>6</sub>f* complex (*Cytb<sub>6</sub>f*) and photosystem I (PSI) embedded in the thylakoid membrane and which act in series. Both PSII and PSI are associated with their light-harvesting systems LHCII and LHCI, respectively, which collect and transfer the light excitation energy to the reaction centers of the two photosystems. In both cases a chlorophyll dimer is oxidized and a charge transfer occurs across the thylakoid membrane. PSII creates thereby a strong oxidant capable of splitting water on its donor side with concomitant evolution of molecular

\*Author for correspondence, e-mail: [Jean-David.Rochaix@unige.ch](mailto:Jean-David.Rochaix@unige.ch)

oxygen and the release of protons in the lumen while electrons are transferred along the photosynthetic electron transport chain through PSII to the plastoquinone pool and *Cytb<sub>6</sub>f*. This complex pumps protons from the stroma to the lumen side of the thylakoid membrane while transferring electrons to plastocyanin and PSI. Ultimately the electrons are transferred to ferredoxin and NADP(H), the final acceptor. As a result of this photosynthetic electron flow, a proton-motive force is generated across the thylakoid membrane consisting of a proton gradient and membrane potential. A fourth complex, the ATP synthase complex, is functionally linked to the other three by using the proton gradient to produce ATP (Fig. 4.1). Both ATP and NADPH fuel the Calvin-Benson-Bassham cycle for CO<sub>2</sub> assimilation. Besides linear electron flow (LEF), cyclic electron flow (CEF) occurs in which elec-

trons are transferred from the PSI acceptor ferredoxin to the plastoquinone pool either through a type I/II thylakoid-bound NADH dehydrogenase (Burrows et al. 1998) or the antimycin-sensitive pathway involving Pgr5 and Pgr11 (Munekage et al. 2002; Hertle et al. 2013). Analysis of a *pgr5* mutant of *Chlamydomonas* revealed that the loss of Pgr5 leads to a reduced proton gradient across the thylakoid membrane and to diminished CEF activity (Johnson et al. 2014). Pgr11 has been proposed to act as a ferredoxin-plastoquinone reductase (Hertle et al. 2013). In contrast to linear electron flow which generates both reducing power and ATP, CEF produces exclusively ATP. The NADPH/ATP ratio can thus be modulated through regulation of CEF versus LEF. It should be noted however that *pgr5* and *pgr11* mutants are still able to perform CEF under specific conditions such as high light or CO<sub>2</sub>

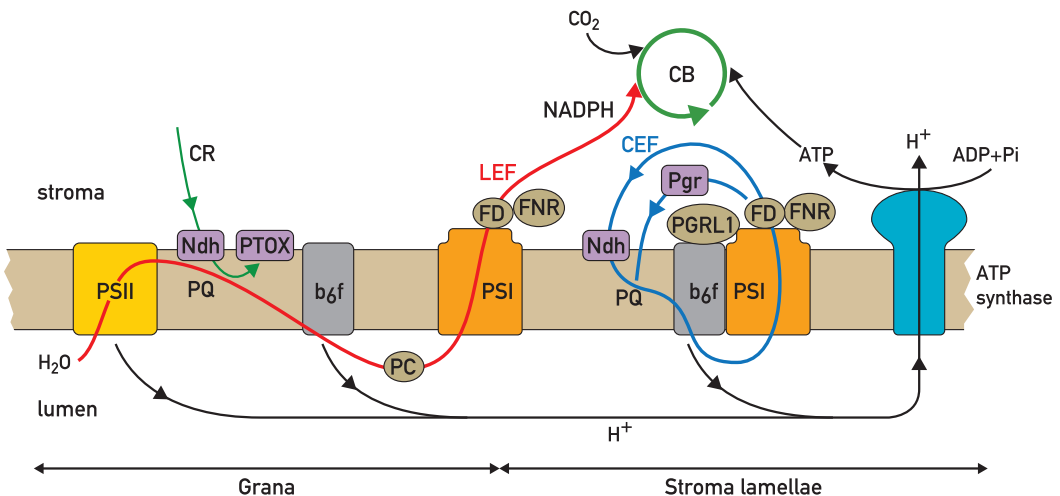


Fig. 4.1. Scheme of the photosynthetic electron transport chain with PSII, *Cytb<sub>6</sub>f*, PSI and ATP synthase. Linear electron flow (LEF) and cyclic electron flow (CEF) are shown in red and blue, respectively with arrows indicating the direction of electron flow. The LEF pathway is driven by the two photochemical reactions of PSII and PSI: electrons are extracted by PSII from water and transferred subsequently to the PQ pool, *Cytb<sub>6</sub>f*, plastocyanin (PC), PSI and ferredoxin (Fd). Ferredoxin-NADPH reductase (FNR) catalyzes the formation of NADPH at the expense of reduced Fd. The CEF pathway is driven by PSI in the stroma lamellae. In *Chlamydomonas reinhardtii* PSI forms a supercomplex with *Cytb<sub>6</sub>f*, FNR, PGRL1, PGR5 and additional factors. Upon reduction of Fd, electrons are returned to the PQ pool either through the NADH complex (Ndh) or via PGRL1 which acts as a Fd-PQ oxidoreductase. Both LEF and CEF are associated with proton pumping into the lumen. The resulting proton gradient is used by ATP synthase to produce ATP which together with NADPH drives CO<sub>2</sub> assimilation by the Calvin-Benson-Bassham cycle (CBB). G, grana; SL, stroma lamellae. Reproduced from (Rochaix 2014) with permission

limitation (Joliot and Johnson 2011; Nandha et al. 2007; DalCorso et al. 2008; Kono et al. 2014). This suggests that PGR5 and PGRL1 are not essential for CEF but are important for its regulation and control.

In addition to LEF and CEF, alternative electron transport occurs within the thylakoid membrane in which  $O_2$  is used as electron sink. These pseudo-cyclic electron transport paths involve the plastoquinone terminal oxidase which can oxidize the plastoquinone pool and, on the acceptor side of PSI, flavodiiron proteins which can reduce  $O_2$  directly to water (Allahverdiyeva et al. 2015; Shikanai and Yamamoto 2017). Alternatively reduction of  $O_2$  gives rise to reactive oxygen species (ROS) which are scavenged by superoxide dismutase and ascorbate peroxidase to produce water through the water-water cycle (see Chap. 8). These alternative pathways are important for maintaining a proper redox balance of the electron transfer chain and, in the case of the flavoproteins, for photoprotection of PSII and PSI especially under fluctuating light (Zhang et al. 2012; Shimakawa et al. 2015). Together with CEF they contribute to the formation of the proton-motive force and hence to ATP synthesis without any net accumulation of NADPH (Allen 2003).

A striking feature of the thylakoid membrane is its lateral heterogeneity with two distinct domains consisting of appressed membranes, called grana, and stromal lamellae which connect the grana regions with each other (Andersson and Andersson 1980; Albertsson 2001). Whereas PSII is mainly localized in the grana regions, PSI and the ATP synthase are found in the stromal lamellae and in the margins of the grana (Dekker and Boekema 2005). This is because these two complexes have large domains protruding in the stromal phase which do not fit into the narrow membrane space between the grana lamellae. The organization of thylakoid membranes in grana and stromal regions is determined to a large extent by the resident photosystem complexes. As an example,

mutants deficient in PSI contain mostly grana with few stroma lamellae (Amann et al. 2004; Barneche et al. 2006). In contrast to the photosystems, the *Cytb<sub>6</sub>f* complex is equally distributed between the grana and stromal thylakoid regions. Grana formation appears to be mediated by van der Waals attractive forces and electrostatic interactions in which LHCII plays an important role (Kirchhoff et al. 2008).

The LHCII genes form a large family with each member encoding a protein with three transmembrane domains and up to eight chlorophyll *a*, six chlorophyll *b* and four xanthophyll molecules. In *Chlamydomonas* there are nine major and two minor LHCII and nine LHCI genes (Minagawa and Takahashi 2004). The LHCII antenna comprises LHCII trimers connected to the PSII core through the CP26 and CP29 LHCII monomers. The LHCII trimers bind PSII at three sites named S (strong), M (medium) and L (loose). In vivo, PSII assembles as dimers associated with two S and M LHCII trimers to form the  $C_2S_2M_2$  PSII-LHCII supercomplex in land plants (Dekker and Boekema 2005). Supercomplexes with one to three LHCII trimers per monomeric PSII core have also been detected in *Chlamydomonas* (Drop et al. 2014; Nield et al. 2000; Tokutsu et al. 2012). In eukaryotic algae the PSI complex is monomeric with a core consisting of the PsaA/PsaB heterodimer and additional subunits as well as up to 10 LHCI proteins in *Chlamydomonas* based on biochemical studies and single-particle electron microscopy (Ozawa et al. 2018; Steinbeck et al. 2018). It is noticeable that in contrast to the conserved core photosynthetic complexes, the antenna systems are considerably more diverse with hydrophobic membrane-embedded LHCs in plants, green and red algae, and extrinsic hydrophilic phycobilisomes in red algae and cyanobacteria. Moreover in most green algae thylakoid membranes are not differentiated in grana and stroma regions (Gunning and Schwartz 1999).

The aim of this chapter is to provide a description of the remarkable dynamics and flexibility of the photosynthetic apparatus of algae in response to changes in environmental conditions, and to compare these responses with those of land plants. They include changes in light quality and quantity and in nutrient availability. These responses involve a reorganization of some of the photosynthetic complexes often mediated by post-translational modifications of their subunits through an extensive signalling network in chloroplasts and between chloroplasts and nucleus which modulates nuclear and plastid gene expression.

## II. Adaptation to Changes in Light Conditions

A distinctive feature of photosynthetic organisms is the presence of light-harvesting systems that funnel the absorbed light energy to the corresponding reaction centers and thereby considerably increase their absorption cross-section. Several regulatory mechanisms operate on these antenna systems for controlling the energy flux to the reaction centers. This is particularly important under changing environmental conditions when the photosynthetic apparatus needs to adapt quickly. Under limiting light it optimizes its light absorption efficiency by adjusting the relative size of its antenna systems through the reversible allocation of a portion of LHCII between PSII and PSI, a process referred to as state transitions which occurs in algae, plants and cyanobacteria (for reviews see (Lemeille and Rochaix 2010; Wollman 2001)). In contrast when the absorbed light energy exceeds the capacity of the photosynthetic apparatus, it dissipates the excess excitation energy through non-photochemical quenching as heat thereby avoiding photodamage (for reviews see (Niyogi 1999; Niyogi and Truong 2013)).

### A. State Transitions

Because the antenna systems of PSII and PSI have a different pigment composition, their relative light absorption properties change when the light quality varies. This is especially important for aquatic algae because the penetration of light in water changes depending on its wavelength; in particular, red light is more absorbed than blue light. Another example is provided by photosynthetic organisms growing under a canopy where far red light is enriched. These changes in light quality can result in an unequal excitation of PSII and PSI and thereby perturb the redox poise of the plastoquinone pool. Over-excitation of PSII relative to PSI leads to increased reduction of the plastoquinone pool and favours thereby docking of plastoquinol to the Q<sub>o</sub> site of the Cytb<sub>6</sub>f complex (Vener et al. 1997; Zito et al. 1999). This process leads to activation of the chloroplast protein kinase Stt7/STN7 and to the phosphorylation of several proteins from LHCII (Depège et al. 2003; Bellafiore et al. 2005). Although the direct phosphorylation of LHCII by the Stt7/STN7 kinase has not yet been demonstrated, this kinase is the best candidate for the LHCII kinase because it is firmly associated with the Cytb<sub>6</sub>f complex and in its absence state transitions do no longer occur (Lemeille et al. 2009). Furthermore it is widely conserved in land plants, mosses and algae. As a result of this phosphorylation, part of the LHCII antenna is detached from PSII and moves and binds to PSI thereby rebalancing the light excitation of PSII and PSI and enhancing photosynthetic yield. This process is reversible as overexcitation of PSI leads to the inactivation of the kinase and to dephosphorylation of LHCII by the PPH1/TAP38 protein phosphatase and its return to PSII (Pribil et al. 2010; Shapiguzov et al. 2010). Thus two different states can be defined, state 1 and state 2 corresponding to the association of the mobile

LHCII antenna to PSII and PSI, respectively. However, a strict causal link between LHCII phosphorylation and its migration from PSII to PSI has been questioned recently by the finding that some phosphorylated LHCII remains associated with PSII supercomplexes and that LHCII serves as antenna for both photosystems under most natural light conditions (Drop et al. 2014; Wientjes et al. 2013a, b). In plants, the LHCII S trimers comprise Lhcb1 and Lhcb2 whereas the M trimers contain Lhcb1 and Lhcb3 (Galka et al. 2012). Both the S and M trimers are most likely not involved in state transitions because the PSII-LHCII supercomplex is unchanged upon phosphorylation (Wientjes et al. 2013a) and PSI does not bind Lhcb3 in state 2 (Galka et al. 2012). Thus LHCII phosphorylation is not sufficient to dissociate all LHCII trimers from PSII. It has therefore been proposed that peripherally bound L trimers associate with PSI in state 2 (Galka et al. 2012). Moreover although Lhcb1 and Lhcb2 display similar phosphorylation kinetics during a state1 to state 2 transition, only phosphorylated Lhcb2 but not Lhcb1 is part of the PSI-LHCII supercomplex (Longoni et al. 2015). In this regard, it was shown recently by cryo-electron microscopy that the first three conserved residues of Lhcb2 including phosphorylated Thr3, interact with specific residues from PsaL, PsaO and PsaH (Pan et al. 2018). A PSI supercomplex has been isolated and characterized in *Chlamydomonas* (Steinbeck et al. 2018; Iwai et al. 2010). It consists of PSI, Cyt *b<sub>6</sub>f*, LHCII, FNR (ferredoxin-NADPH reductase), PGRL1, a protein involved in CEF (Hertle et al. 2013) and additional factors (Fig. 4.1). The correlation between the occurrence of state transitions and CEF raised the possibility that state transitions may act as a switch between LEF and CEF in *Chlamydomonas* (Finazzi et al. 2002). This interpretation is however not compatible with recent studies which indicate that CEF is activated in the *stt7* mutant when the metabolic demand for ATP increases during the

induction of the carbon concentrating mechanism when CO<sub>2</sub> is limiting (Lucker and Kramer 2013). Also, analysis of the *stt7* and *ptox2* mutants, locked in state 1 and 2, respectively, independent of the redox conditions led to similar conclusions (Takahashi et al. 2013). The *ptox2* mutant is deficient in the plastid terminal oxidase which controls the redox state of the PQ pool in the dark (Houille-Vernes et al. 2011). Whereas the accumulation of reducing power and transition to state 2 correlated well with the enhancement of CEF in the wild type, this was not the case for *ptox2*. In this mutant, CEF was not enhanced under aerobic conditions in the dark even though it is locked in state2 with phosphorylated LHCII. Moreover, CEF enhancement and formation of the PSI-Cyt***b<sub>6</sub>f*** supercomplex were still observed in the *stt7* mutant when the PQ pool was reduced. It can be concluded that both of these processes occur under reducing conditions with no correlation with state transitions and their associated LHCII reorganization and that it is the redox state of the photosynthetic electron transport rather than state transitions that controls CEF (Takahashi et al. 2013).

State transitions do not occur under high light because the LHCII kinase is inactivated (Schuster et al. 1986). The current view is that inactivation of the kinase is mediated by the ferredoxin-thioredoxin system and that a disulfide bond in the kinase rather than in the substrate may be the target site of thioredoxin (Rintamaki et al. 1997, 2000). In this respect the N-terminal region of the kinase contains indeed two Cys residues which are conserved in all species examined (Depège et al. 2003; Bellafiore et al. 2005). Both of these Cys are essential for the kinase activity because changes of either Cys through site-directed mutagenesis abolishes the kinase activity (Lemeille et al. 2009). It is noticeable that these Cys are located on the lumen side of the thylakoid membrane whereas the kinase catalytic domain is on the stromal side where the substrate sites of the LHCII

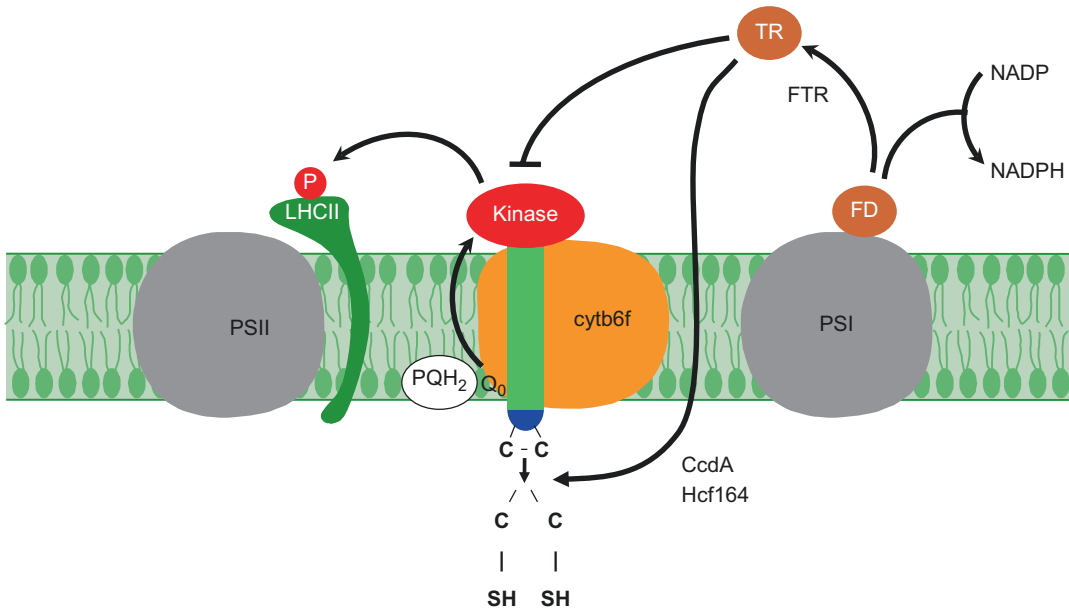


Fig. 4.2. Regulation of the Stt7/STN7 kinase

proteins are located (Depège et al. 2003; Lemeille et al. 2009) (Fig. 4.2). Although the conserved Cys residues in the lumen are on the opposite side of the stromal thioredoxin according to this model, one possibility is that thiol reducing equivalents are transferred across the thylakoid membrane through the CcdA and Hcf164 proteins which operate in this way during heme and Cyt *b*<sub>6</sub>f assembly (Page et al. 2004; Lennartz et al. 2001). Alternatively, transfer of thiol reducing equivalents across the thylakoid membrane could also be mediated by Lto1 (Lumen Thiol Oxidoreductase 1) which catalyzes the formation of disulfide bonds in the thylakoid lumen and is required for PSII assembly (Karamoko et al. 2011; Du et al. 2015) (Fig. 4.2). Its sulfhydryl oxidizing activity is linked to the reduction of plastoquinone, a redox component of PSI. It is not clear whether plastoquinone is involved in other electron transfer processes besides those in PSI. Although the two luminal Cys are prime candidates for the redox control of the activity of the Stt7/STN7 kinase, high light treatment did not change the redox state of these Cys (Shapiguzov et al. 2016).

Another possibility is that high light affects the folding of the kinase in the thylakoid membrane, in particular through reactive oxygen species generated by the high light treatment.

The Stt7/STN7 kinase is associated with the Cyt *b*<sub>6</sub>f complex. This kinase contains a transmembrane domain connecting its N-terminus on the lumen side with two conserved Cys residues to the catalytic domain on the stromal side of the thylakoid membrane. The major substrates of this kinase are the LHCII proteins of the PSII antenna. The LHCII kinase is known to be inactivated by high light through the Fd/Trx system. This system could modulate the redox state of the two luminal Cys through CcdA and Hcf164, two proteins known to mediate the transfer of thiol reducing equivalents across the thylakoid membrane. Another possibility is that this process is catalyzed by Lto1, the luminal thiol oxidoreductase 1.

It is known that the activation of the kinase is intimately connected to the docking of plastoquinone to the Q<sub>o</sub> site of the Cyt *b*<sub>6</sub>f complex (Vener et al. 1997; Zito et al. 1999) (Fig. 4.2). Electron transfer from plasto-

quinol to Cyt *f* is mediated by the Rieske protein and involves the movement of this protein from the proximal to distal position within the Cyt *b<sub>6</sub>f* complex (Darrouzet et al. 2001; Breyton 2000). Recent studies have revealed that the two conserved Cys residues of the Stt7/STN7 kinase form an intramolecular disulfide bridge which appears to be essential for kinase activity (Shapiguzov et al. 2016). However no change in the redox state of these Cys could be detected during state transitions. It is only under prolonged anaerobic conditions that this disulfide bridge was reduced but at a significantly slower pace than transition from state 1 to state 2 which occurs under anaerobiosis in *Chlamydomonas* (Bulté et al. 1990). In wild-type *Arabidopsis* plants the STN7 kinase is only observed as a monomer both under state 1 and state 2 conditions although the dimer could be detected in plants overexpressing STN7 or in mutants with changes in either of the two luminal Cys of STN7 (Wunder et al. 2013). However these results do not exclude the possibility of rapid and transient changes in the redox state of these two Cys. In fact such changes were proposed to occur to accommodate all the known features of the Stt7/STN7 kinase (Shapiguzov et al. 2016). A transient change from an intra- to intermolecular disulfide bond may occur which would activate the kinase and be coupled to the movement of the Rieske protein during electron transfer from plastoquinol to Cyt *f*. Moreover it is interesting to note that an interaction site between the kinase and the Cyt *b<sub>6</sub>f* complex has been located close to the flexible glycine-rich hinge connecting the membrane anchor to the large head of the Rieske protein in the lumen (Shapiguzov et al. 2016). Given that a single chlorophyll *a* molecule with its phytol tail close to the Qo site exists in Cyt *b<sub>6</sub>f* (Stroebel et al. 2003), it is also possible that the kinase senses PQH<sub>2</sub> binding to the Qo site through this chlorophyll *a* molecule of the Cyt *b<sub>6</sub>f* complex (Stroebel et al. 2003; Kurisu et al. 2003). It was proposed that this molecule may play a

role in the activation of the Stt7/STN7 kinase based on site-directed mutagenesis of the chlorophyll *a* binding site (de Lacroix de Lavalette et al. 2008). The activation of the kinase would be triggered through the transient formation of a STN7 dimer with two intermolecular disulfide bridges which would transduce the signal to the catalytic domain on the stromal side of the thylakoid membrane (Shapiguzov et al. 2016).

Besides the luminal side of the Cyt *b<sub>6</sub>f* complex, its stromal side also appears to be involved in the activation of the Stt7/STN7 kinase. Analysis of mutants affected in the chloroplast *petD* gene encoding subunit IV of Cyt *b<sub>6</sub>f* revealed that several residues of the stromal loop connecting helices F and G interact directly with the Stt/STN7 kinase and are critical for state transitions (Dumas et al. 2017).

Another proposal for the mechanism of activation of the Stt7/STN7 kinase is that hydrogen peroxide may be involved by oxidizing the luminal C1 and C2 to form intra and/or intermolecular disulfide bridges. It is based on the observation that singlet oxygen generated by PSII can oxidize plastoquinol with concomitant production of hydrogen peroxide in the thylakoid membranes (Khorobrykh et al. 2015). However this proposal is difficult to reconcile with the observation that these Cys exist mostly in the oxidized form and the conversion from intra- to inter-molecular disulfide bridges appears to be only transient (Shapiguzov et al. 2016).

State transitions involve remodeling of the antenna system of PSII within the thylakoid membranes. This poses a challenging problem especially considering the fact that amongst biological membranes, the thylakoid membrane is very crowded with 70% of the surface area of grana membranes occupied by proteins and 30% by lipids (Kirchhoff et al. 2008). Light-induced architectural changes in the folding of the thylakoid membrane are induced at least partly by changes in phosphorylation of thylakoid proteins catalyzed by the protein kinases Stt7/STN7 and

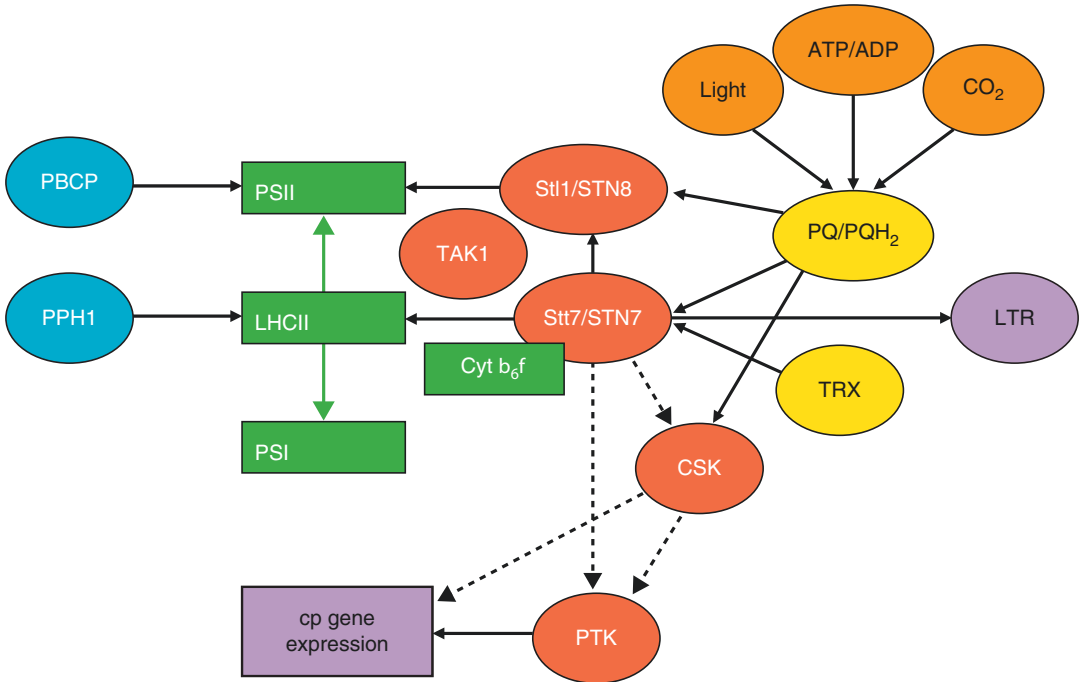


Fig. 4.3. Chloroplast signaling

Stt1/STN8 which most likely facilitate mobility of proteins in these membranes (Fristedt et al. 2009; Samol et al. 2012). These two kinases appear to play an important role in chloroplast signaling in response to changing environmental conditions (Fig. 4.3). Light irradiance, ambient CO<sub>2</sub> level and the cellular ATP/ADP ratio modulate the redox state of the plastoquinone pool of the electron transport chain which is sensed by the Stt7/STN7 kinase. Together with the Stt1/STN8 kinase and the two corresponding protein phosphatases PPH1/TAP38 and PBCP, Stt7/STN7 forms a central quartet which orchestrates the phosphorylation of the LHCII and the PSII core proteins (Fig. 4.3). PTK is another chloroplast Ser/Thr kinase of the casein kinase II family which is associated with the plastid RNA polymerase and acts as a global regulator of chloroplast transcription (Link 2003; Ogrzewalla et al. 2002). The CSK kinase shares structural features with cyanobacterial sensor histidine kinases and is conserved

in all major plant and algal lineages except *C. reinhardtii* (Puthiyaveetil et al. 2008). Upon oxidation of the PQ pool, autophosphorylation of CSK occurs, an event which correlates with phosphorylation of the chloroplast  $\sigma$  factor Sig1 and the decrease of *psaAB* gene expression. Furthermore, CSK interacts with PTK and SIG1 in yeast two hybrid assays. Based on these results it was proposed that CSK is regulated by the redox state of the PQ pool through the STN7 kinase (Fig. 4.3) (Puthiyaveetil et al. 2008). However how the different kinases are linked within this chloroplast signaling network shown in Fig. 4.3 is still unclear.

The redox state of the PQ pool is modulated by the light irradiance, ATP/ADP ratio and ambient CO<sub>2</sub> level. The protein kinases Stt1/STN8, Stt7/STN7, CSK, PTK and TAK1 (Snyders and Kohorn 1999, 2001) are shown with their targets indicated by arrows. Broken arrows indicate putative targets. LTR long term response involving retrograde signaling is mediated through Stt7/STN7.



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### B. *Non Photochemical Quenching (NPQ)*

While state transitions are mainly involved in low light responses through an extensive reorganization of the antenna systems, other mechanisms for the regulation of light-harvesting operate when oxygenic photosynthetic organisms are suddenly exposed to large and sudden changes in light intensity in their natural habitat. In the case of aquatic algae even moderate water mixing can bring algae from full darkness to high light within minutes (MacIntyre et al. 2000; Schubert and Forster 1997). Under these conditions increased electron flow along the electron transport chain generates a large proton gradient. The resulting acidification of the thylakoid lumen leads to the de-excitation of singlet excited light-harvesting pigments and is measured as non-photochemical quenching of chlorophyll fluorescence (NPQ). NPQ comprises several components; the major one is the high energy state quenching qE which leads to the harmless heat dissipation of the absorbed excess light energy (Niyogi and Truong 2013; Ruban et al. 2012; Chap. 12). The other components which also contribute to fluorescence quenching are the photoinhibitory quenching qI and state transitions qT although qT is not associated with thermal dissipation of excitation energy. The qE mechanism occurs in all major algal taxa and land plants. However the underlying molecular mechanisms of heat dissipation of excess excitation energy differ. The qE process involves both the xanthophyll cycle and the PsbS protein in plants. Another protein, LhcsR, has been shown to mediate qE in algae (Niyogi 1999; Peers et al. 2009; Chaps. 3 & 12).

The proton gradient acts as a sensor of the state of the photosynthetic electron transport chain. The magnitude of this gradient is low under low light illumination and high under illumination with high light especially when

it exceeds the capacity of the photosynthetic apparatus. The resulting acidification of the thylakoid lumen activates the xanthophyll cycle in which violaxanthin is de-epoxidized to zeaxanthin, a reaction catalyzed by violaxanthin de-epoxidase (VDE) which has an acidic pH optimum (Demmig-Adams and Adams 1992). The reverse reaction is catalyzed by zeaxanthin epoxidase with a broad pH optimum and which in contrast to VDE is active both in the dark and in the light. Because the turnover of this enzyme is considerably lower than that of VDE, zeaxanthin accumulates rapidly during high light illumination. The zeaxanthin-dependent NPQ depends greatly on the grana structure as unstacking of the membranes abolishes qE. It was proposed that the organization of LHCII in an aggregated state within the stacked grana region is essential for efficient qE (Horton et al. 2008). Both high proton concentration in the lumen and accumulation of zeaxanthin promote not only aggregation of LHCII but also that of the minor PSII antenna proteins CP29, CP26 and CP24 (Phillip et al. 1996; Wentworth et al. 2001). In plants qE occurs in the LHC proteins at multiple sites of the antenna system (Holwarth et al. 2009). These proteins have the ability to switch from an efficient light-harvesting mode to a light energy dissipating state (Kruger et al. 2012). Several mechanisms have been proposed including excitonic coupling, charge transfer and energy transfer between carotenoids and chlorophylls as well as chlorophyll-chlorophyll charge transfer states (for review see (Niyogi and Truong 2013)).

Another important player involved in NPQ is PsbS, a four-helix member of the LHC protein family (Li et al. 2000; Chaps. 3 & 10). However this protein does not bind pigments although a chlorophyll molecule was detected at the dimer interface in the PsbS crystals (Fan et al. 2015). This protein appears to act as a sensor of the lumen pH most likely through protonation of its acidic lumen residues which in turn induces a rearrangement of the light-harvesting system

required for induction of NPQ (Li et al. 2004; Betterle et al. 2009; Goral et al. 2012). In this sense PsbS would act as an antenna organizer, a view which is further supported by the fact that it is mobile in the thylakoid membrane (Teardo et al. 2007), and it is able to associate with both the PSII core complex and LHCII (Bergantino et al. 2003). Moreover, qE can be switched on without PsbS protein if the lumen pH is very low (Johnson and Ruban 2011). It thus appears that protonated PsbS allows for a fast and efficient rearrangement of the PSII antenna which is still possible in its absence but requires a longer time.

In *Chlamydomonas reinhardtii* and *Phaeodactylum tricoratum*, two representatives of green algae and diatoms, respectively, qE is mediated by Lhcsr, another three helix member of the LHC protein family (Peers et al. 2009; Chaps. 3 & 16). In high light, most Lhcsr genes are up-regulated in contrast to the light-harvesting genes which are down-regulated. Recent studies reveal that Lhcsr binds chlorophylls and xanthophylls *in vitro* and that it has a basal quenching activity associated with chlorophyll-xanthophyll charge transfer (Bonente et al. 2011). Its chlorophyll fluorescence lifetime is remarkably short and even shorter at low pH suggesting that this protein has some quenching activity even in low light which is enhanced at low pH. It was proposed that these properties could explain the low expression of Lhcsr under low light when constitutive quenching would be wasteful (Niyogi and Truong 2013). The *Chlamydomonas* Lhcsr is bound to PSII where it may interact with the LHC proteins, especially Lhcbm1 which is known to be involved in thermal dissipation (Allorent et al. 2013; Elrad et al. 2002; Ferrante et al. 2012). Interestingly, as several other LHC proteins, Lhcsr is phosphorylated by the Stt7 kinase and moves from PSII to PSI during a state 1 to state 2 transition (Allorent et al. 2013). This observation is particularly interesting with regard to chlorophyll fluorescence lifetime measurements which reveal two dif-

ferent kinetic components suggesting the existence of two underlying mechanisms (Amarnath et al. 2012).

It is noteworthy that although PsbS is also present in green algae, there is no evidence that it is involved in qE in these organisms under increased illumination. This is in contrast to the moss *Physcomitrella patens* that has both Lhcsr- and PsbS-dependent NPQ which operate independently and additively (Alboresi et al. 2010; Gerotto et al. 2012). The maintenance of these two mechanisms in mosses may be linked to a greater need for inducible NPQ in these organisms (Gerotto et al. 2011).

Two PsbS genes are present in the nuclear genome of the green alga *Chlamydomonas reinhardtii* that are expressed under specific conditions such as nitrogen deprivation (Miller et al. 2010), during a dark to light shift (Zones et al. 2015) and upon light stress (Correa-Galvis et al. 2016). PsbS is also strongly induced by UV-B light together with Lhcsr1 both of which contribute to qE under these conditions. UV irradiation leads to the monomerization of the cytoplasmic dimeric UVR8 receptor which then interacts with the E3 ubiquitin ligase COP1 (Constitutively Photomorphogenic 1), moves to the nucleus and induces changes in gene expression (Rizzini et al. 2011; Christie et al. 2012; Favory et al. 2009; Kaiserli and Jenkins 2007). Amongst the proteins upregulated in this response, PsbS and Lhcsr1 are prominent (Tilbrook et al. 2016) and they provide a direct mechanistic link between UVR8 receptor signaling and acclimation and photoprotection of the photosynthetic machinery of *Chlamydomonas* (Allorent et al. 2016; Allorent and Petroustos 2017).

NPQ has also been investigated in diatoms, an ubiquitous group of unicellular marine algae which make an important contribution to the global carbon assimilation (Geider et al. 2001). Diatoms acquired their chloroplast through secondary endosymbiosis from a red algal ancestor (Keeling 2013). In these organisms, similar to plants and green algae, qE relies on three interacting

components, the light-induced proton gradient across the thylakoid membrane, the conversion of the xanthophyll diadinoxanthin (Dd) to diatoxanthin (Dt) catalyzed by the enzyme Dd-de-epoxidase which depends on a trans-thylakoid proton gradient and the LhcX antenna proteins (for review see (Goss and Lepetit 2015)). Amongst these, LhcX1 appears to play a major role in qE as changes in its level are directly related to the quenching of light energy (Bailleul et al. 2010). LhcX1 also plays an important general role in light responses in diatoms as it accumulates in different amounts in ecotypes originating from different latitudes. In contrast to land plants the proton gradient is unable to induce NPQ on its own in diatoms. It is only required to activate the de-epoxidation of Dd. The qE process represents an important photoprotective mechanism and involves a reorganization of the antenna complexes of diatoms (Goss and Lepetit 2015). However the quenching sites within the antenna systems of these organisms have not yet been precisely determined.

Another original feature of diatoms is the way they adjust the ATP/NADPH ratio which is important for proper carbon assimilation by the Calvin-Benson-Bassam cycle and for optimal growth. In plants and green algae this ratio is mainly set by the relative contributions of LEF and CEF and by the water-to-water cycles (Allen 2003) whereas in diatoms this ratio relies principally on energetic exchanges between plastids and mitochondria (Bailleul et al. 2015). These bidirectional organellar interactions involve the re-routing of reducing power generated by photosynthesis in the plastids to mitochondria and the import of ATP produced in the mitochondria to the plastids.

An additional remarkable feature of microalgae is the presence of flavodiiron (Flv) proteins which are involved in light-dependent electron flow. They catalyze O<sub>2</sub> reduction and thereby prevent oxidative damage when the photosynthetic electron transport chain is over-reduced. These pro-

teins are also found in cyanobacteria and mosses but not in angiosperms. Analysis of *Chlamydomonas* mutants with deficiencies in Flv proteins by chlorophyll fluorescence and oxygen exchange measurement using [<sup>18</sup>O]-labeled O<sub>2</sub> and mass spectrometry revealed that these proteins indeed participate in the photoreduction of oxygen during the induction phase of photosynthesis following a transition from the dark to the light when the Calvin-Benson-Bassham cycle is not yet activated (Chaux et al. 2017).

### C. PSII Repair Cycle

Water splitting by PSII is one of the strongest oxidizing reactions which occurs in living organisms. As a result, photodamage to PSII is unavoidable. A remarkable feature of this system is that it is efficiently repaired (Nixon et al. 2010). PSII exists as a dimer in which each monomer consists of 28 subunits generally associated with two LHCII trimers in a supercomplex (Dekker and Boekema 2005). The PSII core consists of the two reaction center polypeptides D1 and D2 which form a central heterodimer which acts as ligand for the chlorophyll dimer P680 and the other redox components including the quinones Q<sub>A</sub> and Q<sub>B</sub>, the primary and secondary electron acceptors. Amongst all PSII subunits, D1 is the major target of photodamage and needs to be specifically replaced. This process, called PSII repair cycle, involves the partial disassembly of the PSII supercomplex, the removal and degradation of the damaged D1 protein, its replacement by a newly synthesized copy and the reassembly of the PSII complex (Fig. 4.4) (Aro et al. 1993). An important feature of this repair cycle is that it is compartmentalized within the crowded thylakoid membrane (Puthiyaveetil et al. 2014). Whereas damage of D1 occurs in the stacked grana region where most of PSII is located, the replacement of this protein takes place in the stroma lamellae. Although the exact role of phosphorylation

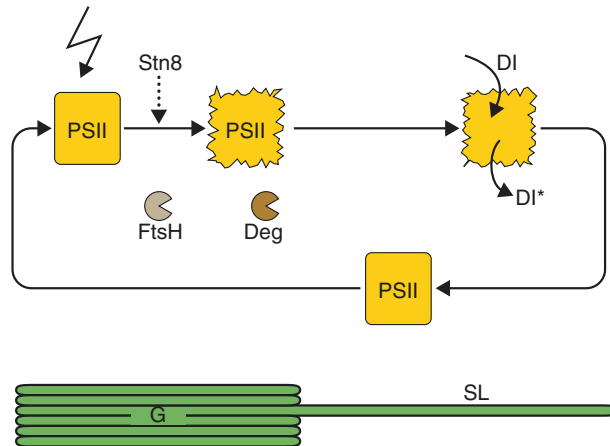


Fig. 4.4. PSII repair cycle

is not fully understood, the current view is that the PSII repair cycle starts with phosphorylation of the PSII core subunits D1, D2, CP43 and PsbH mediated by the STN8 kinase (Vainonen et al. 2005; Bonardi et al. 2005) which leads to the disassembly of the PSII-LHCII supercomplex thereby allowing PSII to move to the grana margins and stroma lamellae (Tikkanen et al. 2008; Herbstova et al. 2012). Dephosphorylation by the PSII core phosphatase Pbcp (Samol et al. 2012) and by other unknown phosphatases is followed by the degradation of D1 by the FtsH and Deg proteases and a newly synthesized D1 is co-translationally inserted into the PSII complex (Nixon et al. 2005). Finally the reassembled PSII complex moves back to the grana and reforms a supercomplex with LHCII. To make this cycle efficient, it is essential that the enzymes involved are confined to distinct thylakoid membrane subcompartments. Thus, the protein degradation occurs on the grana margins and protein synthesis on the stroma lamellae. In addition partial conversion of grana stacks to grana margins allows the proteases to access PSII (Puthiyaveetil et al. 2014).

High light illumination leads to photo-oxidative damage of the PSII reaction center, especially the D1 protein. The PSII core proteins are phosphorylated and the damaged

complex migrates from the grana (G) to the stroma lamellae (SL). The D1 protein is degraded by the FtsH and Deg proteases and upon its removal from the PSII reaction center a newly synthesized D1 protein is inserted co-translationally into the complex which moves back to the grana and thereby completes the repair cycle. Reproduced from (Rochaix 2014) with permission.

D1 degradation is significantly retarded in *stn8* and *stn7 stn8* mutants of *Arabidopsis* in which the thylakoid membrane architecture is affected (Fristedt et al. 2009). In the absence of STN8, grana diameter is increased and there are fewer grana stacks. This observation is particularly intriguing as it suggests that PSII core phosphorylation is important for maintaining grana size, a parameter which is highly conserved in land plants and algae (Kirchhoff et al. 2008). Loss of STN8 also affects partitioning of FtsH between grana and stroma membranes and limits its access to the grana, and migration of D1 from the grana to the stroma lamellae is slowed down during the PSII repair cycle (Fristedt et al. 2009). These observations are thus compatible with the view that PSII core phosphorylation has a strong impact on thylakoid membrane folding and architecture mediated most likely by electrostatic repulsion between membrane layers as proposed earlier (Barber 1982).

*Chlamydomonas* is able to acclimate specifically to singlet oxygen stress which occurs inevitably as a result of PSII activity. Genetic analysis identified a key regulator of the gene expression response of this acclimation process (Wakao et al. 2014). This protein, Sak1 (singlet oxygen acclimation knocked-out 1) encodes a protein of unknown function with a domain conserved in some bZIP transcription factors of chlorophytes. It is located in the cytosol, and induced and phosphorylated after exposure to singlet oxygen. It could thus represent an important intermediate component of the retrograde signal transduction pathway underlying singlet oxygen acclimation.

### III. Response of the Photosynthetic Apparatus to Micronutrient Depletion

The photosynthetic machinery comprises several protein-pigment complexes with specific cofactors including iron, copper, manganese and iron-sulfur centers. Under conditions of limitation of one of these micronutrients, the photosynthetic machinery displays a remarkable plasticity and ability to adapt to its new environment.

#### A. Copper Deficiency

When *Chlamydomonas* cells face copper deficiency, the copper-binding protein plastocyanin which acts as an essential electron carrier between the Cyt $b_6/f$  complex and PSI, is degraded and replaced with Cyt *c6* (Merchant and Bogorad 1987). In this way the cells can maintain photosynthetic electron flow. Besides Cyt *c6*, Cpx (coproporphyrinogen oxidase) is also induced by copper deficiency at the transcriptional level (Moseley et al. 2002). The increase of Cpx1 expression may meet the demand for heme, the cofactor of Cyt *c6*. Crd1, another target besides Cyt *c6* and Cpx1 of this signal transduction pathway responsive to copper deple-

tion, was identified through a genetic screen for a copper-conditional phenotype. Crd1 is a thylakoid diiron membrane protein which is required for the maintenance of PSI and LHCI in copper-deficient cells. It has an isoform, Cth1, which accumulates in copper-sufficient oxygenated cells whereas Crd1 accumulates in a reciprocal manner in copper-deficient cells or under anaerobiosis (Moseley et al. 2002). Crd1 and Cth1 are two isoforms of a subunit of the aerobic cyclase in chlorophyll biosynthesis with overlapping functions in the biosynthesis of Chl proteins (Tottey et al. 2003).

#### B. Iron Deficiency

Iron deficiency occurs often in nature and poses a challenge for photosynthetic organisms because of the abundance and importance of iron in the primary photosynthetic reactions. With its three 4Fe-4S centers PSI is a prime target under these conditions. Under conditions of iron limitation the level of PSI decreases when *Chlamydomonas* cells are grown in the presence of a carbon source such as acetate. Eventually, these Fe-deficient cells become chlorotic because of proteolytically-induced loss of both photosystems and Cyt *b<sub>6</sub>f* (Moseley et al. 2000). Before chlorosis occurs, a graded response is induced in which the LHCI antenna is dissociated from PSI. This dissociation appears to be caused by the decrease of the amount of the peripheral chlorophyll-binding PsaK subunit of PSI which is required for the functional connection of LHCI to PSI. Interestingly loss of Crd1, the Fe-requiring aerobic oxidative cyclase in copper-sufficient cells, also leads to a lower accumulation of PsaK and to uncoupling of LHCI from PSI. It was proposed that a change in plastid iron content is sensed by the diiron enzyme Crd1 through the occupancy of its Fe-containing active site which determines its activity (Moseley et al. 2000). In turn this would affect the flux through the chlorophyll biosynthetic pathway and PsaK

stability. This response to Fe deficiency and also to light quantity and quality further involves a remodeling of the antenna complexes with the degradation of specific subunits and the synthesis of new ones leading to a new state of the photosynthetic apparatus which allows for optimal photosynthetic function and minimal photooxidative damage. The protective value of this antenna remodelling is further confirmed by the observation that the light sensitivity of a PsaF-deficient mutant (Farah et al. 1995) is alleviated in a *psaF-crd1* double mutant (Moseley et al. 2002). The proposed mechanism can be placed within a general framework for explaining the causal link between chlorosis induced by iron deficiency and loss of photosynthetic function.

Marine organisms can face iron limitation in the oceans. A deep-sea/low light strain of the marine green alga *Ostreococcus* has lower photosynthetic activity due to the limited accumulation of PSI (Cardol et al. 2008). Interestingly in this strain electron flow from PSII is shuttled to a plastid plastoquinol terminal oxidase thereby bypassing electron transfer through the *Cyt<sub>b</sub><sub>6</sub>f* complex. This water-to-water cycle allows for the pumping of additional protons to the lumen thylakoid space and thus facilitates ATP production and enhances qE in the case of absorption of excess light excitation energy.

Micronutrient limitation can also act at the level of the biosynthesis of the photosynthetic apparatus which is mediated by the concerted action of the nuclear and chloroplast genetic systems. It is well established that subunits of the photosynthetic complexes originate from these two systems. In addition a large number of nucleus-encoded factors are required for chloroplast gene expression that act at various plastid post-transcriptional steps comprising RNA processing and stability, translation and assembly of the photosynthetic complexes. Many of these factors have unique gene targets in the plastid and often interact directly or indirectly with specific 5'-untranslated

RNA sequences (Eberhard et al. 2008). One of these factors, Taa1 is specifically required for the translation of the PsaA PSI reaction center subunit in *C. reinhardtii* (Lefebvre-Legendre et al. 2015). Under iron limitation, this protein is down-regulated through a post-transcriptional process and it re-accumulates upon restoration of iron. Another recently identified factor is Mac1 which is necessary for stabilization of the *psaC* mRNA (Douchi et al. 2016). Under iron limitation both Mac1 protein and *psaC* mRNA are reduced two-fold and PsaC and PSI are destabilized. Thus PSI abundance appears to be regulated by iron availability through at least two of these nucleus-encoded plastid factors specifically involved in PSI biosynthesis. Another intriguing observation is that Mac1 is differentially phosphorylated in response to changes in the redox state of the electron transport chain raising questions to what extent post-translational protein changes modulate the assembly of photosynthetic complexes.

Similar findings have been reported for Mca1 and Tca1, two nucleus-encoded proteins that are required for the stability and translation of the *petA* mRNA encoding the Cyt *f* subunit in *C. reinhardtii*. Nitrogen deprivation leads to the proteolytic degradation of these factors and in turn to the loss of the Cyt *b<sub>6</sub>f* complex (Boulouis et al. 2011; Raynaud et al. 2007). The response to nitrogen starvation also involves other factors required for the assembly of the Cyt *b<sub>6</sub>f* complex and its hemes (Wei et al. 2014).

### C. Sulfur Deprivation and Hydrogen Production

Many soil-dwelling algae like *Chlamydomonas* experience anoxic conditions especially during the night and are able to rapidly acclimate to anaerobiosis by shifting from aerobic to fermentative metabolism and can thus sustain energy production in the absence of photosynthesis (Gfeller and Gibbs 1984, 1985; Grossman et al. 2010). These anaero-

bic conditions lead to the expression of the oxygen-sensitive hydrogenase which catalyzes the production of hydrogen from protons and electrons derived from the photosynthetic electron transport chain. Sulfur deprivation of *Chlamydomonas* cells leads to a significant decline in photosynthetic activity within 24 h although there is no proportional concomitant decline in the levels of the major photosynthetic complexes (Yildiz et al. 1994; Davies et al. 1994). This decline in electron transport activity is due to the conversion of PSII centers from the  $Q_B$ -reducing to a  $Q_B$  non-reducing center (Wykoff et al. 1998). This system has been used for improving hydrogen production in *Chlamydomonas* cells (Melis et al. 2000). These cells as well other microalgal species possess a chloroplast (FeFe)-hydrogenase which acts as an additional sink when the photosynthetic electron transport chain is over-reduced under anaerobic conditions. Upon sulfur deprivation photosynthetic oxygen evolution decreases whereas respiration is maintained resulting in an anaerobic environment in a closed culture system. Although the exact physiological role of algal hydrogenases is not known, they are likely to play a significant role in redox poise, photoprotection and fermentative energy production (Grossman et al. 2010).

#### D. Nitrogen Deprivation

In contrast to sulphur deprivation which results in PSII deficiency in *Chlamydomonas* (Wykoff et al. 1998), nitrogen deprivation under heterotrophic growth conditions leads to the specific depletion of Rubisco and the *Cytb<sub>6</sub>f* complex due to active proteolysis of this complexes by the Clp and FtsH proteases (Bulte and Wollman 1992; Majeran et al. 2000). These conditions lead to a significant increase in photorespiratory enzymes and to the conversion of thylakoid membranes into a matrix for oxidative catabolism of reductants. Besides *Cytb<sub>6</sub>f* several factors involved in its biogenesis are also degraded

under nitrogen deprivation. These protein degradations were proposed to be triggered by the intracellular production of nitric oxide (NO) generated by rerouting of nitrite during nitrogen starvation (Wei et al. 2014). Indeed, addition of NO donors or of nitrite, the most likely donor of NO, during nitrogen starvation enhanced *Cytb<sub>6</sub>f* degradation whereas NO scavengers had the opposite effect (Wei et al. 2014). These proteolytic processes only occur in the presence of a reduced carbon source such as acetate, but not when respiration is impaired or under phototrophic growth conditions which require a functional photosynthetic apparatus. An important future task will be to elucidate the underlying signalling pathway especially as the overall process has implications for biofuel production in microalgae.

#### IV. Long Term Response: Changes in Nuclear and Chloroplast Gene Expression

While short term responses of the photosynthetic apparatus involve mostly post-translational mechanisms such as phosphorylation or changes in pH and ion levels, long term responses are mediated through changes in the expression of specific chloroplast and nuclear genes and their products. Environmental changes such as changes in light quantity and quality lead to changes in the state of the chloroplast which are perceived by the nucleus through a signalling chain referred to as retrograde signalling. The components of this signalling chain are still largely unknown although a few potential retrograde signals have been identified (Woodson and Chory 2012). Amongst these, tetrapyrroles appear to play a significant role. These compounds are involved in the chlorophyll biosynthetic pathway which needs to be tightly regulated to avoid photo-oxidative damage. Mg-protoporphyrin IX (Mg-Proto) was first shown to be involved in the repression of the LHCII genes in retrograde signal-

ling in *Chlamydomonas* (Johanningmeier and Howell 1984). However such a role for this tetrapyrrole in land plants gave rise to contradictory results and has been questioned (Strand et al. 2003; Mochizuki et al. 2008; Moulin et al. 2008). In contrast feeding experiments with Mg-Proto and hemin in *Chlamydomonas* induce expression of the gene of HemA (glutamyl-tRNA reductase) and of the heat shock proteins Hsp70A, Hsp70B and Hsp70E (Kropat et al. 1997, 2000; von Gromoff et al. 2008). In this alga both Mg-Proto and hemin are exclusively synthesized in the chloroplast. Genome-wide transcriptional profiling revealed that their exogenous addition to *Chlamydomonas* cells elicit transient changes in the expression of almost 1000 genes (Voss et al. 2011). They include only few genes of photosynthetic proteins but several genes of enzymes of the tricarboxylic acid cycle, heme-binding proteins, stress-responsive proteins and proteins involved in protein folding and degradation. Because these tetrapyrroles are not present in the natural environment of the algae, it is likely that these two tetrapyrroles act as secondary messengers for adaptive responses affecting not only organellar proteins but the entire cell. It is noticeable that these large changes in mRNA levels are not matched by similar changes in protein amount (Voss et al. 2011).

The synthesis of tetrapyrroles needs to be tightly controlled because some of these chlorophyll or heme precursors are very photodynamic and can cause serious photo-oxidative damage. In land plants the conversion of protochlorophyllide (PChlide) to chlorophyllide (Chlide) is light-dependent. In the dark, overaccumulation of PChlide is prevented through a negative feedback mediated by the Flu protein which inhibits glutamyl-tRNA reductase at an early step of the tetrapyrrole pathway (Fig. 4.5) (Meskauskiene et al. 2001). Although *Chlamydomonas* is able to synthesize chlorophyll in the dark, it also contains a Flu-like gene called Flp which gives rise to two tran-

scripts by alternative splicing (Falciatore et al. 2005). The relative levels of the two corresponding Flp proteins correlates with the accumulation of specific porphyrin intermediates some of which have been implicated in a signalling chain from the chloroplast to the nucleus. Moreover, decreased levels of the Flp proteins lead to the accumulation of several porphyrin intermediates and to photobleaching when *Chlamydomonas* cells are transferred from the dark to the light. These Flp proteins therefore appear to act as regulators of chlorophyll synthesis and their expression is controlled both by light and plastid signals.

The heme and chlorophyll biosynthetic pathway branch at protoporphyrin IX (Prot IX). GTR, glutamyl tRNA reductase, is subjected to feedback inhibition by heme and FLU. In most land plants conversion of PChlide (protochlorophyllide) to Chlide is light-dependent (in *Chlamydomonas* this conversion also occurs in the dark (*D*)). Through its negative feedback on GTR, FLU prevents overaccumulation of PChlide in the dark. The steps affected by the *gun* and *hy* mutations which affect retrograde signaling, are indicated. *GSA*, glutamate 1-semialdehyde; *ALA*, 5-aminolevulinic acid; *Chl*, chlorophyll; *FC*, ferrochelatase, *HMOX1*, heme oxygenase; *BV*, biliverdin; *PCY*, bilin reductase; *PCB*, phytocyanobilin. Reproduced from (Rochaix 2013) with permission.

Additional evidence for the involvement of the tetrapyrrole biosynthetic pathway in retrograde signalling comes from the identification of a functional bilin biosynthesis pathway in *Chlamydomonas* (Duanmu et al. 2013). In this pathway protoporphyrin IX is converted to protoheme and Mg-Proto by Fe- and Mg-chelatase, respectively. While heme is used as prosthetic group for many hemoproteins, a portion of heme is converted to biliverdin IXa by heme oxygenase (Hmox1) and in the next step by a ferredoxin-dependent phytychromobilin synthase (PcyA) to phytychromobilin, which serves as chromophore of phytychromes (Fig. 4.5). However,



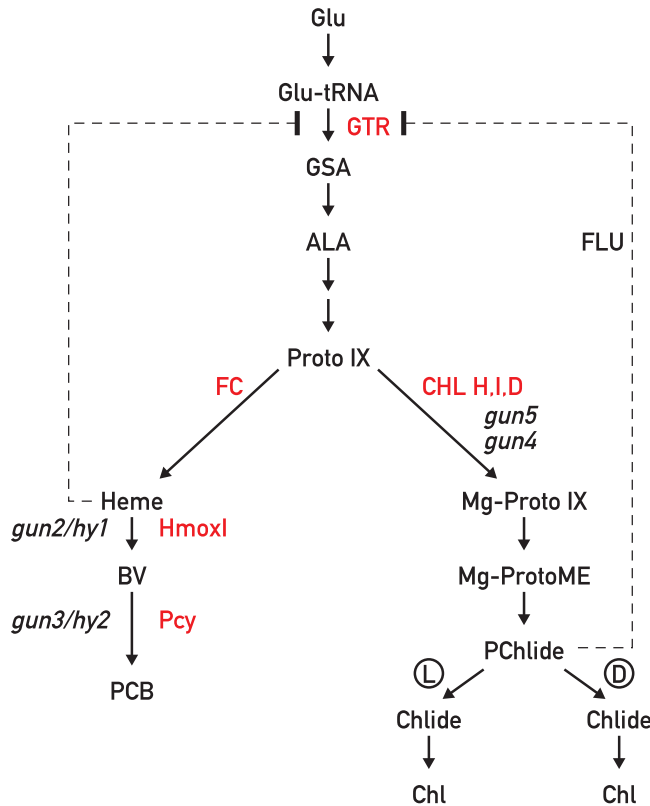


Fig. 4.5. Tetrapyrrole pathway

since *Chlamydomonas* as well as other chlorophytes do not produce phytochromes, the question arises about the role of this pathway in these algae. Some clues came from the analysis of a mutant of *Chlamydomonas* deficient in Hmox1 whose phototrophic growth is compromised and photoacclimates poorly upon a dark to light transition (Duanmu et al. 2013). Comparative transcriptomic studies of wild-type and *hmox1* cells revealed a set of nuclear genes that are up-regulated by bilins and that comprise members of the SOUL heme binding protein family and stress-activated genes, but not genes involved in the biosynthesis of the photosynthetic system or of tetrapyrroles. Further analysis revealed that the poor photoacclimation of the *hmox1* mutant is due to the decreased light-dependent accumulation of PSI reaction center and of the light-harvesting antennae of PSII and PSI (Wittkopp

et al. 2017). Moreover the *hmox1* mutant could be rescued by exogenous biliverdin IXa, the bilin generated by Hmox1 through a blue light-dependent process that is independent of photosynthesis (Wittkopp et al. 2017). These results point to the existence of a bilin-based blue light-sensing system including a still unknown regulatory chromoprotein that operates together with a retrograde signaling pathway. This system appears to have evolved in chlorophytes for the detoxification of reactive oxygen species and conveys robustness to the photosynthetic apparatus during photoacclimation. It remains to be seen whether bilins assume additional roles in chlorophytes besides ensuring smooth daily transitions from dark to light with minimal photo-oxidative damage.

A further striking example of the action of tetrapyrroles as mediators for plastid-to-

nucleus-communication is the identification of a tetrapyrrole-regulated ubiquitin ligase for cell cycle coordination from organelle to nuclear DNA replication in the red alga *Cyanidioschyzon merolae* (Kobayashi et al. 2011, 2009; Tanaka and Hanaoka 2012).

Redox changes within the photosynthetic electron transport chain occur upon changes in light quality and quantity, CO<sub>2</sub> levels, nutrient availability and elevated temperature. As a result of unequal excitation of PSI and PSII or of insufficient electron acceptor capacity on the PSI acceptor side, the redox state of the plastoquinone pool is altered. In this case chloroplast gene expression is affected in land plants (Pfannschmidt et al. 1999) although the evidence is less convincing in algae. However in these organisms there is unambiguous evidence that nuclear gene expression is affected (Escoubas et al. 1995). A possible candidate for sensing the redox state of the plastoquinone pool is the chloroplast protein kinase Stt7/STN7 which is known to be activated when plastoquinol occupies the Qo site of the Cytb6f complex (Vener et al. 1997; Zito et al. 1999). During experiments in which plants were shifted from light preferentially absorbed by PSI to light preferentially absorbed by PSII, the expression levels of 937 genes changed significantly in *Arabidopsis* (Brautigam et al. 2009). 800 of these changes were dependent on Stn7 indicating that most of these genes are under redox control.

In all situations in which the redox poise of the plastoquinone pool is affected, the relative sizes of the PSII and PSI antennae play an important role. Several factors involved in antenna size were identified through a genetic screen in *Chlamydomonas* (Mitra et al. 2012). One of these factors Tla1 functions as a regulator of chlorophyll content and antenna size and is localized in the chloroplast envelope. In the *tla1* mutant, thylakoid membranes were disorganized, appressed grana membranes were lost and accumulation of the PSII core proteins was reduced (Mitra et al. 2012). The second iden-

tified factor Tla2 corresponds to FtsY required for insertion of proteins into thylakoid membranes (Kirst et al. 2012) and the third, Tla3 corresponds to SRP43, a component of chloroplast SRP, known to be essential for the integration of LHCII proteins into the thylakoid membrane (Mitra et al. 2012).

Another protein regulating antenna size in *Chlamydomonas* is Nab1, a cytoplasmic repressor of translation of specific Lcbm isoforms (Wobbe et al. 2009). By binding selectively to the mRNAs of these proteins with Lhcm6 mRNA as its principal target, it sequesters the RNA in translationally silent nucleoprotein complexes. The activity of Nab1 is regulated through a cysteine-based redox control and also by arginine methylation (Wobbe et al. 2009; Blifernez et al. 2011). This protein apparently senses the increased or decreased demand for LHCII protein synthesis through changes in the cytosolic redox state although the underlying molecular mechanisms are still unknown.

## V. Conclusions and Perspectives

The photosynthetic apparatus is a complex machinery consisting of several large protein-pigment complexes whose components are encoded by both nuclear and chloroplast genes. Thus, the biosynthesis of this system involves two distinct genetic systems which act in a coordinate manner. In nature photosynthetic organisms are subjected to continuous environmental changes and need to adapt so as to maintain optimal photosynthetic activity and to protect themselves from photo-oxidative damage. These processes can be grouped in short term and long-term responses. The first occur in the second to minute range and involve light-induced protein conformational changes, post-translational protein modifications, cell compartment-specific pH changes and ion fluxes across the chloroplast and thylakoid membranes. The second occur in the minute to hour range and involve changes in gene expression and protein accu-

mulation which depend on an intricate bilateral communication system between chloroplasts and nucleus. Many nuclear genes encoding chloroplast proteins have been identified which are required for chloroplast gene expression and act mainly at post-transcriptional steps. Some of these factors appear to act constitutively while others assume a regulatory role because they have short half-lives and their level varies greatly upon changes in environmental cues including light, temperature and nutrient availability. However the molecular mechanisms underlying the inter-compartmental communication between chloroplast, mitochondria and nucleus are still largely unknown although several retrograde signals have been identified. They involve specific compounds such as tetrapyrroles and isoprenoids as well as plastid protein synthesis, the redox state of the photosynthetic electron transport chain and ROS generated under specific stress conditions. Moreover, a complex signalling network is operating within chloroplasts comprising several protein kinases and phosphatases, ion channels, and specific metabolites which act as signals and for the communication between chloroplast and nucleus. However the signalling chains connecting these different components are still largely unknown and their identification remains an important challenge for future research.

The flexibility of the thylakoid membrane is truly remarkable. Although it is crowded with proteins, it still allows for efficient remodeling of the photosynthetic complexes especially in response to changes in the quality and quantity of light. Among these responses state transitions and non-photochemical quenching have been studied extensively and some of the underlying molecular mechanisms have been elucidated. However many questions remain open. We still do not fully understand how the Stt7/STN7 kinase which plays a central role in state transitions and chloroplast signaling is activated and inactivated as a result of per-

turbations of the chloroplast redox poise. From an evolutionary point of view, it is particularly interesting to compare these adaptive responses in different photosynthetic organisms such as plants, fresh water and marine algae and cyanobacteria. In this respect NPQ, the dissipation of excess excitation energy as heat in the light-harvesting systems of the photosystems is of great importance and it is widely used in the plant kingdom. Recent studies on NPQ in different photosynthetic organisms raise several questions regarding the evolution of this essential photoprotective mechanism. For example it is not clear why the Lhcsr proteins were lost during the transition from aquatic to land plants. Moreover the qE process in most algae derived by secondary endosymbiosis from a red algal ancestor differs from that in extant red algae. All of these derived algae possess a xanthophyll cycle and Lhcsr-related proteins which are apparently absent in red algae (Goss and Lepetit 2015) and which have been suggested to be derived from green algae (Frommolt et al. 2008; Moustafa et al. 2009). It will clearly be important and challenging to elucidate these evolutionary puzzles.

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## References

- Albertsson P (2001) A quantitative model of the domain structure of the photosynthetic membrane. *Trends Plant Sci* 6(8):349–358
- Alboresi A, Gerotto C, Giacometti GM, Bassi R, Morosinotto T (2010) *Physcomitrella patens* mutants affected on heat dissipation clarify the evolution of photoprotection mechanisms upon land colonization. *Proc Natl Acad Sci U S A* 107(24):11128–11133

- Allahverdiyeva Y, Isojarvi J, Zhang P, Aro EM (2015) Cyanobacterial oxygenic photosynthesis is protected by flavodiiron proteins. *Life (Basel)* 5(1):716–743
- Allen JF (2003) Cyclic, pseudocyclic and noncyclic photophosphorylation: new links in the chain. *Trends Plant Sci* 8:15–19
- Allouret G, Petroustos D (2017) Photoreceptor-dependent regulation of photoprotection. *Curr Opin Plant Biol* 37:102–108
- Allouret G, Tokutsu R, Roach T, Peers G, Cardol P, Girard-Bascou J et al (2013) A dual strategy to cope with high light in *Chlamydomonas reinhardtii*. *Plant Cell* 25(2):545–557
- Allouret G, Lefebvre-Legendre L, Chappuis R, Kuntz M, Truong TB, Niyogi KK et al (2016) UV-B photoreceptor-mediated protection of the photosynthetic machinery in *Chlamydomonas reinhardtii*. *Proc Natl Acad Sci U S A* 113(51):14864–14869
- Amann K, Lezhneva L, Wanner G, Herrmann RG, Meurer J (2004) Accumulation of photosystem one1, a member of a novel gene family, is required for accumulation of [4Fe-4S] cluster-containing chloroplast complexes and antenna proteins. *Plant Cell* 16(11):3084–3097
- Amarnath K, Zaks J, Park SD, Niyogi KK, Fleming GR (2012) Fluorescence lifetime snapshots reveal two rapidly reversible mechanisms of photoprotection in live cells of *Chlamydomonas reinhardtii*. *Proc Natl Acad Sci U S A* 109(22):8405–8410
- Andersson B, Andersson J (1980) Lateral heterogeneity in the distribution of chlorophyll-protein complexes of the thylakoid membranes of spinach chloroplasts. *Biochim Biophys Acta* 593(2):427–440
- Aro EM, Virgin I, Andersson B (1993) Photoinhibition of photosystem II. Inactivation, protein damage and turnover. *Biochim Biophys Acta* 1143(2):113–134
- Bailleul B, Rogato A, de Martino A, Coesel S, Cardol P, Bowler C et al (2010) An atypical member of the light-harvesting complex stress-related protein family modulates diatom responses to light. *Proc Natl Acad Sci U S A* 107(42):18214–18219
- Bailleul B, Berne N, Murik O, Petroustos D, Prihoda J, Tanaka A et al (2015) Energetic coupling between plastids and mitochondria drives CO<sub>2</sub> assimilation in diatoms. *Nature* 524(7565):366–369
- Barber J (1982) The control of membrane organization by electrostatic forces. *Biosci Rep* 2(1):1–13
- Barneche F, Winter V, Crevecoeur M, Rochaix JD (2006) ATAB2 is a novel factor in the signalling pathway of light-controlled synthesis of photosystem proteins. *EMBO J* 25(24):5907–5918. Epub 2006 Nov 30
- Bellaïre S, Barneche F, Peltier G, Rochaix JD (2005) State transitions and light adaptation require chloroplast thylakoid protein kinase STN7. *Nature* 433:892–895
- Bergantino E, Segalla A, Brunetta A, Teardo E, Rigoni F, Giacometti GM et al (2003) Light- and pH-dependent structural changes in the PsbS subunit of photosystem II. *Proc Natl Acad Sci U S A* 100(25):15265–15270
- Betterle N, Ballottari M, Zorzan S, de Bianchi S, Cazzaniga S, Dall’osto L et al (2009) Light-induced dissociation of an antenna hetero-oligomer is needed for non-photochemical quenching induction. *J Biol Chem* 284(22):15255–15266
- Blifernez O, Wobbe L, Niehaus K, Kruse O (2011) Protein arginine methylation modulates light-harvesting antenna translation in *Chlamydomonas reinhardtii*. *Plant J* 65(1):119–130
- Bonardi V, Pesaresi P, Becker T, Schleiff E, Wagner R, Pfannschmidt T et al (2005) Photosystem II core phosphorylation and photosynthetic acclimation require two different protein kinases. *Nature* 437(7062):1179–1182
- Bonente G, Ballottari M, Truong TB, Morosinotto T, Ahn TK, Fleming GR et al (2011) Analysis of LhcSR3, a protein essential for feedback de-excitation in the green alga *Chlamydomonas reinhardtii*. *PLoS Biol* 9(1):e1000577
- Boulouis A, Raynaud C, Bujaldon S, Aznar A, Wollman FA, Choquet Y (2011) The nucleus-encoded transacting factor MCA1 plays a critical role in the regulation of cytochrome f synthesis in *Chlamydomonas* chloroplasts. *Plant Cell* 23(1):333–349
- Brautigam K, Dietzel L, Kleine T, Stroher E, Wormuth D, Dietz KJ et al (2009) Dynamic plastid redox signals integrate gene expression and metabolism to induce distinct metabolic states in photosynthetic acclimation in *Arabidopsis*. *Plant Cell* 21(9):2715–2732
- Breyton C (2000) Conformational changes in the cytochrome b6f complex induced by inhibitor binding. *J Biol Chem* 275(18):13195–13201
- Bulte L, Wollman FA (1992) Evidence for a selective destabilization of an integral membrane protein, the cytochrome b6/f complex, during gametogenesis in *Chlamydomonas reinhardtii*. *Eur J Biochem* 204(1):327–336
- Bulté L, Gans P, Rebeille F, Wollman FA (1990) ATP control on state transitions in *Chlamydomonas*. *Biochim Biophys Acta* 1020:72–80
- Burrows PA, Sazanov LA, Svab Z, Maliga P, Nixon PJ (1998) Identification of a functional respiratory complex in chloroplasts through analysis of tobacco mutants containing disrupted plastid *ndh* genes. *EMBO J* 17(4):868–876

- Cardol P, Bailleul B, Rappaport F, Derelle E, Beal D, Breyton C et al (2008) An original adaptation of photosynthesis in the marine green alga *Ostreococcus*. *Proc Natl Acad Sci U S A* 105(22):7881–7886
- Chaux F, Burlacot A, Mekhalfi M, Auroy P, Blangy S, Richaud P et al (2017) Flavodiiron proteins promote fast and transient O<sub>2</sub> photo-reduction in *Chlamydomonas*. *Plant Physiol* 174(3):1825–1836
- Christie JM, Arvai AS, Baxter KJ, Heilmann M, Pratt AJ, O'Hara A et al (2012) Plant UVR8 photoreceptor senses UV-B by tryptophan-mediated disruption of cross-dimer salt bridges. *Science* 335(6075):1492–1496
- Correa-Galvis V, Poschmann G, Melzer M, Stuhler K, Jahns P (2016) PsbS interactions involved in the activation of energy dissipation in *Arabidopsis*. *Nat Plants* 2:15225
- DalCorso G, Pesaresi P, Masiero S, Aseeva E, Schunemann D, Finazzi G et al (2008) A complex containing PGRL1 and PGR5 is involved in the switch between linear and cyclic electron flow in *Arabidopsis*. *Cell* 132(2):273–285
- Darrouzet E, Moser CC, Dutton PL, Daldal F (2001) Large scale domain movement in cytochrome bc(1): a new device for electron transfer in proteins. *Trends Biochem Sci* 26(7):445–451
- Davies JP, Yildiz F, Grossman AR (1994) Mutants of *Chlamydomonas* with aberrant responses to sulfur deprivation. *Plant Cell* 6(1):53–63
- de Lacroix de Lavalette A, Finazzi G, Zito F (2008) b6f-Associated chlorophyll: structural and dynamic contribution to the different cytochrome functions. *Biochemistry* 47:5259–5265
- Dekker JP, Boekema EJ (2005) Supramolecular organization of thylakoid membrane proteins in green plants. *Biochim Biophys Acta* 1706(1–2):12–39
- Demmig-Adams B, Adams W-W (1992) Photoprotection and other responses of plants to high light stress. *Annu Rev Plant Physiol Plant Mol Biol* 43:599–626
- Depège N, Bellafiore S, Rochaix JD (2003) Role of chloroplast protein kinase Stt7 in LHCII phosphorylation and state transition in *Chlamydomonas*. *Science* 299:1572–1575
- Douchi D, Qu Y, Longoni P, Legendre-Lefebvre L, Johnson X, Schmitz-Linneweber C et al (2016) A nucleus-encoded chloroplast phosphoprotein governs expression of the photosystem I subunit PsuC in *Chlamydomonas reinhardtii*. *Plant Cell* 28(5):1182–1199
- Drop B, Webber-Birungi M, Yadav SK, Filipowicz-Szymanska A, Fusetti F, Boekema EJ et al (2014) Light-harvesting complex II (LHCII) and its supra-molecular organization in *Chlamydomonas reinhardtii*. *Biochim Biophys Acta* 1837(1):63–72
- Du JJ, Zhan CY, Lu Y, Cui HR, Wang XY (2015) The conservative cysteines in transmembrane domain of AtVKOR/LTO1 are critical for photosynthetic growth and photosystem II activity in *Arabidopsis*. *Front Plant Sci* 6:238
- Duanmu D, Rockwell NC, Casero D, Dent RM, Gallaher S, Yang W et al (2013) Retrograde bilin signaling enables *Chlamydomonas* greening and phototrophic survival. *Proc Natl Acad Sci U S A* 110(9):3621–3626
- Dumas L, Zito F, Blangy S, Auroy P, Johnson X, Peltier G et al (2017) A stromal region of cytochrome b6f subunit IV is involved in the activation of the Stt7 kinase in *Chlamydomonas*. *Proc Natl Acad Sci U S A* 114(45):12063–12068
- Eberhard S, Finazzi G, Wollman FA (2008) The dynamics of photosynthesis. *Annu Rev Genet* 42:463–515
- Elrad D, Niyogi KK, Grossman AR (2002) A major light-harvesting polypeptide of photosystem II functions in thermal dissipation. *Plant Cell* 14(8):1801–1816
- Escoubas JM, Lomas M, LaRoche J, Falkowski PG (1995) Light intensity regulation of cab gene transcription is signaled by the redox state of the plastoquinone pool. *Proc Natl Acad Sci U S A* 92(22):10237–10241
- Falciatore A, Merendino L, Barneche F, Ceol M, Meskauskiene R, Apel K et al (2005) The FLP proteins act as regulators of chlorophyll synthesis in response to light and plastid signals in *Chlamydomonas*. *Genes Dev* 19(1):176–187
- Fan M, Li M, Liu Z, Cao P, Pan X, Zhang H et al (2015) Crystal structures of the PsbS protein essential for photoprotection in plants. *Nat Struct Mol Biol* 22(9):729–735
- Farah J, Rappaport F, Choquet Y, Joliet P, Rochaix JD (1995) Isolation of a psaF-deficient mutant of *Chlamydomonas reinhardtii*: efficient interaction of plastocyanin with the photosystem I reaction center is mediated by the PsuF subunit. *EMBO J* 14(20):4976–4984
- Favory JJ, Stec A, Gruber H, Rizzini L, Oravecz A, Funk M et al (2009) Interaction of COP1 and UVR8 regulates UV-B-induced photomorphogenesis and stress acclimation in *Arabidopsis*. *EMBO J* 28(5):591–601
- Ferrante P, Ballottari M, Bonente G, Giuliano G, Bassi R (2012) LHCBM1 and LHCBM2/7 polypeptides, components of major LHCII complex, have distinct functional roles in photosynthetic antenna system of *Chlamydomonas reinhardtii*. *J Biol Chem* 287(20):16276–16288

- Finazzi G, Rappaport F, Furia A, Fleischmann M, Rochaix JD, Zito F et al (2002) Involvement of state transitions in the switch between linear and cyclic electron flow in *Chlamydomonas reinhardtii*. *EMBO Rep* 3(3):280–285. Epub 2002 Feb 15
- Fristedt R, Willig A, Granath P, Crèvecoeur M, Rochaix JD, Vener A (2009) Phosphorylation of photosystem II controls functional macroscopic folding of plant photosynthetic membranes. *Plant Cell*
- Frommolt R, Werner S, Paulsen H, Goss R, Wilhelm C, Zauner S et al (2008) Ancient recruitment by chromists of green algal genes encoding enzymes for carotenoid biosynthesis. *Mol Biol Evol* 25(12):2653–2667
- Galka P, Santabarbara S, Khuong TT, Degand H, Morsomme P, Jennings RC et al (2012) Functional analyses of the plant photosystem I-light-harvesting complex II supercomplex reveal that light-harvesting complex II loosely bound to photosystem II is a very efficient antenna for photosystem I in state II. *Plant Cell* 24(7):2963–2978
- Geider RJ, Delucia EH, Falkowski PG, Finzi J (2001) Primary productivity of planet earth: biological determinants and physical constraints in terrestrial and aquatic habitats. *Glob Chang Biol* 7:849–882
- Gerotto C, Alboresi A, Giacometti GM, Bassi R, Morosinotto T (2011) Role of PSBS and LHCSR in *Physcomitrella patens* acclimation to high light and low temperature. *Plant Cell Environ* 34(6):922–932
- Gerotto C, Alboresi A, Giacometti GM, Bassi R, Morosinotto T (2012) Coexistence of plant and algal energy dissipation mechanisms in the moss *Physcomitrella patens*. *New Phytol* 196(3):763–773
- Gfeller RP, Gibbs M (1984) Fermentative metabolism of *Chlamydomonas reinhardtii*: I. Analysis of fermentative products from starch in dark and light. *Plant Physiol* 75(1):212–218
- Gfeller RP, Gibbs M (1985) Fermentative metabolism of *Chlamydomonas reinhardtii*: II. Role of plastoquinone. *Plant Physiol* 77(2):509–511
- Goral TK, Johnson MP, Duffy CD, Brain AP, Ruban AV, Mullineaux CW (2012) Light-harvesting antenna composition controls the macrostructure and dynamics of thylakoid membranes in *Arabidopsis*. *Plant J* 69(2):289–301
- Goss R, Lepetit B (2015) Biodiversity of NPQ. *J Plant Physiol* 172:13–32
- Grossman AR, Catalanotti C, Yang W, Dubini A, Magneschi L, Subramanian V (2010) Multiple facets of anoxic metabolism and hydrogen production in the unicellular green alga *Chlamydomonas reinhardtii*. *New Phytol.* <https://doi.org/10.1111/j.1469-8137.2010.03534.x>
- Gunning EBS, Schwartz OM (1999) Confocal microscopy of thylakoid autofluorescence in relation to origin of grana and phylogeny in the green algae. *Aust J Plant Physiol* 26:695–708
- Herbstova M, Tietz S, Kinzel C, Turkina MV, Kirchhoff H (2012) Architectural switch in plant photosynthetic membranes induced by light stress. *Proc Natl Acad Sci U S A* 109(49):20130–20135
- Hertle AP, Blunder T, Wunder T, Pesaresi P, Pribil M, Armbruster U et al (2013) PGRL1 is the elusive ferredoxin-plastoquinone reductase in photosynthetic cyclic electron flow. *Mol Cell* 49(3):511–523
- Holwarth AR, Miloslavina Y, Nilkens M, Jahns P (2009) Identification of two quenching sites active in the regulation of photosynthetic light-harvesting studies by time-resolved fluorescence. *Chem Phys Lett* 483:262–267
- Horton P, Johnson MP, Perez-Bueno ML, Kiss AZ, Ruban AV (2008) Photosynthetic acclimation: does the dynamic structure and macro-organisation of photosystem II in higher plant grana membranes regulate light harvesting states? *FEBS J* 275(6):1069–1079
- Houille-Vernes L, Rappaport F, Wollman FA, Alric J, Johnson X (2011) Plastid terminal oxidase 2 (PTOX2) is the major oxidase involved in chlororespiration in *Chlamydomonas*. *Proc Natl Acad Sci U S A* 108(51):20820–20825
- Iwai M, Takizawa K, Tokutsu R, Okamuro A, Takahashi Y, Minagawa J (2010) Isolation of the elusive supercomplex that drives cyclic electron flow in photosynthesis. *Nature* 464(7292):1210–1213
- Johanningmeier U, Howell SH (1984) Regulation of light-harvesting chlorophyll-binding protein mRNA accumulation in *Chlamydomonas reinhardtii*. Possible involvement of chlorophyll synthesis precursors. *J Biol Chem* 259(21):13541–13549
- Johnson MP, Ruban AV (2011) Restoration of rapidly reversible photoprotective energy dissipation in the absence of PsbS protein by enhanced DeltapH. *J Biol Chem* 286(22):19973–19981
- Johnson X, Steinbeck J, Dent RM, Takahashi H, Richaud P, Ozawa S et al (2014) Proton gradient regulation 5-mediated cyclic electron flow under ATP- or redox-limited conditions: a study of DeltaATPase pgr5 and DeltarbcL pgr5 mutants in the green alga *Chlamydomonas reinhardtii*. *Plant Physiol* 165(1):438–452
- Joliet P, Johnson GN (2011) Regulation of cyclic and linear electron flow in higher plants. *Proc Natl Acad Sci U S A* 108(32):13317–13322
- Kaiserli E, Jenkins GI (2007) UV-B promotes rapid nuclear translocation of the *Arabidopsis* UV-B spe-

- cific signaling component UVR8 and activates its function in the nucleus. *Plant Cell* 19(8):2662–2673
- Karamoko M, Cline S, Redding K, Ruiz N, Hamel PP (2011) Lumen Thiol Oxidoreductase1, a disulfide bond-forming catalyst, is required for the assembly of photosystem II in Arabidopsis. *Plant Cell* 23(12):4462–4475
- Keeling PJ (2013) The number, speed, and impact of plastid endosymbioses in eukaryotic evolution. *Annu Rev Plant Biol* 64:583–607
- Khorobrykh SA, Karonen M, Tyystjarvi E (2015) Experimental evidence suggesting that H<sub>2</sub>O<sub>2</sub> is produced within the thylakoid membrane in a reaction between plastoquinol and singlet oxygen. *FEBS Lett* 589(6):779–786
- Kirchhoff H, Haferkamp S, Allen JF, Epstein DB, Mullineaux CW (2008) Protein diffusion and macromolecular crowding in thylakoid membranes. *Plant Physiol* 146(4):1571–1578
- Kirst H, Garcia-Cerdan JG, Zurbriggen A, Melis A (2012) Assembly of the light-harvesting chlorophyll antenna in the green alga *Chlamydomonas reinhardtii* requires expression of the TLA2-CpF<sub>TSY</sub> gene. *Plant Physiol* 158(2):930–945
- Kobayashi Y, Kanesaki Y, Tanaka A, Kuroiwa H, Kuroiwa T, Tanaka K (2009) Tetrapyrrole signal as a cell-cycle coordinator from organelle to nuclear DNA replication in plant cells. *Proc Natl Acad Sci U S A* 106(3):803–807
- Kobayashi Y, Imamura S, Hanaoka M, Tanaka K (2011) A tetrapyrrole-regulated ubiquitin ligase controls algal nuclear DNA replication. *Nat Cell Biol* 13(4):483–487
- Kono M, Noguchi K, Terashima I (2014) Roles of the cyclic electron flow around PSI (CEF-PSI) and O(2)-dependent alternative pathways in regulation of the photosynthetic electron flow in short-term fluctuating light in Arabidopsis thaliana. *Plant Cell Physiol* 55(5):990–1004
- Kropat J, Oster U, Rudiger W, Beck CF (1997) Chlorophyll precursors are signals of chloroplast origin involved in light induction of nuclear heat-shock genes. *Proc Natl Acad Sci U S A* 94(25):14168–14172
- Kropat J, Oster U, Rudiger W, Beck CF (2000) Chloroplast signalling in the light induction of nuclear HSP70 genes requires the accumulation of chlorophyll precursors and their accessibility to cytoplasm/nucleus. *Plant J* 24(4):523–531
- Kruger TP, Ilioaia C, Johnson MP, Ruban AV, Papagiannakis E, Horton P et al (2012) Controlled disorder in plant light-harvesting complex II explains its photoprotective role. *Biophys J* 102(11):2669–2676
- Kurusu G, Zhang H, Smith JL, Cramer WA (2003) Structure of the cytochrome b<sub>6</sub>f complex of oxygenic photosynthesis: tuning the cavity. *Science* 302(5647):1009–1014. Epub 2003 Oct 2
- Lefebvre-Legendre L, Choquet Y, Kuras R, Loubery S, Douchi D, Goldschmidt-Clermont M (2015) A nucleus-encoded chloroplast protein regulated by iron availability governs expression of the photosystem I subunit PsaA in *Chlamydomonas reinhardtii*. *Plant Physiol* 167(4):1527–1540
- Lemeille S, Rochaix JD (2010) State transitions at the crossroad of thylakoid signalling pathways. *Photosynth Res* 106:33–46
- Lemeille S, Willig A, Depège-Fargeix N, Delessert C, Bassi R, Rochaix JD (2009) Analysis of the chloroplast protein kinase Stt7 during state transitions. *PLoS Biol* 7(3):e45
- Lennartz K, Plucken H, Seidler A, Westhoff P, Bechtold N, Meierhoff K (2001) HCF164 encodes a thioredoxin-like protein involved in the biogenesis of the cytochrome b(6)f complex in Arabidopsis. *Plant Cell* 13(11):2539–2551
- Li XP, Bjorkman O, Shih C, Grossman AR, Rosenquist M, Jansson S et al (2000) A pigment-binding protein essential for regulation of photosynthetic light harvesting. *Nature* 403(6768):391–395
- Li XP, Gilmore AM, Caffarri S, Bassi R, Golan T, Kramer D et al (2004) Regulation of photosynthetic light harvesting involves intrathylakoid lumen pH sensing by the PsbS protein. *J Biol Chem* 279(22):22866–22874
- Link G (2003) Redox regulation of chloroplast transcription. *Antioxid Redox Signal* 5:79–87
- Longoni P, Douchi D, Cariti F, Fucile G, Goldschmidt-Clermont M (2015) Phosphorylation of the light-harvesting complex II isoform Lhcb2 is central to state transitions. *Plant Physiol* 169(4):2874–2883
- Lucker B, Kramer DM (2013) Regulation of cyclic electron flow in *Chlamydomonas reinhardtii* under fluctuating carbon availability. *Photosynth Res* 117(1–3):449–459
- MacIntyre HL, Kana TM, Geider RJ (2000) The effect of water motion on short-term rates of photosynthesis by marine phytoplankton. *Trends Plant Sci* 5(1):12–17
- Majeran W, Wollman FA, Vallon O (2000) Evidence for a role of ClpP in the degradation of the chloroplast cytochrome b(6)f complex. *Plant Cell* 12(1):137–150
- Melis A, Zhang L, Forestier M, Ghirardi ML, Seibert M (2000) Sustained photobiological hydrogen gas production upon reversible inactivation of oxygen evolution in the green alga *Chlamydomonas reinhardtii*. *Plant Physiol* 122(1):127–136

- Merchant S, Bogorad L (1987) Metal ion regulated gene expression: use of a plastocyanin-less mutant of *Chlamydomonas reinhardtii* to study the Cu(II)-dependent expression of cytochrome c-552. *EMBO J* 6(9):2531–2535
- Meskauskiene R, Nater M, Goslings D, Kessler F, op den Camp R, Apel K (2001) FLU: a negative regulator of chlorophyll biosynthesis in *Arabidopsis thaliana*. *Proc Natl Acad Sci U S A* 98(22):12826–12831
- Miller R, Wu G, Deshpande RR, Vieler A, Gartner K, Li X et al (2010) Changes in transcript abundance in *Chlamydomonas reinhardtii* following nitrogen deprivation predict diversion of metabolism. *Plant Physiol* 154(4):1737–1752
- Minagawa J, Takahashi Y (2004) Structure, function and assembly of Photosystem II and its light-harvesting proteins. *Photosynth Res* 82(3):241–263
- Mitra M, Kirst H, Dewez D, Melis A (2012) Modulation of the light-harvesting chlorophyll antenna size in *Chlamydomonas reinhardtii* by TLA1 gene overexpression and RNA interference. *Philos Trans R Soc Lond Ser B Biol Sci* 367(1608):3430–3443
- Mochizuki N, Tanaka R, Tanaka A, Masuda T, Nagatani A (2008) The steady-state level of Mg-protoporphyrin IX is not a determinant of plastid-to-nucleus signaling in *Arabidopsis*. *Proc Natl Acad Sci U S A* 105(39):15184–15189
- Moseley J, Quinn J, Eriksson M, Merchant S (2000) The *Crd1* gene encodes a putative di-iron enzyme required for photosystem I accumulation in copper deficiency and hypoxia in *Chlamydomonas reinhardtii*. *EMBO J* 19(10):2139–2151
- Moseley JL, Allinger T, Herzog S, Hoerth P, Wehinger E, Merchant S et al (2002) Adaptation to Fe-deficiency requires remodeling of the photosynthetic apparatus. *EMBO J* 21(24):6709–6720
- Moulin M, McCormac AC, Terry MJ, Smith AG (2008) Tetrapyrrole profiling in *Arabidopsis* seedlings reveals that retrograde plastid nuclear signaling is not due to Mg-protoporphyrin IX accumulation. *Proc Natl Acad Sci U S A* 105(39):15178–15183
- Moustafa A, Beszteri B, Maier UG, Bowler C, Valentin K, Bhattacharya D (2009) Genomic footprints of a cryptic plastid endosymbiosis in diatoms. *Science* 324(5935):1724–1726
- Munekage Y, Hojo M, Meurer J, Endo T, Tasaka M, Shikanai T (2002) PGR5 is involved in cyclic electron flow around photosystem I and is essential for photoprotection in *Arabidopsis*. *Cell* 110(3):361–371
- Nandha B, Finazzi G, Joliot P, Hald S, Johnson GN (2007) The role of PGR5 in the redox poisoning of photosynthetic electron transport. *Biochim Biophys Acta* 1767(10):1252–1259
- Nield J, Kruse O, Ruprecht J, da Fonseca P, Buchel C, Barber J (2000) Three-dimensional structure of *Chlamydomonas reinhardtii* and *Synechococcus elongatus* photosystem II complexes allows for comparison of their oxygen-evolving complex organization. *J Biol Chem* 275(36):27940–27946
- Nixon PJ, Barker M, Boehm M, de Vries R, Komenda J (2005) FtsH-mediated repair of the photosystem II complex in response to light stress. *J Exp Bot* 56(411):357–363
- Nixon PJ, Michoux F, Yu J, Boehm M, Komenda J (2010) Recent advances in understanding the assembly and repair of photosystem II. *Ann Bot* 106(1):1–16
- Niyogi KK (1999) PHOTOPROTECTION REVISITED: genetic and molecular approaches. *Annu Rev Plant Physiol Plant Mol Biol* 50:333–359
- Niyogi KK, Truong TB (2013) Evolution of flexible non-photochemical quenching mechanisms that regulate light harvesting in oxygenic photosynthesis. *Curr Opin Plant Biol* 16(3):307–314
- Ogrzewalla K, Piotrowski M, Reinbothe S, Link G (2002) The plastid transcription kinase from mustard (*Sinapis alba* L.). A nuclear-encoded CK2-type chloroplast enzyme with redox-sensitive function. *Eur J Biochem* 269(13):3329–3337
- Ozawa SI, Bald T, Onishi T, Xue H, Matsumura T, Kubo R et al (2018) Configuration of ten light-harvesting chlorophyll a/b complex I subunits in *Chlamydomonas reinhardtii* photosystem I. *Plant Physiol* 178(2):583–595
- Page ML, Hamel PP, Gabilly ST, Zegzouti H, Perea JV, Alonso JM et al (2004) A homolog of prokaryotic thiol disulfide transporter CcdA is required for the assembly of the cytochrome b6f complex in *Arabidopsis* chloroplasts. *J Biol Chem* 279(31):32474–32482
- Pan X, Ma J, Su X, Cao P, Chang W, Liu Z et al (2018) Structure of the maize photosystem I supercomplex with light-harvesting complexes I and II. *Science* 360(6393):1109–1113
- Peers G, Truong TB, Ostendorf E, Busch A, Elrad D, Grossman AR et al (2009) An ancient light-harvesting protein is critical for the regulation of algal photosynthesis. *Nature* 462(7272):518–521
- Pfannschmidt T, Nilsson A, Tullberg A, Link G, Allen JF (1999) Direct transcriptional control of the chloroplast genes *psbA* and *psaAB* adjusts photosynthesis to light energy distribution in plants. *IUBMB Life* 48:271–276
- Phillip D, Ruban AV, Horton P, Asato A, Young AJ (1996) Quenching of chlorophyll fluorescence in the major light-harvesting complex of photosystem II:



- a systematic study of the effect of carotenoid structure. *Proc Natl Acad Sci U S A* 93(4):1492–1497
- Pribil M, Pesaresi P, Hertle A, Barbato R, Leister D (2010) Role of plastid protein phosphatase TAP38 in LHCII dephosphorylation and thylakoid electron flow. *PLoS Biol* 8(1):e1000288
- Puthiyaveetil S, Kavanagh TA, Cain P, Sullivan JA, Newell CA, Gray JC et al (2008) The ancestral symbiont sensor kinase CSK links photosynthesis with gene expression in chloroplasts. *Proc Natl Acad Sci U S A* 105(29):10061–10066
- Puthiyaveetil S, Tsabari O, Lowry T, Lenhart S, Lewis RR, Reich Z et al (2014) Compartmentalization of the protein repair machinery in photosynthetic membranes. *Proc Natl Acad Sci U S A* 111(44):15839–15844
- Raynaud C, Loiselay C, Wostrickoff K, Kuras R, Girard-Bascou J, Wollman FA et al (2007) Evidence for regulatory function of nucleus-encoded factors on mRNA stabilization and translation in the chloroplast. *Proc Natl Acad Sci U S A* 104(21):9093–9098
- Rintamaki E, Salonen M, Suoranta UM, Carlberg I, Andersson B, Aro EM (1997) Phosphorylation of light-harvesting complex II and photosystem II core proteins shows different irradiance-dependent regulation in vivo. Application of phosphothreonine antibodies to analysis of thylakoid phosphoproteins. *J Biol Chem* 272(48):30476–30482
- Rintamaki E, Martinsuo P, Pursiheimo S, Aro EM (2000) Cooperative regulation of light-harvesting complex II phosphorylation via the plastoquinol and ferredoxin-thioredoxin system in chloroplasts. *Proc Natl Acad Sci U S A* 97(21):11644–11649
- Rizzini L, Favory JJ, Cloix C, Faggionato D, O'Hara A, Kaiserli E et al (2011) Perception of UV-B by the Arabidopsis UVR8 protein. *Science* 332(6025):103–106
- Rochaix JD (2013) Redox regulation of thylakoid protein kinases and photosynthetic gene expression. *Antioxid Redox Signal* 18(16):2184–2201
- Rochaix JD (2014) Regulation and dynamics of the light-harvesting system. *Annu Rev Plant Biol* 65:287–309
- Ruban AV, Johnson MP, Duffy CD (2012) The photoprotective molecular switch in the photosystem II antenna. *Biochim Biophys Acta* 1817(1):167–181
- Samol I, Shapiguzov A, Ingelsson B, Fucile G, Crevecoeur M, Vener AV et al (2012) Identification of a photosystem II phosphatase involved in light acclimation in Arabidopsis. *Plant Cell* 24(6):2596–2609
- Schubert H, Forster RM (1997) Sources of variability in the factors used for modelling primary productivity in eutrophic waters. *Hydrobiologia* 349:75–85
- Schuster G, Dewit M, Staehelin LA, Ohad I (1986) Transient inactivation of the thylakoid photosystem II light-harvesting protein kinase system and concomitant changes in intramembrane particle size during photoinhibition of *Chlamydomonas reinhardtii*. *J Cell Biol* 103(1):71–80
- Shapiguzov A, Ingelsson B, Samol I, Andres C, Kessler F, Rochaix JD et al (2010) The PPH1 phosphatase is specifically involved in LHCII dephosphorylation and state transitions in Arabidopsis. *Proc Natl Acad Sci U S A* 107(10):4782–4787
- Shapiguzov A, Chai X, Fucile G, Longoni P, Zhang L, Rochaix JD (2016) Activation of the Stt7/STN7 kinase through dynamic interactions with the cytochrome b6f complex. *Plant Physiol* 171(1):82–92
- Shikanai T, Yamamoto H (2017) Contribution of cyclic and pseudo-cyclic electron transport to the formation of proton motive force in chloroplasts. *Mol Plant* 10(1):20–29
- Shimakawa G, Shaku K, Nishi A, Hayashi R, Yamamoto H, Sakamoto K et al (2015) FLAVODIIRON2 and FLAVODIIRON4 proteins mediate an oxygen-dependent alternative electron flow in *Synechocystis* sp. PCC 6803 under CO<sub>2</sub>-limited conditions. *Plant Physiol* 167(2):472–480
- Snyders S, Kohorn BD (1999) TAKs, thylakoid membrane protein kinases associated with energy transduction. *J Biol Chem* 274(14):9137–9140
- Snyders S, Kohorn BD (2001) Disruption of thylakoid-associated kinase 1 leads to alteration of light harvesting in Arabidopsis. *J Biol Chem* 276(34):32169–32176
- Steinbeck J, Ross IL, Rothnagel R, Gabelein P, Schulze S, Giles N et al (2018) Structure of a PSI-LHCI-cyt b6f supercomplex in *Chlamydomonas reinhardtii* promoting cyclic electron flow under anaerobic conditions. *Proc Natl Acad Sci U S A* 115(41):10517–10522
- Strand A, Asami T, Alonso J, Ecker JR, Chory J (2003) Chloroplast to nucleus communication triggered by accumulation of Mg-protoporphyrin IX. *Nature* 421(6918):79–83
- Stroebel D, Choquet Y, Popot JL, Picot D (2003) An atypical haem in the cytochrome b(6)f complex. *Nature* 426(6965):413–418
- Takahashi H, Clowez S, Wollman FA, Vallon O, Rappaport F (2013) Cyclic electron flow is redox-controlled but independent of state transition. *Nat Commun* 4:1954–1961
- Tanaka K, Hanaoka M (2012) The early days of plastid retrograde signaling with respect to replication and transcription. *Front Plant Sci* 3:301
- Teardo E, de Laureto PP, Bergantino E, Dalla Vecchia F, Rigoni F, Szabo I et al (2007) Evidences for

- interaction of PsbS with photosynthetic complexes in maize thylakoids. *Biochim Biophys Acta* 1767(6):703–711
- Tikkanen M, Nurmi M, Suorsa M, Danielsson R, Mamedov F, Styring S et al (2008) Phosphorylation-dependent regulation of excitation energy distribution between the two photosystems in higher plants. *Biochim Biophys Acta* 1777(5):425–432
- Tilbrook K, Dubois M, Crocco CD, Yin R, Chappuis R, Allorent G et al (2016) UV-B perception and acclimation in *Chlamydomonas reinhardtii*. *Plant Cell* 28(4):966–983
- Tokutsu R, Kato N, Bui KH, Ishikawa T, Minagawa J (2012) Revisiting the supramolecular organization of photosystem II in *Chlamydomonas reinhardtii*. *J Biol Chem* 287(37):31574–31581
- Tottey S, Block MA, Allen M, Westergren T, Albriex C, Scheller HV et al (2003) Arabidopsis CHL27, located in both envelope and thylakoid membranes, is required for the synthesis of protochlorophyllide. *Proc Natl Acad Sci U S A* 100(26):16119–16124
- Vainonen JP, Hansson M, Vener AV (2005) STN8 protein kinase in *Arabidopsis thaliana* is specific in phosphorylation of photosystem II core proteins. *J Biol Chem* 280(39):33679–33686
- Vener AV, van Kan PJ, Rich PR, Ohad II, Andersson B (1997) Plastoquinol at the quinol oxidation site of reduced cytochrome *b<sub>f</sub>* mediates signal transduction between light and protein phosphorylation: thylakoid protein kinase deactivation by a single-turnover flash. *Proc Natl Acad Sci U S A* 94(4):1585–1590
- von Gromoff ED, Alawady A, Meinecke L, Grimm B, Beck CF (2008) Heme, a plastid-derived regulator of nuclear gene expression in *Chlamydomonas*. *Plant Cell* 20(3):552–567
- Voss B, Meinecke L, Kurz T, Al-Babili S, Beck CF, Hess WR (2011) Hemin and magnesium-protoporphyrin IX induce global changes in gene expression in *Chlamydomonas reinhardtii*. *Plant Physiol* 155(2):892–905
- Wakao S, Chin BL, Ledford HK, Dent RM, Casero D, Pellegrini M et al (2014) Phosphoprotein SAK1 is a regulator of acclimation to singlet oxygen in *Chlamydomonas reinhardtii*. *elife* 3:e02286
- Wei L, Derrien B, Gautier A, Houille-Vernes L, Boulouis A, Saint-Marcoux D et al (2014) Nitric oxide-triggered remodeling of chloroplast bioenergetics and thylakoid proteins upon nitrogen starvation in *Chlamydomonas reinhardtii*. *Plant Cell* 26(1):353–372
- Wentworth M, Ruban AV, Horton P (2001) Kinetic analysis of nonphotochemical quenching of chlorophyll fluorescence. 2. Isolated light-harvesting complexes. *Biochemistry* 40(33):9902–9908
- Wientjes E, van Amerongen H, Croce R (2013a) LHCII is an antenna of both photosystems after long-term acclimation. *Biochim Biophys Acta* 1827(3):420–426
- Wientjes E, Drop B, Kouril R, Boekema EJ, Croce R (2013b) During state 1 to state 2 transition in *Arabidopsis thaliana*, the photosystem II supercomplex gets phosphorylated but does not disassemble. *J Biol Chem* 288(46):32821–32826
- Wittkopp TM, Schmollinger S, Saroussi S, Hu W, Zhang W, Fan Q et al (2017) Bilin-dependent photoacclimation in *Chlamydomonas reinhardtii*. *Plant Cell* 29(11):2711–2726
- Wobbe L, Blifernez O, Schwarz C, Mussgnug JH, Nickelsen J, Kruse O (2009) Cysteine modification of a specific repressor protein controls the translational status of nucleus-encoded LHCII mRNAs in *Chlamydomonas*. *Proc Natl Acad Sci U S A* 106(32):13290–13295
- Wollman FA (2001) State transitions reveal the dynamics and flexibility of the photosynthetic apparatus. *EMBO J* 20(14):3623–3630
- Woodson JD, Chory J (2012) Organelle signaling: how stressed chloroplasts communicate with the nucleus. *Curr Biol* 22(17):R690–R692
- Wunder T, Liu Q, Aseeva E, Bonardi V, Leister D, Pribil M (2013) Control of STN7 transcript abundance and transient STN7 dimerisation are involved in the regulation of STN7 activity. *Planta* 237(2):541–558
- Wykoff DD, Davies JP, Melis A, Grossman AR (1998) The regulation of photosynthetic electron transport during nutrient deprivation in *Chlamydomonas reinhardtii*. *Plant Physiol* 117(1):129–139
- Yildiz FH, Davies JP, Grossman AR (1994) Characterization of sulfate transport in *Chlamydomonas reinhardtii* during sulfur-limited and sulfur-sufficient growth. *Plant Physiol* 104(3):981–987
- Zhang P, Eisenhut M, Brandt AM, Carmel D, Silen HM, Vass I et al (2012) Operon *flv4-flv2* provides cyanobacterial photosystem II with flexibility of electron transfer. *Plant Cell* 24(5):1952–1971
- Zito F, Finazzi G, Delosme R, Nitschke W, Picot D, Wollman FA (1999) The Qo site of cytochrome *b<sub>6f</sub>* complexes controls the activation of the LHCII kinase. *EMBO J* 18(11):2961–2969
- Zones JM, Blaby IK, Merchant SS, Umen JG (2015) High-resolution profiling of a synchronized diurnal transcriptome from *Chlamydomonas reinhardtii* reveals continuous cell and metabolic differentiation. *Plant Cell* 27(10):2743–2769