Protein kinases and phosphatases involved in the acclimation of the photosynthetic apparatus to a changing light environment

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Photosynthetic organisms are subjected to frequent changes in light quality and quantity and need to respond accordingly. These acclimatory processes are mediated to a large extent through thylakoid protein phosphorylation. Recently, two major thylakoid protein kinases have been identified and characterized. The Stt7/STN7 kinase is mainly involved in the phosphorylation of the LHCII antenna proteins and is required for state transitions. It is firmly associated with the cytochrome b6f complex, and its activity is regulated by the redox state of the plastoquinone pool. The other kinase, Stl1/STN8, is responsible for the phosphorylation of the PSII core proteins. Using a reverse genetics approach, we have recently identified the chloroplast PPH1/TAP38 and PBPC protein phosphatases, which counteract the activity of STN7 and STN8 kinases, respectively. They belong to the PP2C-type phosphatase family and are conserved in land plants and algae. The picture that emerges from these studies is that of a complex regulatory network of chloroplast protein kinases and phosphatases that is involved in light acclimation, in maintenance of the plastoquinone redox poise under fluctuating light and in the adjustment to metabolic needs.

Keywords: photosynthesis; chloroplast; protein kinase; protein phosphatase; acclimation; state transitions

1. INTRODUCTION

Photosynthetic organisms have the remarkable ability to adapt rapidly to changes in light conditions. This is particularly true for plants and algae, which have developed specific mechanisms that allow them to act either as energy dissipators (when the light energy absorbed by the light-harvesting system exceeds the capacity of the photosynthetic system) or as energy collectors (when the absorbed light is limiting). In these organisms, the primary reactions of photosynthesis occur in the thylakoid membranes and are catalyzed by the photosynthetic complexes photosystem II (PSII), the cytochrome b6f complex (cyt b6f) and photosystem I (PSI). These three complexes form the photosynthetic electron transport chain in which two serially connected photochemical reactions in PSII and PSI lead to the oxidation of water, followed by electron flow through the plastoquinone pool, cyt b6f and plastocyanin, and ultimately to the reduction of ferredoxin. These processes are responsible for the formation of both reducing power and a proton gradient across the thylakoid membrane that is used to produce ATP through the ATP synthase complex. Thylakoid membranes are compartmentalized in grana regions consisting of appressed membranes and stromal lamellae that often connect grana stacks to one another. It has been known since the pioneering studies of Andersson & Andersson [1] that while PSII and LHCII (light-harvesting complex II) are mostly confined to the grana regions, PSI and ATP synthase are localized exclusively in the stroma lamellae because of their stromal domains, which prevent these complexes from entering the grana [2]. Cyt b6f is distributed equally between these two thylakoid domains.

When plants and algae are subjected to an irradiance that is in excess of that which can be used by photosynthesis, a large proton gradient is generated across the thylakoid membrane, which leads to the dissipation of the excess absorbed light energy into heat through non-photochemical quenching (for review see [3]). As an unavoidable consequence of its photochemical activity that generates one of the most oxidizing reactions observed in biological systems, PSII is damaged mainly at the level of the D1 reaction centre protein and needs to be repaired. Current evidence favours a model in which the damaged PSII moves out of the grana to the stromal lamellae where the damaged D1 protein is degraded through the...
concerted action of the FtsH and Deg proteases [4–6]. A newly synthesized D1 protein is then inserted into the PSII complex, which moves back to the grana region. This repair cycle requires a series of phosphorylation and dephosphorylation events at the level of the PSII core proteins D1, D2, CP43 and PsbH [7,8].

By contrast, when the light is limiting for growth, the photosynthetic machinery optimizes light capture and photosynthetic yield. Because the light-harvesting systems of PSII and PSI have distinct sizes and light absorption properties, their relative performance in light capture depends on both light quality and quantity. In particular, red and far-red light are preferentially absorbed by PSII and PSI, respectively. Thus, changes in the red/far-red ratio that occur under a canopy or through shading affect the relative yields of PSII and PSI. Under conditions that promote a preferential excitation of PSII relative to PSI, the redox state of the plastoquinone pool is more reduced. Binding of plastoquinol to the Qo site of cyt b$_6$f leads to the activation of a protein kinase that phosphorylates LHCII. The latter dissociates from PSII and migrates to PSI. If PSI is preferentially excited, the plastoquinone pool is oxidized, the kinase is inactivated and a phosphatase dephosphorylates the mobile LHCII, which moves back to PSII. (a) State 1 and (b) state 2 refer to the states in which the mobile LHCII is associated with PSII and PSI, respectively. Fd, ferredoxin; PC, plastocyanin. Reproduced with permission from Rochaix [9].

![Figure 1. Scheme of state transitions. State transitions involve a redistribution of the mobile light-harvesting antenna of PSII (LHCII) between PSII and PSI. Upon preferential excitation of PSII, the plastoquinone pool (PQ) is reduced and plastoquinol docks to the Qo site of cyt b$_6$f. This leads to the activation of a protein kinase that phosphorylates LHCII. The latter dissociates from PSII and migrates to PSI. If PSI is preferentially excited, the plastoquinone pool is oxidized, the kinase is inactivated and a phosphatase dephosphorylates the mobile LHCII, which moves back to PSII. (a) State 1 and (b) state 2 refer to the states in which the mobile LHCII is associated with PSII and PSI, respectively. Fd, ferredoxin; PC, plastocyanin. Reproduced with permission from Rochaix [9].](http://rstb.royalsocietypublishing.org/)

2. GENETIC DISSECTION OF STATE TRANSITIONS

Although phosphorylation of LHCII had already been demonstrated by Bennett [20] in the seventies, the biochemical hunt for the protein kinase involved in...
this phosphorylation was not successful in spite of numerous attempts [21–24]. We and others therefore decided to use a genetic approach in *Chlamydomonas*, on the basis of the observation that a large fraction of the LHCII antenna is mobile during state transitions, resulting in a large decrease in PSII fluorescence after a transition from state 1 to state 2 [25,26]. This property was used in a screen for mutants deficient in state transitions with a fluorescence video imaging system. Several mutants were isolated in this screen and are listed in table 1. One mutant of particular interest, *stt7-7*, was deficient in a protein kinase called Stt7 [27]. Besides being unable to perform state transitions, this mutant was deficient in LHCII phosphorylation under conditions favouring state 2 and blocked in state 1. Subsequently, two additional allelic mutants (called *stt7-6* and *stt7-9*) were isolated. Among the mutants identified, some affect state transitions indirectly. This is the case for *stt10* and *stt2*, which are deficient in the expression of *MenC* and *MenD*, two genes involved in the phylloquinone biosynthetic pathway [28]. In at least one of these mutants, phylloquinone, which normally acts as an electron carrier within PSI, is replaced by plastoquinone. This replacement leads to decreased performance of PSI and is the cause for the state transition phenotype. Two other mutants that have been partially characterized are *C11* and *HCM*, which are locked in state 1 (S. Miras, S. Lobre`aux and J.-D. Rochaix 2008, unpublished results). Because these mutants are leaky, it has not yet been possible to identify the genes responsible for the mutant phenotype.

### Table 1. State transition mutants of *Chlamydomonas reinhardtii*.

<table>
<thead>
<tr>
<th>mutant</th>
<th>TAP-LLa</th>
<th>TAP-HLb</th>
<th>HSMc</th>
<th>product</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>stt7-7</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Stt7 kinase</td>
</tr>
<tr>
<td><em>stt7-9</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><em>stt7-6</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><em>stt10</em></td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>MenC</td>
</tr>
<tr>
<td><em>stt2</em></td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>MenD</td>
</tr>
<tr>
<td><em>C11</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><em>HCM</em></td>
<td>+</td>
<td>−</td>
<td>−</td>
<td></td>
</tr>
</tbody>
</table>

*a*Tris-acetate-phosphate medium—low light.

*b*TAP—high light.

*c*High salt minimal medium.

3. ROLE OF STT7/STN7 KINASE

The Stt7 protein kinase is related to the Stl1 kinase in *C. reinhardtii*. Together, they form a duo of kinases that is conserved in land plants where the orthologues of Stt7 and Stl1 are called STN7 and STN8, respectively [29]. Although these two kinases appear to have distinct substrates and functions with Stt7/STN7 required for LHCII phosphorylation and state transitions [27,29] and Stl1/STN8 for PSII core protein phosphorylation [30,31], there is some overlap between these two kinases as seen by the comparison of the protein phosphorylation patterns of *stn7* and *stn8* with that of the *stn7–stn8* double mutant. While several proteins are still phosphorylated both in the *stn7* or *stn8* mutant, most of these phosphorylations are lost in the double mutant.

The Stt7/STN7 kinase contains a transmembrane domain that separates its N-terminal end in the lumen and the catalytic domain on the stromal side of the thylakoid membrane. This kinase is firmly associated with the cyt *b*6*f* complex and interacts with LHCII and PSI, based on co-immunoprecipitations. The kinase probably exists as a dimer. Two conserved Cys between Stt7 and STN7 are located on the lumen side and could form an intersubunit disulphide bridge. One possibility is that inactivation of the kinase under conditions that lead to reduction of ferredoxin and thior- edoxin occurs through a trans-thylakoid thiol pathway, including CcdA and Hcf164, which would reduce the disulphide bond (indicated with a broken line). TR, thioreredoxin; FTR, ferredoxin-thioreredoxin reductase, Fd, ferredoxin.

![Figure 2. Model of Stt7/STN7 kinase in the thylakoid membrane.](image-url)
presence of two Cys residues within the N-terminal domain. Among all Cys residues, these are the only two that are conserved between Stt7 and STN7 [27]. Site-directed mutagenesis of either of these two residues abolished kinase activity, indicating that they are essential for state transitions although they are not part of the catalytic kinase domain [32].

The LHClII kinase has been shown to be inactivated under high light through the ferredoxin-thioredoxin system (figure 2) [7]. One possibility is that this inactivation occurs through the opening of a disulphide bridge formed between the two luminal Cys. At first sight, the fact that the Cys and the thioredoxins are located on opposite sides of the thylakoid membrane seems to exclude such a mechanism. However, a trans-thylakoid trans-membrane thiol pathway has been identified across the thylakoid membrane, which seems to exclude such a mechanism. However, under state 1 conditions, the kinase is unstable and degraded [32,43]. While site-directed mutagenesis of the unique phosphorylation site of Stt7 did not have any impact on state transitions, the LHCII phosphorylation or protein stability, in the case of STN7 when the four phosphorylated residues were changed to Asp the kinase was stable under state 1 conditions indicating that the phosphorylation influences the accumulation of STN7 [43].

Another substrate of Stt7 is its paralogue Stl1. This kinase forms a dimer that is sensitive to reductants and that could involve two disulphide bridges between the luminal Cys pair (G. Fucile and J.-D. Rochaix 2011, unpublished results). The recently determined atomic structure of the cyt b6/f complex reveals a large cavity on the lumen side, which could accommodate the site of interaction of the kinase dimer [35].

The major substrates of the Stt7 kinase are the LHClII proteins. To identify the corresponding phosphorylated residues, thylakoid membranes from the wild-type and the stt7 mutant were isolated and digested with trypsin to release the LHClII phosphopeptides that are located on the stromal side of the membrane [36]. These peptides were then enriched by metal affinity chromatography and subjected to mass-spectrometric analysis. In this way, the comparative analysis between wild-type and stt7 revealed several Stt7-dependent LHClII phosphorylation sites (table 2). Besides LHClII, additional protein substrates were identified, notably Stt7 itself. A recent analysis of recombinant Stt7 kinase reveals that the kinase is capable of autophosphorylation (G. Fucile and J.-D. Rochaix 2011, unpublished results). Moreover, four phosphorylation sites have been identified in STN7 [42]. These phosphorylation sites are located in the C-terminal part of the kinase, which is poorly conserved between Stt7 and STN7 (figure 3). Under state 2 conditions, the Stt7/STN7 kinase is stable. However, under state 1 conditions, the kinase is unstable and degraded [32,43]. While site-directed mutagenesis of the unique phosphorylation site of Stt7 did not have any impact on state transitions, LHClII phosphorylation or protein stability, in the case of STN7 when the four phosphorylated residues were changed to Asp the kinase was stable under state 1 conditions indicating that the phosphorylation influences the accumulation of STN7 [43].

Table 2. Phosphorylation substrates of the Stt7/STN kinase.

<table>
<thead>
<tr>
<th>protein</th>
<th>peptide</th>
<th>stt7 state 1</th>
<th>stt7 state 2</th>
<th>wt state 1</th>
<th>wt state 2</th>
<th>ref.</th>
</tr>
</thead>
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<td>photosystem II D2 protein</td>
<td>Ac-tIAAGTYQEK</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>[25,37]</td>
</tr>
<tr>
<td>Lhcbm1</td>
<td>tVKPASK</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>[37]</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lhcbm4</td>
<td>Ac-KAtGKKKtAAK</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>[37]</td>
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<td></td>
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<tr>
<td>PsbR</td>
<td>VGLNsIEDPVVK</td>
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<tr>
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<tr>
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<tr>
<td></td>
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<tr>
<td></td>
<td>NNKGrSVEAIVQATPDEVSENRA</td>
<td>−</td>
<td>−</td>
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</tr>
<tr>
<td>CP43</td>
<td>Ac-tLFNGTTLTGGGR</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>[37]</td>
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<tr>
<td>LC15</td>
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<td>+</td>
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<tr>
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<tr>
<td>RuBisCO activase</td>
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<td>−</td>
<td>−</td>
<td>+</td>
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<td>[36]</td>
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<tr>
<td>protein kinase Sl1</td>
<td>LQADDQGIVQR</td>
<td>−</td>
<td>−</td>
<td>−</td>
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<td>[36]</td>
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<tr>
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<td>DAGLAsMEEAILK</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>[36]</td>
</tr>
<tr>
<td>PsbH</td>
<td>AtGtSKAKPSK</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>[36]</td>
</tr>
<tr>
<td>calcium sensing receptor</td>
<td>TGTSTSTR</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>[36,41]</td>
</tr>
</tbody>
</table>

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for translation of the \textit{psaB} mRNA) and Nac2 (involved in the stability/processing of the \textit{psbD} RNA). These data raise the possibility that the Stt7/STN7 kinase has a wider role and is also involved in chloroplast gene expression, although the phosphorylation of these proteins needs to be confirmed by phosphoproteomics.

4. STN8 KINASE AND ITS ROLE IN PSII TURNOVER AND IN THE FOLDING OF THYLAKOID MEMBRANES

The principal substrates of STN8 are the PSII core proteins D1, D2, CP43 and PsbH, and the Ca$^{2+}$-sensitive thylakoid phosphoprotein, CAS [30,31]. Although phosphorylation of the PSII core proteins has been studied intensively, its precise role has not yet been fully elucidated. A possible role for D1 phosphorylation and dephosphorylation has been proposed for the PSII repair cycle in which damaged D1 is replaced by newly synthesized D1. In both \textit{stn8} and the \textit{stn7-stn8} double mutant, degradation of D1 was markedly retarded [45]. In the double mutant, only residual light-independent phosphorylation of D2 and PsbH proteins of PSII was detected at ten times lower levels than in the wild-type thylakoids [46]. Moreover, folding of the thylakoid membranes was affected. In the absence of STN8, grana size was increased, and there were less grana stacks. It should be noted that grana size is...
Remarkably conserved in land plants and algae [47]. Interestingly, comparison of the amount of FtsH in isolated grana and stromal membranes from wild-type and the \textit{stn7-stn8} mutant revealed two important features linked to the absence of STN8 [46]. First, the access of the FtsH protease to the grana region was restricted. This protease is known to play a key role in the PSII repair cycle that occurs during photosynthesis [48]. It is responsible to a large extent for the degradation of D1, together with the Deg proteases [5,49,50]. Second, the migration of damaged D1 from the grana to the stromal membranes was hindered, and degradation of D1 was significantly reduced during photoinhibitory light treatment [46]. Taken together, these results further confirm that phosphorylation of the PSII reaction centre proteins plays a key role in protein migration within the thylakoid membranes. A unique feature of the thylakoid membrane is that its protein content is the highest among biological membranes. Diffusion of proteins or other membrane components in this crowded environment is therefore limited. A possible role of PSII protein phosphorylation could be to loosen the membrane structure so as to facilitate diffusion.

A proteome-wide screen for STN8 substrates by comparative quantitative phosphoproteomics with WT and STN8-deficient plants confirmed the presence of D2, CAS and PsbH among the substrates of this kinase [44]. However, in this screen, no differences were detected in the phosphorylation state of D1 and CP43 between wild-type and \textit{stn8}. Moreover, this screen identified four new substrates of STN8: a putative envelope transporter, CP29, the minor antenna of PSII and PGRL1. This protein is involved in cyclic electron flow [51] and is part of a large supercomplex comprising PSI, cyt \textit{b\text{-}f} and LHClI in \textit{C. reinhardtii} [52]. Spectroscopic analysis with \textit{stn7}, \textit{stn8} and the \textit{stn7/stn8} double mutant revealed a Stn8-specific effect on the transition between linear and cyclic electron flow [44]. In the absence of STN8, the stability of cyclic electron flow is reduced. This leads to a change in the relative efficiency of cyclic and linear electron flow suggesting a possible interplay between protein phosphorylation and cyclic electron flow.

The role of STN8 is not limited to thylakoid protein phosphorylation, as the loss of STN8 affects the expression of several nuclear and chloroplast genes encoding proteins involved in photosynthesis [30].

5. PHOSPHATASES INVOLVED IN THYLAKOID PROTEIN DEPHOSPHORYLATION

Surprisingly, among the mutants deficient in state transitions, none was affected in the phosphatase required for the dephosphorylation of LHClI. Possible reasons are an overlap of substrate sites among different phosphatases or that the mutant screen is still far from saturation. We therefore turned to \textit{Arabidopsis} using a bioinformatic approach. First, phosphatase subunits targeted at the chloroplast were identified and a sublist including phosphatases coexpressed with STN7 was established. Homozygous TDNA insertion lines from these lists were then tested using a dephosphorylation screen in which the phosphorylation status of LHClI of young seedlings was first estimated under blue light corresponding to state 2. Dephosphorylation was assessed under far-red illumination after 20 and 40 min corresponding to state 1 conditions. In this way, two allelic mutants were identified which were unable to dephosphorylate LHClI upon transfer from state 2 to state 1. The gene inactivated in these mutants encodes a PP2C phosphatase.
called PPH1/TAP38 [53,54]. The same reverse genetic screen identified another PP2C phosphatase dubbed PBCP, which is required for the dephosphorylation of the PSII core proteins [55]. This phosphatase represents the counterpart of the STN8 kinase. It is not yet known whether the PPH1/TAP38 and PPBC phosphatases are constitutively active or whether they are regulated, possibly by the redox state of the plastocyanine pool. An intriguing feature of the PPBC phosphatase is that its activity is Mn²⁺-dependent. Both phosphatases are conserved in land plants and orthologues exist in C. reinhardtii.

6. CONCLUSIONS AND PERSPECTIVES

The picture that emerges from these studies is that of a complex signalling network in the chloroplast, which is modulated by the light environment, CO₂ availability and the cellular ATP levels (figure 4) [60,61]. These parameters impact the redox state of the photosynthetic plastocyanine pool, which in turn controls the activity of the Stt7/STN7 and STN8 kinases. The Stt7/STN7 kinase plays an important role in several processes. It is involved in the redistribution of the light excitation energy between the two photosystems and in the readjustment of the ATP/NADPH ratio during state transitions. It also plays a key role in maintaining the proper redox poise of the plastocyanine pool upon changes in light conditions. Moreover, Stt7/STN7 is also involved in retrograde signalling. Other chloroplast protein kinases have been identified and characterized, including the casein kinase CKII (involved in the control of chloroplast gene expression [56]), the CSK kinase (which has also been proposed to control gene expression through redox control [57] although the evidence is still sparse) and the TAK1 kinase [58,59] (which has been proposed to phosphor- ylate LHCII). Unfortunately, no further studies have been performed with this kinase. In at least one case, it is known that phosphorylation of Stl1 is dependent on the Stt7 kinase, suggesting a signalling cascade between these two kinases. This network has been further extended by the identification of the PPH1/TAP38 and PPBC phosphatases, which act mainly on the LHCCI and the PSII core proteins, respectively, and thus represent the counterparts of Stt7/STN7 and Stt1/STN8 kinases [53–55]. Like both of these kinases, the two phosphatases are conserved in land plants and green algae. Other phosphatases must be responsible for achieving reversible phosphorylation/dephosphorylation of several thylakoid membrane proteins, depending on the environmental conditions. A challenging task will be to elucidate the links between these different kinases and phosphatases, and the signalling chains that operate in the plastocyanin.

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