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Review

Dynamics of reversible protein phosphorylation in thylakoids of flowering plants: The roles of STN7, STN8 and TAP38☆

Paolo Pesaresi [a], Mathias Pribil [b,c], Tobias Wunder [b], Dario Leister [b,*]

[a] Dipartimento di Scienze Biomolecolari e Biotecnologie, Università degli studi di Milano, I-20133 Milano, Italy
[b] Lehrstuhl für Molekularbiologie der Pflanzen (Botanik), Department Biologie I, Ludwig-Maximilians-Universität München, D-82152 Planegg-Martinsried, Germany
[c] Mass Spectrometry Unit, Department Biologie I, Ludwig-Maximilians-Universität München, D-82152 Planegg-Martinsried, Germany

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ABSTRACT

Phosphorylation is the most common post-translational modification found in thylakoid membrane proteins of flowering plants, targeting more than two dozen subunits of all multiprotein complexes, including some light-harvesting proteins. Recent progress in mass spectrometry-based technologies has led to the detection of novel low-abundance thylakoid phosphoproteins and localised their phosphorylation sites. Three of the enzymes involved in phosphorylation/dephosphorylation cycles in thylakoids, the protein kinases STN7 and STN8 and the phosphatase TAP38/PPH1, have been characterised in the model species *Arabidopsis thaliana*. Differential protein phosphorylation is associated with changes in illumination and various other environmental parameters, and has been implicated in several acclimation responses, the molecular mechanisms of which are only partly understood. The phenomenon of State Transitions, which enables rapid adaptation to short-term changes in illumination, has recently been shown to depend on reversible phosphorylation of LHCII by STN7-TAP38/PPH1. STN7 is also necessary for long-term acclimation responses that counteract imbalances in energy distribution between PSII and PSI by changing the rates of accumulation of their reaction-centre and light-harvesting proteins. Another aspect of photosynthetic acclimation, the modulation of thylakoid ultrastructure, depends on phosphorylation of PSII core proteins, mainly executed by STN8. Here we review recent advances in the characterisation of STN7, STN8 and TAP38/PPH1, and discuss their physiological significance within the overall network of thylakoid protein phosphorylation. This article is part of a Special Issue entitled: Regulation of Electron Transport in Chloroplasts.

1. Introduction

Post-translational phosphorylation of proteins serves as a regulatory mechanism in almost all basic cellular processes in eukaryotic cells, and plays a central role in signal transduction pathways. Phosphorylation of amino acid side-chains can modulate enzymatic activity, protein conformation, and protein–protein interactions, and alter the localisation and stability of proteins. It is estimated that about one third of all eukaryotic proteins are subject to reversible phosphorylation mediated by the action of protein kinases (PKs) and phosphatases (PPs) [1]. Various types of PKs and PPs exist in plants, and are classified according to the presence of domains other than their catalytic sites. Based on the latest version of the Arabidopsis genome annotation (TAIR9) and the PlantP database, the nuclear genome of the model plant *Arabidopsis thaliana* encodes 989 PKs and 217 PPs. Of these, 45 PKs and 21 PPs are predicted to be located in chloroplasts [2]. However, the set of enzymes known to be involved in the reversible phosphorylation of chloroplast proteins in *A. thaliana* comprises altogether only 7 chloroplast PKs and 10 chloroplast PPs [2-5].

Recent advances in mass spectrometry (MS)-based technologies and methods for phosphopeptide enrichment have paved the way for large-scale, high-throughput *in vivo* mapping of phosphorylation sites, and several phosphoproteome studies in plants and algae have been reported [6-14]. These define the "phosphorylome"—i.e. the set of proteins within the proteome or a sub-proteome of an organism that can be reversibly modified by phosphorylation *in vivo*. These studies revealed high levels of protein phosphorylation in thylakoid membranes isolated from light-adapted leaves that had been exposed to various stress conditions, including drought, low temperature, and limiting CO_2 concentration, implicating thylakoid protein phosphorylation in adapting the photosynthetic process to environmental changes. The identification of thylakoid PKs and PPs, together with

Abbreviations: STN7/STN8, State TransitioN 7/State TransitioN 8; TAP38/PPH1, Thylakoid-Associated Phosphatase of 38 kDa/Protein PHosphatase 1; LTR, Long-Term Response; LHCII, Light Harvesting Complex of photosystem II

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* Corresponding author. Großhaderner Str. 2, D-82152 Planegg-Martinsried, Germany. Tel:. +49 89 2180 74550; fax: +49 89 2180 74599.
E-mail address: leister@lrz.uni-muenchen.de (D. Leister).

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analyses of corresponding loss-of-function lines, has revealed important aspects of how plants cope with imbalances in photosynthesis, both in the short and the long term [4,5,15-20].

In this review, we report on recent advances in the expanding field of reversible phosphorylation of thylakoid proteins. The characteristics and physiological roles of the thylakoid PKs STN7 and STN8, as well as the newly identified thylakoid PP TAP38/PPH1, will be discussed. Particular emphasis will be given to the involvement of these enzymes and their substrates in the acclimation of plants to changes in the spectral composition and intensity of incident light.

2. The thylakoid phosphorylome

Experiments in the mid-1970s first established the occurrence of protein phosphorylation in thylakoids, when Bennet and colleagues demonstrated that PKs phosphorylate subunits of the light-harvesting complexes [21]. Since then, phosphoproteomic studies in plants have been performed on subcellular fractions, such as thylakoids, stroma, plastids and, more recently, also on Arabidopsis cell suspensions [10], leaves [14] and whole shoots [13]. Based on these studies, 197 chloroplast phosphoproteins have been identified so far. Chloroplast phosphoproteins have diverse functions in carbohydrate, lipid, nucleotide and amino acid metabolism, and several enzymes involved in tetrapyrrole biosynthesis are phosphorylated. Furthermore, 28 thylakoid proteins involved at different levels of photosynthetic electron transport and ATP generation are also subject to phosphorylation (Table 1).

2.1. The LHCII antenna phosphoproteins

In the thylakoids of higher plants the antenna complex of photosystem II (PSII), light-harvesting complex II (LHCII), contains highly phosphorylated proteins [22]. Phosphorylation of Lhcb1 and Lhcb2 reaches a maximum under low-level light, when 15–25% of these polypeptides are phosphorylated [23]. This fraction decreases drastically at higher irradiances or when plants are kept in darkness [24]. Specific phosphorylation of another antenna protein of PSII, Lhcb4 (also named CP29), has been observed in maize exposed to cold stress [25-27], in winter rye subjected to high-intensity light and cold temperatures simultaneously [28] and in barley exposed to water stress [29]. High levels of Lhcb4 phosphorylation have also been observed in Arabidopsis leaves adapted to low-light conditions [30,31], implying that a certain degree of variability exists in protein phosphorylation patterns, even among closely related species (Table 1). More recently, phosphorylation of Lhcb5 (also named CP26) and Lhcb6 (also named CP24) has been reported [13].

2.2. The PSII phosphoproteins

In contrast to LHCII polypeptides, up to 80% of the PSII subunits D1, D2, CP43 and PsbH are phosphorylated at their N-termini [32-35] in plants exposed to high light intensities [36] or drought stress [37]. The light-dependent phosphorylation of CP47 has also been reported, recently [13]. Thus, PSII core protein phosphorylation must have a major role in photoprotection, because light that is needed to drive photosynthesis is also the main cause of damages to the photosynthetic apparatus, with PSII as the main target. In addition to light, PSII reaction-centre subunits retain their phosphate groups even in the dark when plants are deprived of magnesium and sulphur simultaneously [38], or maintained at subfreezing temperatures [39] or under high light stress [40]. Taking these observations together, it appears that PSII core protein phosphorylation might be regarded as a sort of memory of the experienced stress conditions, thus priming PSII for oxidative damage protection over prolonged periods. Conversely, exposure of spinach leaves to elevated temperatures accelerated dephosphorylation of D1, D2 and CP43 [41], implying the existence of a heat-shock induced thylakoid phosphatase that triggers accelerated repair of photodamaged PSII.

Light-dependent phosphorylation has been also observed for PsbP, PsbQ and PsbR, components of the oxygen evolving complex [10,13,14]. Also in their cases, phosphorylation is maintained after prolonged dark exposure [13].

2.3. The LHCI-PSI phosphoproteins

Recent studies have suggested that subunits of photosystem I (PSI) and its light-harvesting complex (LHCI) can also be targeted for phosphorylation [10,13,30,42,43] (Table 1). Thus, the stroma-exposed D and E subunits of PSI are phosphorylated in light-exposed leaves [13,30], together with PsaF, PsaL, and PsaN [10,13]. Phosphorylation of the tentative PSI subunit P (also known as TMP14) [13,30,43] has also been detected in both light- and dark-exposed leaves. In addition, investigation of *psae1-3* and *psad1-1* mutants, which are characterised by decreased levels of PSI-E and -D subunits, respectively [44,45], and overreduction of the plastoquinone pool thought to activate the LHCII kinase (see below), revealed that Lhca4, an antenna subunit associated with PSI, is phosphorylated at an N-terminal threonine residue [42]. Lhca4 phosphorylation has also been obtained for wild-type (WT) Arabidopsis seedlings exposed to light (100 μmol photons m^{-2} s^{-1}) under short-day conditions, although here an N-terminal serine residue resulted to be phosphorylated [13].

2.4. Other thylakoid phosphoproteins

Light-dependent phosphorylation has been also reported for the Cytochrome b_6/f (Cyt b_6/f) complex and the chloroplast ATP synthase (cpATPase), albeit at lower levels than in PSII or LHCII proteins. Phosphoproteomics analysis of stroma membranes isolated from light-adapted spinach leaves has, for the first time, demonstrated that a subunit of the Cyt b_6/f complex, the Rieske Fe–S protein, is phosphorylated [34]. Moreover, the α-, β- and γ-subunits of the chloroplast ATPase complex from Arabidopsis seedlings have now been shown to be phosphorylated [13,14]. Phosphorylation of the β-subunit of chloroplast ATPase has been also observed in barley [46]. Besides components of the major thylakoid photosynthetic complexes, light-dependent phosphorylation of the soluble 9-kDa thylakoid phosphoprotein TSP9 [13,47], the calcium-sensing receptor CaS [48], the PGRL1 protein and the STN7 kinase [13] has been reported (Table 1).

2.5. Characteristics of thylakoid phosphoproteins

One feature that is common to most thylakoid phosphoproteins identified so far is the phosphorylation of threonine residues at their stroma-exposed N-termini (Table 1). However, N-terminal phosphorylation at serine residues has also been reported [10,13,14]. Furthermore, phosphorylation is not restricted to N-terminal regions, and may even encompass the stroma-exposed C-termini of proteins such as CP43, TSP9, CaS and STN7 [10,13,48,49]. Interestingly, in the case of CP47, PsbP, PsbQ, PsbR and PsaF phosphorylation takes place on the luminal side of thylakoid membranes (Table 1), implying the existence of luminal protein kinase activity. Moreover, in several cases phosphorylation is observed at multiple sites within each subunit, which would enable such thylakoid phosphoproteins to act as integrators of multiple signal transduction pathways.

3. The kinases STN7 and STN8 and their substrates

The fact that so many proteins can become phosphorylated in thylakoids raises the question of how many different PKs are involved. A survey of thylakoid phosphorylation sites clearly indicates that they have different physicochemical properties [10,13,14]. This, together with the highly variable pattern of thylakoid protein phosphorylation

Table 1
Thylakoid phosphoproteins identified in higher plants, together with the corresponding PKs and PPs, phosphorylated residues and environmental conditions favouring phosphorylation.

Phospho-protein[a]	PK/PP[b]	Phosphorylated residue[c]	Thylakoid side[d]	Environmental conditions[e]	Reference
D1	STN8/STN7 TAP38/?	Thr-2 (A; S)	stroma	−Mg −S/cold and drought stress/ darkness/low- and high-light	[4,16,18,32,33,36-40,49]
D2	STN8/STN7/?TAP38/?	Thr-2 (A; S)	stroma	−Mg −S/drought stress/darkness/ low- and high-light	[4,13,16,18,33,36-38,40,49]
CP43	STN8/STN7	Thr-15 (A;S), Thr-20 (S), Thr-22 (S), Thr-346 (S), Ser-468 (A)	stroma	−Mg −S/drought stress/darkness/ low- and high-light	[10,13,16,18,33,34,36-38,49]
CP47	nd	Ser-329 (A)	lumen	darkness/light	[13]
PsbH	nd STN8	Thr-3 (A;S) Thr-5 (A)	stroma	darkness/light darkness/light	[13,18,35,49]
PsbP	nd	Thr-133 (PsbP-1) (A), Thr-141 (PsbP-1) (A), Thr-143 (PsbP-1) (A), Ser-147 (PsbP-1) (A), Ser-159 (PsbP-1) (A), Ser-145 (PsbP-2) (A) , Ser-157 (PsbP-2) (A)	lumen	darkness/light	[13,14]
PsbQ	nd	Ser-125 (A)	lumen	darkness/light	[13,14]
PsbR	nd	Ser-58 (A)	lumen	darkness/light	[10,13]
Lhcb1	STN7/STN8/?TAP38	Thr-36 (Lhcb1.5) (A), Thr-38 (Lhcb1.1/1.2/1.3) (A; S), Ser-30, Ser-38 (Lhcb1.1) (S),(Lhcb1.1/1.2/1.3) (A), Ser-47 (Lhcb1.4) (A), Ser-46 (Lhcb1.5) (A) Ser-48 (Lhcb1.1/1.2/1.3) (A)	stroma	low-light	[4,10,13,15,16,18,23,49,54]
Lhcb2	STN7/STN8/?TAP38	Thr-41 (Lhcb2.3) (A), Thr-40 (Lhcb2.1/2.2) (A; S)	stroma	low-light	[4,15,16,18,23,54]
Lhcb4/CP29	STN7	Thr-37 (Lhcb4.2) (A), Thr-109 (Lhcb4.2) (A), Thr-111 (Lhcb4.2) (A), Thr-112 (Lhcb4.1) (A, M), Thr-114 (Lhcb4.1) (A), Ser-34 (Lhcb4.3) (A)	stroma	cold and drought stress/low- and high-light	[13,25-31]
Lhcb5/CP26	nd	nd (A)	nd	darkness	[13]
Lhcb6/CP24	nd	Thr-42 (A)	stroma	light	[13]
PsaD	nd	Thr-48 (PsaD-1) (A), Thr-46 (PsaD-2) (A)	stroma	light	[13,30]
PsaE	nd	nd (PsaE-1) (A)	stroma	light	[13]
PsaF	nd	Ser-94 (A)	lumen	darkness/light	[10]
PsaL	nd	nd (A)	nd	light	[13]
PsaN	nd	nd (A)	nd	light	[13]
PsaP/TMP14	nd	Thr-65 (A), Thr-66 (A)	stroma	darkness/light	[13,30,43]
Lhca4	STN7	Thr-68 (A), Ser-35 (A)	stroma	*psad1-1* and *psae1-3* mutants/light	[13,42]
Rieske Fe–S	nd	Thr-70 (S), Ser-71 (S)	stroma	light	[34]
cpATPase α subunit	nd	Ser-125 (A)	stroma	light	[13]
cpATPase β subunit	nd	Thr-62 (A), Ser-8 (A), Ser-13 (A), Ser-286 (A), Ser-79/Thr-82 (B), Thr-252/255 (B), Thr-454 (B), Thr-489 (B)	stroma	darkness/light	[13,14,46]
cpATPase γ subunit 1	nd	Ser-347 (A)	stroma	darkness/light	[13]
TSP9	STN7	Thr-43 (A), Thr-64 (A), Thr-71 (A), Ser-25 (A), Ser-96 (A), Thr-66 (S), Thr-73 (S), Thr-80 (S)	stroma	light	[13,47,53]
CaS	STN8/TAP38	Thr-380 (A), Ser-373 (A), Ser-378 (A)	stroma	low- and high-light/darkness	[4,13,48]
PGRL1	nd	nd (A)	nd	light	[13]
STN7	nd	Thr-537 (A), Thr-539 (A), Thr-541 (A), Ser-526 (A)	nd	light	[13]

nd, not determined.
[a] Thylakoid phosphoproteins from higher plants.
[b] PKs and PPs required for the phosphorylation. The symbol "?" indicates the additional involvement of unknown kinases and phosphatases.
[c] The phosphorylated amino acid residues are numbered according to their positions in the primary translation products. Additionally, the species in which the phosphoaminoacid residues have been identified are reported: Arabidopsis (A), spinach (S), barley (B), maize (M).
[d] Orientation of phosphoaminoacids.
[e] Condition described to favour and maintain phosphorylation of the respective protein. −Mg −S, magnesium and sulphur deficiency.

observed under different environmental conditions, suggests that various PKs should act on thylakoid proteins (Table 1). Significant advances have been made in the identification and characterisation of thylakoid PKs, thanks to genetic and biochemical studies in *A. thaliana* and *Chlamydomonas reinhardtii* [15-18]. Mutant analyses imply that phosphorylation in Arabidopsis thylakoids depends mostly on the highly homologous PKs STN7 and STN8 [15,16,18]. Each of these comprises a large C-terminal catalytic domain characteristic of Ser-Thr PKs, a single transmembrane domain, and an N-terminal stretch that, in the case of STN7 and its Chlamydomonas homologue STT7, contains two cysteine residues not found in the N-terminal portion of STN8 [15-17].

The topology of STN7 and STN8 within the thylakoid membranes is not clear. However, important information has emerged from studies performed in Chlamydomonas. STT7 is characterised by a transmembrane helix that separates its stroma-exposed catalytic domain from its lumen-located N-terminal end [50]. This is consistent with the stroma exposure of STT7-dependent phosphorylation sites, and is likely to represent the topology of STN7 and STN8 also.

3.1. The substrates of STN7

Considerable progress has been made in elucidating the substrate specificity of STN7 and STN8. Thylakoid phosphorylation patterns of WT and *stn* mutant Arabidopsis plants have been monitored by different approaches, including immunoblot analyses with different anti-phosphothreonine antibodies, in vivo phosphate (^{33}P) labelling and mass-spectrometric analyses [12,15,16,18,51,52]. Arabidopsis mutants devoid of STN7 displayed much less light-induced phosphorylation of LHCII, the linker protein Lhcb4, the regulatory protein TSP9 and the PSI antenna protein Lhca4 [15,16,31,42,53] (Table 1). MS-based analysis revealed phosphorylation of threonine and serine residues at the N-termini of LHCII proteins in Arabidopsis and spinach [10,13,18,49,54]. Interestingly, two phosphorylated peptides–RKtVAKPK ("t" indicates phosphorylated Thr) and RRtVK–corresponding to the N-termini of Lhcb1 and Lhcb2 were characterised, suggesting that phosphorylated threonine residues flanked by the basic residues Lys and Arg could be a signature for the substrates of STN7. A similar motif in STT7-dependent phosphorylation sites has recently been identified in *C. reinhardtii* Lhcbm proteins [55]. However, it should be noted that phosphorylation sites in Lhcb4 [30,31] and TSP9 proteins do not show these characteristics [13,47].

3.2. The substrates of STN8

The substrate specificity of STN8 is very different from that of STN7. Loss of STN8 causes a marked decrease in the phosphorylation of D1, D2, CP43 and PsbH subunits of PSII in plants exposed to light, as detected by using anti-phosphothreonine antibodies [16,18] (Table 1). Immunoblotting analyses have also revealed the STN8-dependent phosphorylation of a 40-kDa protein in photosynthetic thylakoid membranes exposed to high levels of light [48]. The protein has been identified as a calcium-sensing receptor (CaS), that localizes to chloroplasts and is enriched in stroma thylakoids [48]. More detailed MS analyses, based on phosphopeptide quantification, indicated that disruption of *STN8* causes a 50–60% decrease in phosphorylation of D1 (tAILER) and D2 (tIALGK) at their N-terminal threonine residues [18,49]. A similar decrease in phosphorylation was also observed for CP43 (tLFNGTLALAGR), whereas phosphorylation of Thr-5, but not of Thr-3, of the PsbH subunit (AtQtVEDSSR) was abolished [18,49]. In addition, the phosphopeptide SGtKFLPSSD, which derives from CaS, could not be detected in *stn8* thylakoids [48]. Taken together, the data indicate that, unlike STN7, the STN8 phosphopeptides do not show any obvious consensus amino acid sequence that might define the substrates of the kinase. Moreover, it appears likely that there are other PKs besides STN8 in Arabidopsis that phosphorylate the N-terminal threonines of D1, D2, and CP43, as well as Thr-3 of PsbH. Interestingly, phosphorylation of Thr-5 in PsbH by STN8 requires prior phosphorylation of Thr-3 by another PK [49], which again implies that thylakoid phosphoproteins can serve as integrators of multiple signal transduction pathways.

3.3. Overlaps between substrate sets

The idea that STN8 is not the only kinase involved in phosphorylation of PSII core proteins was initially suggested by comparisons of thylakoid phosphorylation patterns in *stn7*, *stn8* and *stn7 stn8* double mutants [16]. Based on *in-vivo* labelling experiments with [^{33}P] orthophosphate and immunoblot analyses with anti-phosphothreonine antibodies, thylakoids from the *stn7 stn8* double mutant were found to show a complete lack of phosphorylation of LHCII and PSII core proteins under all light regimes tested, whereas residual phosphorylation of PSII core proteins was detectable in *stn8* plants [16]. This clearly indicates that STN7 and STN8 show some degree of overlap in their substrate specificities (Table 1). Similar results have been obtained more recently from an MS-based analysis of *stn7 stn8* thylakoids isolated from light-adapted plants [12]. Thus, the phosphopeptides corresponding to the N-terminal stretches of D1 and CP43 proteins of PSII were completely absent in *stn7 stn8* mutant thylakoids. Nevertheless, residual phosphorylation, equivalent to 5–10% of WT levels, could be detected in D2 and LHCII proteins, and phosphorylation of Thr-3 in PsbH was unaffected. Interestingly, the levels of residual phosphorylation were almost identical under light and dark conditions, indicating that a third, light-independent, thylakoid PK might exist.

4. The phosphatase TAP38/PPH1 and its substrates

Extensive efforts to identify the PPs responsible for dephosphorylation have been going since the first thylakoid phosphoproteins were discovered. Based on biochemical approaches, it was shown that soluble and membrane-bound PPs of different families are involved in the dephosphorylation of thylakoid phosphoproteins [56-61]. Eukaryotic Ser/Thr protein phosphatases are typically classified according to their substrate specificity, requirement for divalent cations, and susceptibility to inhibitors [62,63]. By these criteria, two major families have been identified: the Mg^{2+}-dependent PPM family which includes PP2C, and the Mg^{2+}-independent PPP family which includes subfamilies PP1, PP2A, and PP2B. In particular, dephosphorylation of PSII core proteins was postulated to be catalyzed by a PP2A-like PP [64], whereas dephosphorylation of LHCII was shown to be dependent on the presence of divalent cations and to be insensitive to microcystin and okadaic acid [59,61], suggesting the involvement of a PP2C-type PP [63]. The latter inference was confirmed when a PP2C-type PP called TAP38/PPH1 was independently identified by two groups and found to be required for the dephosphorylation of LHCII in *A. thaliana* [4,5]. Two alternative *TAP38/PPH1* mRNA variants have been detected in leaves, although only one translation product could be found in chloroplasts. Immunoblot analysis detected the TAP38/PPH1 phosphatase in the thylakoid membranes, preferentially located within the stroma lamellae [5]. A transmembrane helix was predicted in the C-terminal portion of TAP38/PPH1, whereas the amino acids that constitute the PP2C signature are in the N-terminal region, most probably exposed to the stroma, like the catalytic domains of STN kinases [4]. Plants devoid of TAP38/PPH1 phosphatase showed aberrantly high levels of phosphorylated LHCII (pLHCII) under all lighting conditions tested. Moreover, *tap38/pph1* mutants were characterised by high levels of the phosphorylated thylakoid protein CaS. In contrast, overexpression of TAP38/PPH1 (oeTAP38/PPH1) results in constitutively reduced levels of pLHCII and thus mimics the *stn7* phenotype. Furthermore, oeTAP38/PPH1 plants showed reduced levels of phosphorylated CaS, D1 and D2 proteins, possibly revealing a substrate overlap with an unknown PSII-core PP [4] (Table 1).

5. The acclimation responses controlled by STN7, STN8 and TAP38/PPH1

Light-dependent reversible phosphorylation of thylakoid proteins by the STN kinases and TAP38/PPH1 is implicated in a number of responses to changes in light quality. Together, these responses act to maintain optimal activity of the photosynthetic apparatus (Fig. 1). The process appears not only to have a crucial role in short-term acclimation mechanisms (State Transitions, D1 turnover), but is also essential for long-term responses (LTR), as it is required to initiate alterations in the ultrastructure of pigment–protein supercomplexes and is the key reaction in signalling cascades [12,19,20,52,65].

5.1. STN7-dependent LHCII phosphorylation induces State 2

During linear photosynthetic electron flow, PSI and PSII are connected in series via the Cyt b_6/f complex to drive the vectorial electron transfer through thylakoids that ultimately leads to the production of NADPH and ATP. However, the antenna systems of PSII and PSI are tuned to light of different wavelengths—680 and 700 nm, respectively [66,67]. Because of these differences in light absorption properties, changes in the quality of incident light can lead to unequal excitation of the two photosystems, and thus to a decrease in photosynthetic yield. Plants and green algae are able to balance the light energy absorbed, on a timescale of minutes, by varying the antenna cross-sections of the two photosystems, a process that was discovered more than 40 years ago and is referred to as State Transitions [68,69]. This adjustment is achieved in land plants by the light- and STN7-dependent phosphorylation of Lhcb1 and Lhcb2 (Fig. 1 and Table 1). Specifically, when PSII is over-stimulated relative to PSI, the redox state of the PQ pool is shifted to a more reduced state. These conditions favour docking of plastoquinol (PQH_2) to the Qo site of Cyt b_6/f, which in turn leads to the activation of the STN7 kinase and phosphorylation of LHCII [70]. A two-step process for kinase activation has been proposed, involving a conformational change in the Cyt b_6/f complex caused by movement of the Rieske protein from a distal to a proximal position upon binding of PQH_2 to Cyt b_6/f [71]. This model is supported by recent data from coimmunoprecipitation assays in Chlamydomonas, which showed that the STT7 kinase interacts with Cyt b_6/f, PSI and LHCII [50]. However, maximum phosphorylation of LHCII occurs in vivo at low light intensities, whereas LHCII phosphorylation is down-regulated at high irradiance although the PQ pool is reduced [16,72,73]. Therefore LHCII phosphorylation cannot be regulated solely by the redox state of PQ. Instead, a complex network involving cooperative redox control by PQ and the Cyt b_6/f complex, as well as by the ferredoxin/thioredoxin system in the stroma of the chloroplasts, must be invoked. In this context, the two cysteines of the STN7/STT7 exposed to the thylakoid lumen might function as targets of reversible oxidation/reduction. These residues have been shown by site-directed mutagenesis to be essential for kinase activity: replacement of the cysteines in both STT7 and STN7 leads to the loss of State Transitions and LHCII phosphorylation [19]. But the two cysteines are located in the lumen, whereas the ferredoxin and thioredoxin are present in the stroma. How then can the activity of the kinase be regulated under these conditions? Two components of a transthylakoid thiol-reducing pathway that might provide the answer have been identified in chloroplasts. The CcdA protein [74] is able to transfer thiol-reducing equivalents from the stroma to the lumen, and Hcf164 is a transmembrane protein containing a thioredoxin domain in the thylakoid lumen that has disulphide reductase activity [75,76]. Thus, it is conceivable that the ferredoxin-thioredoxin system could regulate the redox state of the two cysteines on the opposite side of the membrane via CcdA/Hcf164. Regulation of LHCII kinase may also occur at the level of kinase availability. Time-course experiments have shown that the level of STT7 protein decreases under prolonged exposure to darkness or high light intensities [50].

Upon activation of STN7, the pLHCII migrates from PSII to PSI, allowing PSI to absorb and utilise some of the light that would otherwise be collected by PSII. This situation corresponds to the so-called State 2. The PSI-LHCI-pLHCII complex has been visualised in native PAGE gels recently [65,77], and was found to be characteristic of State 2-adapted wild-type Arabidopsis thylakoids. It is absent in *stn7* leaves and in plants, such as *psal*-deficient lines, devoid of the PSI docking domain for LHCII [65].

The STN7-dependent phosphorylation of Lhcb4 and TSP9 also appears to be part of the mechanism underlying State Transitions. The phosphorylated Lhcb4 (pLhcb4) protein has been shown to dissociate from PSII and bind to the core domain of PSI after induction of State 2 in Chlamydomonas cells [78,79], thus favouring the migration of pLHCII from PSII to PSI. It appears plausible that pLhcb4 might play a similar role in flowering plants, although evidence for the existence of pLhcb4-PSI protein complexes is not available yet. Similarly, TSP9-less plants have been reported to have lower levels of phosphorylated LHCII polypeptides and show a reduced ability to execute the transition to State 2 [53,80]. In addition, blue native electrophoresis of thylakoid membrane protein complexes revealed that loss of TSP9 increases the stability of PSII dimers and supercomplexes [53]. Therefore, it can be hypothesised that TSP9 and its phosphorylated variants might regulate light utilisation by facilitating the migration of light-harvesting proteins between the two photosystems.

5.2. TAP38/PPH1-dependent dephosphorylation of LHCII induces State 1

State Transitions are reversible, since both preferential excitation of PSI by light enriched in the far-red region and high light intensities are able to inactivate the STN7 kinase, which promotes the dephosphorylation of pLHCII and its return to PSII, thus inducing the so-called State 1. TAP38/PPH1 has been identified as the long-sought phosphatase responsible for LHCII dephosphorylation and the transition to State 1 [4,5] (Fig. 1). Indeed, plants devoid of TAP38/PPH1 are locked into State 2, as shown by the increase in PSI fluorescence emission at low temperature (77 K) under both State 1- and State 2-inducing conditions. In agreement with these observations, pLHCII remains attached to PSI, resulting in the persistence of the PSI-LHCI-pLHCII protein complex even under State 1-inducing conditions [4]. Several aspects of the function of the TAP38/PPH1 phosphatase remain to be elucidated, including whether it physically interacts with LHCII. A direct interaction between STT7 and LHCII has been shown in co-immunoprecipitation experiments [50], but similar evidence has yet to be presented for TAP38/PPH1. However, in vitro dephosphorylation assays have confirmed that TAP38/PPH1 can dephosphorylate pLHCII [4]. The possibility that TAP38/PPH1 activity might also be modulated by light or reducing agents remains to be investigated. Interestingly, unlike STT7, levels of TAP38/PPH1 protein remain unchanged under both State 1 and State 2 conditions [4].

A recent study based on gentle mechanical fractionation of the thylakoid membranes has indicated that the lateral movement of pLHCII, and therefore the interplay of STN7 and TAP38 enzymes, might be confined to a very limited portion of the thylakoid membranes, at the grana margins. This may represent the site at which redistributions of light energy actually occur [51].

5.3. STN7 and the long-term response

Besides inducing short-term acclimation processes such as State Transitions, changes in light conditions are known to lead to changes in amounts of the antenna proteins of PSII and PSI and to readjustment of photosystem stoichiometry in the long term (long-term response or LTR) [81,82]. The LTR is achieved through a signalling network that coordinates gene expression in the nucleus and chloroplast (Fig. 1). Studies on the *stn7* mutant uncovered an intriguing connection between State Transitions and the LTR, when

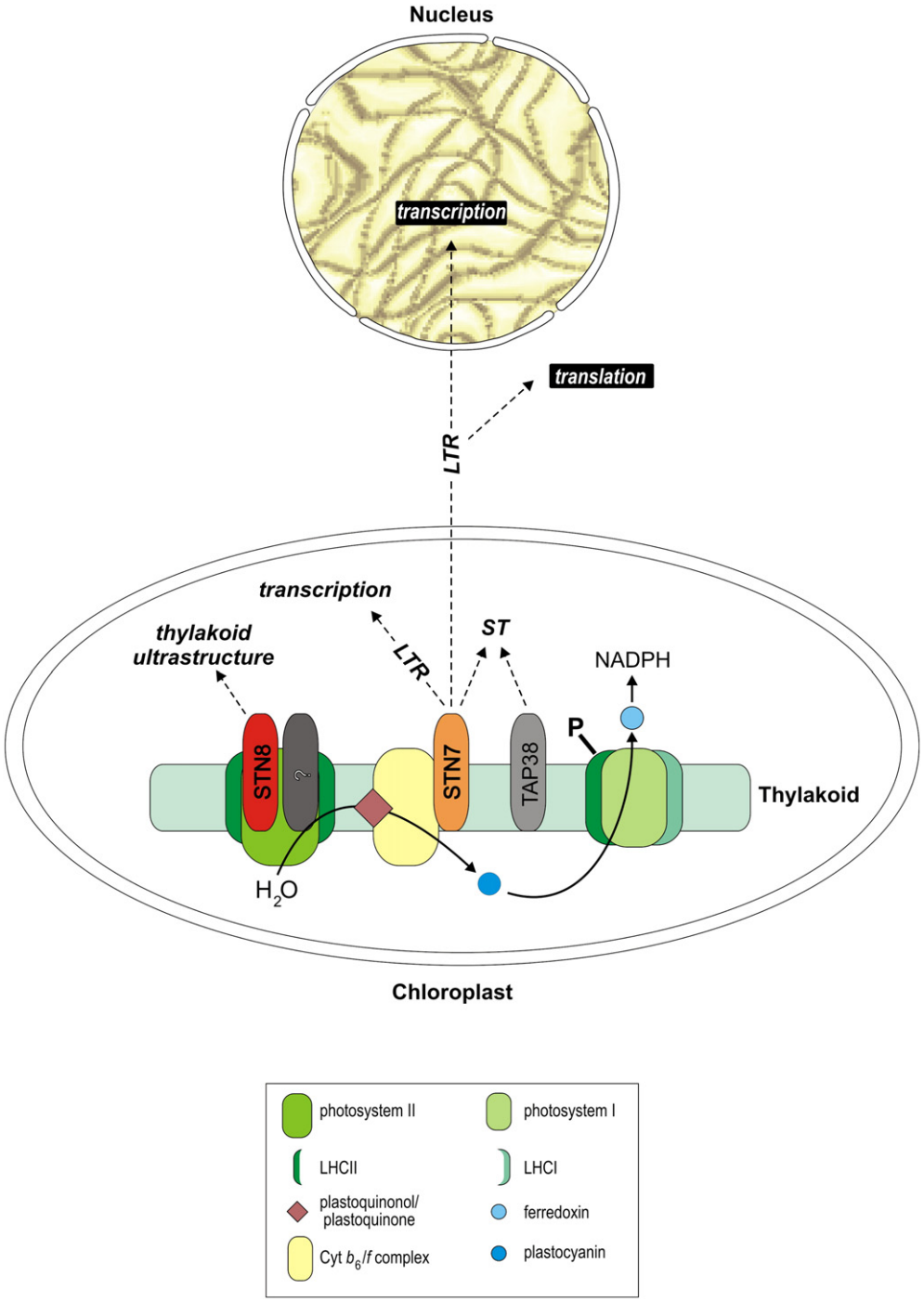

Fig. 1. Overview of thylakoid PKs and PPs and their physiological significance. The STN7 kinase is assumed to be activated by interaction with reduced Cyt b_6/f complex and to phosphorylate the mobile pool of LHCII (pLHCII) directly, thus favouring its migration to PSI and activating the State Transition (ST) 1→2. STN7 has been also reported to be part of the signalling pathway that initiates the long-term response (LTR), acting as a redox sensor aimed to optimise, both at short and long term, the thylakoid electron flow. The LTR involves the transcriptional regulation of chloroplast gene expression as well as the transcriptional and post-transcriptional regulation of nuclear genes [65]. The phosphatase TAP38/PPH1 has been shown to dephosphorylate pLHCII and to promote migration of the complex back to PSII, switching the system from State 2 to State 1. It is not yet known whether TAP38/PPH1 is also required for the LTR signalling pathway. Disruption of TAP38/PPH1 does not cause major changes in the dephosphorylation rate of PSII-core proteins, arguing in favour of the existence of an as yet unknown, PSII-specific, phosphatase (indicated by the symbol "?"). The STN8 kinase has a major role in controlling PSII supercomplex organisation and thylakoid ultrastructure through the phosphorylation of PSII-core proteins. Although STN7 and STN8 kinases account for almost all of the thylakoid protein phosphorylation, residual phosphorylation of thylakoid proteins was reported to occur in the *stn7 stn8* double mutant [12], which might indicate the existence of a third thylakoid-associated PK (not shown).

analyses of photosynthetic parameters, such as the chlorophyll *a*/*b* ratio and steady-state chlorophyll fluorescence, indicated that STN7 is also required for the LTR [16,31]. Thus it appears that the STN7 kinase represents a common redox sensor and/or signal transducer for both responses, supporting earlier suggestions that the two processes are subject to regulatory coupling [83]. In this context, the inability of *psad1-1* and *psae1-3* mutants, which both have reduced linear electron transport rates [44,45], to undergo the LTR appears to be a direct result of the fact that the PQ pool remains permanently reduced and, as a consequence, STN7 is constantly active and no longer subject to regulation by light conditions [65]. The regulatory coupling of State Transitions to the LTR and the dependence of both processes on STN7 activity are, in principle, compatible with the view that the signal pathways leading to State Transitions and LTR represent a

hierarchically organised signalling cascade, with changes in PQ redox state first triggering State Transitions and then the LTR via an STN7-dependent phosphorylation cascade. The LTR signalling pathway clearly does not directly depend on LHCII phosphorylation or on the conformational changes in the thylakoid associated with State Transitions themselves, as shown by the undiminished ability of Arabidopsis mutant lines that lack various components required for State Transitions to perform LTR [65]. Moreover, Arabidopsis RNAi lines devoid of the TSP9 protein, which is thought to dissociate partially from the thylakoid membrane upon phosphorylation and has been tentatively suggested to function in the signalling pathway [47,84], exhibit a normal LTR [65]. However, LTR signalling might involve stromal PKs that phosphorylate metabolic enzymes and proteins that regulate chloroplast gene expression. One attractive candidate is the casein kinase II (CKII), which is known to be regulated by phosphorylation, and is involved in the phosphorylation of proteins that act as transcriptional and post-transcription regulators [85-87]. A direct regulatory role in the transcription of the *psbA* and *psaA* genes was recently demonstrated for the "chloroplast sensor kinase" (CSK), a homologue of bacterial two-component sensor kinases [3]. In vitro, CSK has autophosphorylation activity that is dependent on reducing reagents. In contrast to CKII, no substrates are known for CSK. The latter could control transcription in chloroplasts directly by phosphorylating RNA polymerase subunits or sigma factors, or indirectly via cross-talk with CKII. Moreover, preliminary data on phosphorylation of chloroplast PKs by other kinases have been obtained from phosphoproteomics studies [13], which support the existence of phosphorylation signalling cascades in the organelle. For instance, STN7 itself has four phosphorylation sites near its C-terminus [13] (Table 1). Interestingly, this C-terminal region is not conserved between Chlamydomonas STT7 (where LTR has not been observed yet) and Arabidopsis STN7, suggesting that the C-terminal phosphorylation of STN7 might be part of the signalling pathway that triggers the LTR [20]. Moreover, the STN7 C-terminal phosphorylation sites differ in their sequence contexts, suggesting that multiple PKs–possibly including CKII–may act on STN7 [13]. Interestingly, microarray analyses have revealed no statistically significant differences in the levels of transcripts of photosynthesis genes between *stn7* and WT plants [31,65], indicating that the STN7 kinase activity as such does not directly affect the transcription of nuclear photosynthesis-related genes in Arabidopsis. Thus, it can be concluded that the STN7-dependent phosphorylation cascade might lead to the activation/deactivation of proteins involved in post-transcriptional events, including RNA maturation, RNA editing and protein translation, which could modulate the abundance of nucleus-encoded photosynthesis-related proteins [31,65].

5.4. STN8 activity influences D1 turnover

Apart from State Transitions and the LTR, the reversible phosphorylation of PSII core proteins has been regarded as one of the most important protective mechanisms in the thylakoid membranes, especially at high light intensities [88]. Under these conditions, the PSII kinase(s) should be highly active because (i) the PQ pool is in a highly reduced state and (ii) the enzyme (unlike the LHCII kinase) is intrinsically insensitive to inhibition through the ferredoxin/thioredoxin mechanism [16,18]. In particular, the oxidative chemistry of PSII, which is responsible for the water-splitting process in an environment where chlorophyll molecules carrying highly energised electrons are also present, readily leads to the formation of dangerously reactive radicals [89]. Because of its specific role in binding most of the cofactors required for electron transport in PSII, the D1 subunit is the main target for photodamage [90]. Core protein repair in PSII involves rapid turnover of D1, at a rate that is correlated with the level of PSII core protein phosphorylation. Indeed, it was hypothesised that damaged D1 is marked by phosphorylation, which then functions as a signal for migration of the complete PSII complex from grana to stroma lamellae, where D1 is degraded by the action of the proteases DegP and FtsH [91,92] (Fig. 1). Newly synthesised D1 protein is then cotranslationally inserted into existing PSII complexes in the stroma thylakoids. STN7 and/or STN8 would seem to be ideal candidates for the role of the kinase(s) that mark(s) damaged D1.

Recent studies on Arabidopsis plants devoid of either STN8 or STN7 or both enzymes simultaneously have challenged this model. Despite the dramatic reduction in phosphorylation of D1 and other PSII core proteins, *stn8* and *stn7 stn8* mutant plants did not show any alteration in D1 turnover [16]. In addition, *stn8* plants show WT-like rates of growth and seed production under both optimal greenhouse and field conditions [16,93]. These findings were interpreted as indicating (i) that inability to phosphorylate PSII proteins does not render PSII centres more susceptible to photoinhibition, and (ii) that the phosphorylation/dephosphorylation cycle is not crucial for D1 turnover and PSII repair, but rather might act to fine-tune this process. However, more recent work has demonstrated that, under illumination, the degradation of damaged D1 protein is actually retarded in the *stn8* and the *stn7 stn8* mutants compared to WT or *stn7* mutant plants, and this in turn was shown to result from defects in the disassembly of damaged PSII supercomplexes [52]. Under prolonged high light stress, the *stn7 stn8* mutants accumulate more damaged PSII proteins and undergo more oxidative stress than WT plants. The mechanism that facilitates the repair of PSII in WT relative to the *stn7 stn8* mutant was found to reside in the dynamics of PSII oligomerisation, which are enhanced by PSII core protein phosphorylation and permit rapid migration of photodamaged PSII to stroma membranes for detachment and repair of damaged D1 from the core complex [52]. Similar results were also obtained by Fristedt et al. [12], who showed that D1 turnover in the *stn8* and *stn7 stn8* mutants was slower than in WT plants. In addition, analysis of the ultrastructure of *stn8* and *stn7 stn8* thylakoids exposed to high light revealed a more compact thylakoid organisation with increased grana size in comparison to WT [12]. As a consequence, upon prolonged exposure to high light, the PSII complexes in the *stn7 stn8* mutant remained locked in dimers and even in supercomplexes, whereas they were nearly completely monomerised in the WT. Thus it appears that quantitative phosphorylation of PSII core proteins is required for large-scale rearrangements of the entire membrane network of plant thylakoids and for protein–protein interactions that facilitate the transfer of damaged PSII complexes to specialised sites for repair and recycling. In agreement with this idea, when fluorescence recovery after photobleaching in a laser-scanning confocal microscope was used to track the movement of chlorophyll proteins within thylakoid membranes, it emerged that a fraction of chlorophyll proteins is mobile in wild-type thylakoids and increases its mobility under high light conditions. These findings are compatible with a role for protein diffusion in the PSII repair cycle [94]. In *stn8* and *stn7 stn8* plants PSII proteins were less mobile under low light conditions, and no increase in mobility was observed when light intensities were increased. Clearly, the changes in protein mobility correlate with changes in the packing density and size of thylakoid protein complexes. Thus, protein phosphorylation switches the membrane system to a more fluid state that facilitates the PSII repair cycle.

Taking all the data together, it seems clear that PSII-core protein phosphorylation is not essential for D1 turnover, since this process can take place in plants totally devoid of STN kinases [12,16,52]. However, PSII-core phosphorylation appears to facilitate D1 turnover by increasing thylakoid fluidity, making it easier for damaged PSII cores to move from grana to stroma. This pathway appears to be predominant under the medium light intensities used by Tikkanen et al. [52] and Fristedt et al. [12] to induce photooxidative damages (about 1000 μmol photons m^{-2} s^{-1}), and might involve the FtsH protease, which requires the displacement of damaged PSII cores to stroma lamellae. However, under higher light intensities

(2000 μmol photons m^{-2} s^{-1}; [16]) an alternative D1 proteolysis pathway, controlled by Deg1, Deg5 and Deg8 proteases, might prevail. This alternative pathway should not be dependent on thylakoid structure, because the proteases are localised in the thylakoid lumen, thus explaining the discrepancies among the data reported above.

6. The physiological role of STN- and TAP38/PPH1-dependent regulatory mechanisms

In many respects, the physiological significance of the reversible phosphorylation of most thylakoid proteins remains unclear. This uncertainty largely stems from the absence of clear-cut phenotypes in mutants in which phosphorylation is perturbed when these are grown under optimal conditions.

6.1. State Transitions balance energy distribution in higher plants

While the physiological role of STT7 and State Transitions in *C. reinhardtii* is quite clear [19,66,67], the importance of State Transitions in flowering plants has been a matter of intense debate. Under greenhouse conditions, loss of *STN7* function provokes no obvious phenotype, and the elimination of State Transitions has only marginal effects on the development and fitness of mutant plants grown under fluctuating light or under field conditions, where the ability to adapt to changes in illumination would be expected to have an impact on performance [15,16,93].

The importance of State Transitions in flowering plants has recently been reinvestigated using a genetic approach. Mutations that affect linear electron transport and are associated with an increased pool of reduced PQ, including *psad1-1* and *psae1-3*, were introduced into either the *stn7* or *psal* genetic backgrounds [65]. In all cases, the double mutants exhibited a marked decrease in growth rate relative to the parental single mutants. In addition, a consistent drop in the effective quantum yield of PSII and an increase in the reduction state of the PQ pool are observed, implying that State Transitions become critical for plant performance when linear electron flow is perturbed. Interestingly, in higher plants, unlike the case in *C. reinhardtii*, State Transitions are not strictly linked to cyclic electron flow around PSI. Indeed, under State 2 conditions no increase in cyclic relative to linear electron transport could be observed in Arabidopsis plants, possibly because the relative size of the pLHCII pool that moves between the two photosystems in Arabidopsis is much smaller (20%) than in Chlamydomonas (80%) [65]. The analysis of *tap38/pph1* mutant lines further corroborated the role of State Transitions in light energy redistribution among photosystems and optimisation of linear electron flow. Indeed *tap38/pph1* mutants, which are permanently locked into State 2, are characterised by the fact that a larger fraction of PSI complexes is associated with pLHCII than in WT plants [4]. As a consequence, under State 2-promoting conditions (greenhouse light conditions), *tap38/pph1* plants show improved linear electron transport–a larger fraction of PQ is oxidised and the effective quantum yield of PSII is higher–and an increase in growth rate.

6.2. LTR adjusts photosystem stoichiometry in response to changes in ambient light

Like State Transitions, the LTR alters thylakoid electron flows to accommodate changes in illumination, although it operates over a longer time-scale. Here, STN7-mediated protein phosphorylation is thought to be part of a signalling cascade that ultimately readjusts the stoichiometry of thylakoid protein complexes according to ambient light conditions. Thus, when *stn7* plants were shifted between PSI and PSII light every 2–3 days (a period which allows for LTR), they produced 50% fewer seeds than wild-type plants [82]. Thus it appears that LTR, as well as State Transitions, might provide a degree of metabolic flexibility which allows photosynthetic organisms to acclimate both to long- and short-period changes in light quality, and thus enables them to adapt to the wide range of light fluctuations that occur in a natural environment.

6.3. STN8-mediated phosphorylation of core proteins facilitates the repair of damaged PSII

Although phosphorylation of PSII-core proteins has been shown to influence thylakoid organisation and PSII ultrastructure, thus facilitating turnover of D1, the physiological importance of STN8-dependent PSII phosphorylation remains difficult to assess. Bonardi et al. [16] observed only a marginal decrease in PSII activity in *stn8* and *stn7 stn8* mutants after prolonged exposure to high light levels. A 24-hour exposure of *stn7 stn8* plants to 1000 μmol photons m^{-2} s^{-1} has a similarly mild effect [52]. In addition, disruption of *STN8* in *psae1-3* and *psad1-1* backgrounds did not result in double mutants with exacerbated phenotypes [65], while *stn8* plants grown under natural conditions in the field showed WT-like seed production [93]. These observations, together with the fact that loss of the STN8 kinase does not cause any alteration in growth rate, make it difficult to attribute a physiological role to STN8 kinase activity. Clearly, molecular mechanisms exist that compensate for the absence of STN8 and deserve further investigation.

7. Conclusions

Since 1977, when Bennet for the first time reported the phosphorylation of LHCII subunits [21], the number of phosphorylated thylakoid proteins has been increasing, and currently 28 thylakoid proteins are known to be phosphorylated under different environmental conditions. However, it cannot be excluded that some thylakoid phosphoproteins identified by sensitive MS methods accumulate only in minuscule amounts and represent non-physiological substrates of thylakoid PKs. A large fraction of thylakoid phosphoproteins exhibit multiple phosphorylation sites, suggesting that they might act as integrators of various environmental signals. Despite the large number of phosphoproteins identified, our knowledge of the physiological impact of thylakoid phosphorylation on plant performance is far from complete. Clarification of the physiological relevance of the phosphorylation of thylakoid proteins is certainly the main challenge for researchers in the field. The characterisation of loss-of-function lines lacking the enzymes involved, as well as the mutation of phosphorylatable sites, would seem to be the methods of choice for this task. Indeed, the identification of PKs and PPs responsible for the reversible phosphorylation of thylakoid proteins, together with detailed biochemical and physiological analyses of the corresponding mutants, will provide the direct links between enzymes and substrates, and between phosphoproteins and plant performance.

The characterisation of *stn7* and *tap38/pph1* mutants has revealed the importance of State Transitions in higher plants, whereas the investigation of *stn8* plants has indicated a role for PSII-core phosphorylation in determining thylakoid ultrastructure, although further analyses are needed before its physiological significance can be understood. In addition, phosphoproteome analyses suggest that additional thylakoid PKs and PPs should exist, thus encouraging large-scale reverse genetic approaches for their identification. The major difficulty in attributing a function to protein phosphorylation arises from the fact that plants devoid of a given kinase, phosphatase or phosphorylation site of interest often behave like the WT. The very subtle phenotypic alterations observed in Arabidopsis plants devoid of both STN7 and STN8–which show a drastic decrease in thylakoid phosphorylation–underscore the difficulties in dissecting the physiological significance of the reversible phosphorylation of individual thylakoid phosphoproteins. Characterisation, under field conditions,

of plants that lack the phosphorylation site(s) of specific phosphoproteins appears to offer a promising strategy for dissecting their respective roles. Moreover, the comprehensive characterisation of the chloroplast phosphoproteome from different PK and PP mutants with quantitative proteomics tools should reveal further targets of the enzymes, possibly identifying components of phosphorylation cascades.

The development of more sensitive MS-based technologies for phosphoproteomics studies, together with the identification of further thylakoid-associated PKs and PPs, should further improve our understanding of the signalling networks underlying short- and long-term acclimation processes to changing environmental light conditions. Dissection of the interactions of these signalling components is fundamental for understanding the molecular details of the regulation of photosynthesis and related thylakoid functions.

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References

[1] J.V. Olsen, B. Blagoev, F. Gnad, B. Macek, C. Kumar, P. Mortensen, M. Mann, Global, in vivo, and site-specific phosphorylation dynamics in signaling networks, Cell 127 (2006) 635–648.
[2] I. Schliebner, M. Pribil, J. Zühlke, A. Dietzmann, D. Leister, A survey of chloroplast protein kinases and phosphatases in *Arabidopsis thaliana*, Curr. Genomics 9 (2008) 184–190.
[3] S. Puthiyaveetil, T.A. Kavanagh, P. Cain, J.A. Sullivan, C.A. Newell, J.C. Gray, C. Robinson, M. van der Giezen, M.B. Rogers, J.F. Allen, The ancestral symbiont sensor kinase CSK links photosynthesis with gene expression in chloroplasts, Proc. Natl Acad. Sci. USA 105 (2008) 10061–10066.
[4] M. Pribil, P. Pesaresi, A. Hertle, R. Barbato, D. Leister, Role of plastid protein phosphatase TAP38 in LHCII dephosphorylation and thylakoid electron flow, PLoS Biol. 8 (2010) e1000288.
[5] A. Shapiguzov, B. Ingelsson, I. Samol, C. Andres, F. Kessler, J.D. Rochaix, A.V. Vener, M. Goldschmidt-Clermont, The PPH1 phosphatase is specifically involved in LHCII dephosphorylation and state transitions in Arabidopsis, Proc. Natl Acad. Sci. USA 107 (2010) 4782–4787.
[6] M.B. Goshe, T.P. Conrads, E.A. Panisko, N.H. Angell, T.D. Veenstra, R.D. Smith, Phosphoprotein isotope-coded affinity tag approach for isolating and quantitating phosphopeptides in proteome-wide analyses, Anal. Chem. 73 (2001) 2578–2586.
[7] Y. Oda, T. Nagasu, B.T. Chait, Enrichment analysis of phosphorylated proteins as a tool for probing the phosphoproteome, Nat. Biotechnol. 19 (2001) 379–382.
[8] H. Zhou, J.D. Watts, R. Aebersold, A systematic approach to the analysis of protein phosphorylation, Nat. Biotechnol. 19 (2001) 375–378.
[9] S. de la Fuente, D. van Bentem, I. Dohnal Anrather, E. Roitinger, E. Csaszar, J. Joore, J. Buijnink, A. Carreri, C. Forzani, Z.J. Lorkovic, A. Barta, D. Lecourieux, A. Verhounig, C. Jonak, H. Hirt, Site-specific phosphorylation profiling of Arabidopsis proteins by mass spectrometry and peptide chip analysis, J. Proteome Res. 7 (2008) 2458–2470.
[10] N. Sugiyama, H. Nakagami, K. Mochida, A. Daudi, M. Tomita, K. Shirasu, Y. Ishihama, Large-scale phosphorylation mapping reveals the extent of tyrosine phosphorylation in Arabidopsis, Mol. Syst. Biol. 4 (2008) 193.
[11] A.V. Vener, Environmentally modulated phosphorylation and dynamics of proteins in photosynthetic membranes, Biochim. Biophys. Acta 1767 (2007) 449–457.
[12] R. Fristedt, A. Willig, P. Granath, M. Crevecoeur, J.D. Rochaix, A.V. Vener, Phosphorylation of photosystem II controls functional macroscopic folding of photosynthetic membranes in Arabidopsis, Plant Cell 21 (2009) 3950–3964.
[13] S. Reiland, G. Messerli, K. Baerenfaller, B. Gerrits, A. Endler, J. Grossmann, W. Gruissem, S. Baginsky, Large-scale Arabidopsis phosphoproteome profiling reveals novel chloroplast kinase substrates and phosphorylation networks, Plant Physiol. 150 (2009) 889–903.
[14] K. Lohrig, B. Müller, J. Davydova, D. Leister, D.A. Wolters, Phosphorylation site mapping of soluble proteins: bioinformatical filtering reveals potential plastidic phosphoproteins in *Arabidopsis thaliana*, Planta 229 (2009) 1123–1134.
[15] S. Bellafiore, F. Barneche, G. Peltier, J.D. Rochaix, State transitions and light adaptation require chloroplast thylakoid protein kinase STN7, Nature 433 (2005) 892–895.
[16] V. Bonardi, P. Pesaresi, T. Becker, E. Schleiff, R. Wagner, T. Pfannschmidt, P. Jahns, D. Leister, Photosystem II core phosphorylation and photosynthetic acclimation require two different protein kinases, Nature 437 (2005) 1179–1182.
[17] N. Depege, S. Bellafiore, J.D. Rochaix, Role of chloroplast protein kinase Stt7 in LHCII phosphorylation and state transition in Chlamydomonas, Science 299 (2003) 1572–1575.
[18] J.P. Vainonen, M. Hansson, A.V. Vener, STN8 protein kinase in *Arabidopsis thaliana* is specific in phosphorylation of photosystem II core proteins, J. Biol. Chem. 280 (2005) 33679–33686.
[19] J.D. Rochaix, Role of thylakoid protein kinases in photosynthetic acclimation, FEBS Lett. 581 (2007) 2768–2775.
[20] S. Lemeille, J.D. Rochaix, State transitions at the crossroad of thylakoid signalling pathways, Photosynth Res. 106 (2010) 33–46.
[21] J. Bennett, Phosphorylation of chloroplast membrane polypeptides, Nature 269 (1977) 344–446.
[22] J. Bennett, Protein phosphorylation in green plant chloroplasts, Annu. Rev. Plant Physiol. Plant Mol. Biol. 42 (1991) 281–311.
[23] K. Islam, The rate and extent of phosphorylation of the two light-harvesting chlorophyll a/b binding protein complex (LHC-II) polypeptides in isolated thylakoids, Biochim. Biophys. Acta 893 (1987) 333–341.
[24] E. Rintamaki, M. Salonen, U.M. Suoranta, I. Carlberg, B. Andersson, E.M. Aro, 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 (1997) 30476–30482.
[25] E. Bergantino, P. Dainese, Z. Cerovic, S. Sechi, R. Bassi, A post-translational modification of the photosystem II subunit CP29 protects maize from cold stress, J. Biol. Chem. 270 (1995) 8474–8481.
[26] E. Bergantino, D. Sandona, D. Cugini, R. Bassi, The photosystem II subunit CP29 can be phosphorylated in both C3 and C4 plants as suggested by sequence analysis, Plant Mol. Biol. 36 (1998) 11–22.
[27] M.G. Testi, R. Croce, P. Polverino-De Laureto, R. Bassi, A CK2 site is reversibly phosphorylated in the photosystem II subunit CP29, FEBS Lett. 399 (1996) 245–250.
[28] S. Pursiheimo, P. Mulo, E. Rintamaki, E.M. Aro, Coregulation of light-harvesting complex II phosphorylation and *lhcb* mRNA accumulation in winter rye, Plant J. 26 (2001) 317–327.
[29] W.J. Liu, Y.E. Chen, W.J. Tian, J.B. Du, Z.W. Zhang, F. Xu, F. Zhang, S. Yuan, H.H. Lin, Dephosphorylation of photosystem II proteins and phosphorylation of CP29 in barley photosynthetic membranes as a response to water stress, Biochim. Biophys. Acta 1787 (2009) 1238–1245.
[30] M. Hansson, A.V. Vener, Identification of three previously unknown in vivo protein phosphorylation sites in thylakoid membranes of *Arabidopsis thaliana*, Mol. Cell. Proteomics 2 (2003) 550–559.
[31] M. Tikkanen, M. Piippo, M. Suorsa, S. Sirpio, P. Mulo, J. Vainonen, A.V. Vener, Y. Allahverdiyeva, E.M. Aro, State transitions revisited-a buffering system for dynamic low light acclimation of Arabidopsis, Plant Mol. Biol. 62 (2006) 779–793.
[32] M. Salonen, E.M. Aro, E. Rintamaki, Reversible phosphorylation and turnover of the D1 protein under various redox states of Photosystem II induced by low temperature photoinhibition, Photosynth. Res. 58 (1998) 143–151.
[33] H. Michel, D.F. Hunt, J. Shabanowitz, J. Bennett, Tandem mass spectrometry reveals that three photosystem II proteins of spinach chloroplasts contain N-acetyl-O-phosphothreonine at their NH_2 termini, J. Biol. Chem. 263 (1988) 1123–1130.
[34] S. Rinalducci, M.R. Larsen, S. Mohammed, L. Zolla, Novel protein phosphorylation site identification in spinach stroma membranes by titanium dioxide microcolumns and tandem mass spectrometry, J. Proteome Res. 5 (2006) 973–982.
[35] H.P. Michel, J. Bennet, Identification of the phosphorylation site of an 8.3 kDa protein from photosystem II of spinach, FEBS Lett. 212 (1987) 103–108.
[36] E. Baena-Gonzalez, R. Barbato, E.M. Aro, Role of phosphorylation in the repair cycle and oligomeric structure of photosystem II, Planta 208 (1999) 196–204.
[37] M.T. Giardi, A. Cona, B. Geiken, T. Kucera, J. Masojidek, A.K. Mattoo, Long-term drought stress induces structural and functional reorganization of photosystem II, Planta 199 (1996) 118–125.
[38] H. Dannehl, A. Herbik, D. Godde, Stress-induced degradation of the photosynthetic apparatus is accompanied by changes in thylakoid protein-turnover and phosphorylation, Physiol. Plant. 93 (1995) 179–186.
[39] W.W. Adams 3rd, B. Demmig-Adams, T.N. Rosenstiel, V. Ebbert, Dependence of photosynthesis and energy dissipation activity upon growth form and light environment during the winter, Photosynth. Res. 67 (2001) 51–62.
[40] V. Ebbert, B. Demmig-Adams, W.W. Adams 3rd, K.E. Mueh, L.A. Staehelin, Correlation between persistent forms of zeaxanthin-dependent energy dissipation and thylakoid protein phosphorylation, Photosynth. Res. 67 (2001) 63–78.
[41] A. Rokka, E.M. Aro, R.G. Herrmann, B. Andersson, A.V. Vener, Dephosphorylation of photosystem II reaction center proteins in plant photosynthetic membranes as an immediate response to abrupt elevation of temperature, Plant Physiol. 123 (2000) 1525–1536.
[42] A. Ihnatowicz, P. Pesaresi, K. Lohrig, D. Wolters, B. Müller, D. Leister, Impaired photosystem I oxidation induces STN7-dependent phosphorylation of the light-harvesting complex I protein Lhca4 in *Arabidopsis thaliana*, Planta 227 (2008) 717–722.
[43] A. Khrouchtchova, M. Hansson, V. Paakkarinen, J.P. Vainonen, S. Zhang, P.E. Jensen, H.V. Scheller, A.V. Vener, E.M. Aro, A. Haldrup, A previously found thylakoid membrane protein of 14 kDa (TMP14) is a novel subunit of plant photosystem I and is designated PSI-P, FEBS Lett. 579 (2005) 4808–4812.
[44] A. Ihnatowicz, P. Pesaresi, C. Varotto, E. Richly, A. Schneider, P. Jahns, F. Salamini, D. Leister, Mutants for photosystem I subunit D of *Arabidopsis thaliana*: effects on photosynthesis, photosystem I stability and expression of nuclear genes for chloroplast functions, Plant J. 37 (2004) 839–852.
[45] A. Ihnatowicz, P. Pesaresi, D. Leister, The E subunit of photosystem I is not essential for linear electron flow and photoautotrophic growth in *Arabidopsis thaliana*, Planta 226 (2007) 889–895.

[46] G. del Riego, L.M. Casano, M. Martin, B. Sabater, Multiple phosphorylation sites in the beta subunit of thylakoid ATP synthase, Photosynth. Res. 89 (2006) 11–18.
[47] I. Carlberg, M. Hansson, T. Kieselbach, W.P. Schroder, B. Andersson, A.V. Vener, A novel plant protein undergoing light-induced phosphorylation and release from the photosynthetic thylakoid membranes, Proc. Natl Acad. Sci. USA 100 (2003) 757–762.
[48] J.P. Vainonen, Y. Sakuragi, S. Stael, M. Tikkanen, Y. Allahverdiyeva, V. Paakkarinen, E. Aro, M. Suorsa, H.V. Scheller, A.V. Vener, E.M. Aro, Light regulation of CaS, a novel phosphoprotein in the thylakoid membrane of *Arabidopsis thaliana*, FEBS J. 275 (2008) 1767–1777.
[49] A.V. Vener, A. Harms, M.R. Sussman, R.D. Vierstra, Mass spectrometric resolution of reversible protein phosphorylation in photosynthetic membranes of *Arabidopsis thaliana*, J. Biol. Chem. 276 (2001) 6959–6966.
[50] S. Lemeille, A. Willig, N. Depege-Fargeix, C. Delessert, R. Bassi, J.D. Rochaix, Analysis of the chloroplast protein kinase Stt7 during state transitions, PLoS Biol. 7 (2009) e45.
[51] M. Tikkanen, M. Nurmi, M. Suorsa, R. Danielsson, F. Mamedov, S. Styring, E.M. Aro, Phosphorylation-dependent regulation of excitation energy distribution between the two photosystems in higher plants, Biochim. Biophys. Acta 1777 (2008) 425–432.
[52] M. Tikkanen, M. Nurmi, S. Kangasjarvi, E.M. Aro, Core protein phosphorylation facilitates the repair of photodamaged photosystem II at high light, Biochim. Biophys. Acta 1777 (2008) 1432–1437.
[53] R. Fristedt, I. Carlberg, A. Zygadlo, M. Piippo, M. Nurmi, E.M. Aro, H.V. Scheller, A.V. Vener, Intrinsically unstructured phosphoprotein TSP9 regulates light harvesting in *Arabidopsis thaliana*, Biochemistry 48 (2009) 499–509.
[54] H. Michel, P.R. Griffin, J. Shabanowitz, D.F. Hunt, J. Bennett, Tandem mass spectrometry identifies sites of three post-translational modifications of spinach light-harvesting chlorophyll protein II. Proteolytic cleavage, acetylation, and phosphorylation, J. Biol. Chem. 266 (1991) 17584–17591.
[55] S. Lemeille, M.V. Turkina, A.V. Vener, J.D. Rochaix, Stt7-dependent phosphorylation during state transitions in the green alga *Chlamydomonas reinhardtii*, Mol. Cell. Proteomics 9 (2010) 1281–12895.
[56] M.F. Hammer, G. Sarath, J. Markwell, Dephosphorylation of the thylakoid membrane light-harvesting complex-II by a stromal protein phosphatase, Photosynth. Res. 45 (1995) 195–201.
[57] J. Bennett, Chloroplast phosphoproteins. Evidence for a thylakoid-bound phosphoprotein phosphatase, Eur. J. Biochem. 104 (1980) 85–89.
[58] M.F. Hammer, J. Markwell, G. Sarath, Purification of a protein phosphatase from chloroplast stroma capable of dephosphorylating the light-harvesting complex-II, Plant Physiol. 113 (1997) 227–233.
[59] M.F. Hammer, G. Sarath, J.C. Osterman, J. Markwell, Assesing modulations of stromal and thalkoid light-harvesting complex-II phosphatase activites with phosphopeptide substrates, Photosynth. Res. 44 (1995) 107–115.
[60] T. Hast, H. Follmann, Identification of two thylakoid-associated phosphatases with protein phosphatase activity in chloroplasts of the soybean (*Glycine max*), J. Photochem. Photobiol. B-Biology 36 (1996) 313–319.
[61] G. Sun, D. Bailey, M.W. Jones, J. Markwell, Chloroplast thylakoid protein phosphatase is a membrane surface-associated activity, Plant Physiol. 89 (1989) 238–243.
[62] P.T. Cohen, Novel protein serine/threonine phosphatases: variety is the spice of life, Trends Biochem. Sci. 22 (1997) 245–251.
[63] P. Cohen, The structure and regulation of protein phosphatases, Annu. Rev. Biochem. 58 (1989) 453–508.
[64] A.V. Vener, A. Rokka, H. Fulgosi, B. Andersson, R.G. Herrmann, A cyclophilin-regulated PP2A-like protein phosphatase in thylakoid membranes of plant chloroplasts, Biochemistry 38 (1999) 14955–14965.
[65] P. Pesaresi, A. Hertle, M. Pribil, T. Kleine, R. Wagner, H. Strissel, A. Ihnatowicz, V. Bonardi, M. Scharfenberg, A. Schneider, T. Pfannschmidt, D. Leister, Arabidopsis STN7 kinase provides a link between short- and long-term photosynthetic acclimation, Plant Cell 21 (2009) 2402–2423.
[66] F.A. Wollman, State transitions reveal the dynamics and flexibility of the photosynthetic apparatus, EMBO J. 20 (2001) 3623–3630.
[67] S. Eberhard, G. Finazzi, F.A. Wollman, The dynamics of photosynthesis, Annu. Rev. Genet. 42 (2008) 463–515.
[68] C. Bonaventura, J. Myers, Fluorescence and oxygen evolution from *Chlorella pyrenoidosa*, Biochim. Biophys. Acta 189 (1969) 366–383.
[69] N. Murata, Control of excitation transfer in photosynthesis. I. Light-induced change of chlorophyll a fluorescence in *Porphyridium cruentum*, Biochim. Biophys. Acta 172 (1969) 242–251.
[70] F. Zito, G. Finazzi, R. Delosme, W. Nitschke, D. Picot, F.A. Wollman, The Qo site of cytochrome b_6f complexes controls the activation of the LHCII kinase, EMBO J. 18 (1999) 2961–2969.
[71] G. Finazzi, F. Zito, R.P. Barbagallo, F.A. Wollman, Contrasted effects of inhibitors of cytochrome b_6f complex on state transitions in *Chlamydomonas reinhardtii*—the role of Q_o site occupancy in LHCII kinase activation, J. Biol. Chem. 276 (2001) 9770–9774.
[72] E. Rintamaki, P. Martinsuo, S. Pursiheimo, E.M. Aro, Cooperative regulation of light-harvesting complex II phosphorylation via the plastoquinol and ferredoxin-thioredoxin system in chloroplasts, Proc. Natl Acad. Sci. USA 97 (2000) 11644–11649.
[73] C.X. Hou, E. Rintamaki, E.M. Aro, Ascorbate-mediated LHCII protein phosphorylation–LHCII kinase regulation in light and in darkness, Biochemistry 42 (2003) 5828–5836.
[74] M.L. Page, P.P. Hamel, S.T. Gabilly, H. Zegzouti, J.V. Perea, J.M. Alonso, J.R. Ecker, S.M. Theg, S.K. Christensen, S. Merchant, 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 (2004) 32474–32482.
[75] K. Lennartz, H. Plucken, A. Seidler, P. Westhoff, N. Bechtold, K. Meierhoff, HCF164 encodes a thioredoxin-like protein involved in the biogenesis of the cytochrome b_6f complex in Arabidopsis, Plant Cell 13 (2001) 2539–2551.
[76] K. Motohashi, T. Hisabori, HCF164 receives reducing equivalents from stromal thioredoxin across the thylakoid membrane and mediates reduction of target proteins in the thylakoid lumen, J. Biol. Chem. 281 (2006) 35039–35047.
[77] P. Pesaresi, C. Lunde, P. Jahns, D. Tarantino, J. Meurer, C. Varotto, R.D. Hirtz, C. Soave, H.V. Scheller, F. Salamini, D. Leister, A stable LHCII-PSI aggregate and suppression of photosynthetic state transitions in the *psae1-1* mutant of *Arabidopsis thaliana*, Planta 215 (2002) 940–948.
[78] J. Kargul, M.V. Turkina, J. Nield, S. Benson, A.V. Vener, J. Barber, Light-harvesting complex II protein CP29 binds to photosystem I of *Chlamydomonas reinhardtii* under State 2 conditions, FEBS J. 272 (2005) 4797–4806.
[79] H. Takahashi, M. Iwai, Y. Takahashi, J. Minagawa, Identification of the mobile light-harvesting complex II polypeptides for state transitions in *Chlamydomonas reinhardtii*, Proc. Natl Acad. Sci. USA 103 (2006) 477–482.
[80] M. Hansson, T. Dupuis, R. Stromquist, B. Andersson, A.V. Vener, I. Carlberg, The mobile thylakoid phosphoprotein TSP9 interacts with the light-harvesting complex II and the peripheries of both photosystems, J. Biol. Chem. 282 (2007) 16214–16222.
[81] L. Dietzel, K. Brautigam, T. Pfannschmidt, Photosynthetic acclimation: state transitions and adjustment of photosystem stoichiometry—functional relationships between short-term and long-term light quality acclimation in plants, FEBS J. 275 (2008) 1080–1088.
[82] R. Wagner, L. Dietzel, K. Brautigam, W. Fischer, T. Pfannschmidt, The long-term response to fluctuating light quality is an important and distinct light acclimation mechanism that supports survival of *Arabidopsis thaliana* under low light conditions, Planta 228 (2008) 573–587.
[83] J.F. Allen, T. Pfannschmidt, Balancing the two photosystems: photosynthetic electron transfer governs transcription of reaction centre genes in chloroplasts, Philos. Trans. R. Soc. Lond. B Biol. Sci. 355 (2000) 1351–1359.
[84] H. Zer, I. Ohad, Light, redox state, thylakoid–protein phosphorylation and signaling gene expression, Trends Biochem. Sci. 28 (2003) 467–470.
[85] S. Baginsky, K. Tiller, G. Link, Transcription factor phosphorylation by a protein kinase associated with chloroplast RNA polymerase from mustard (*Sinapis alba*), Plant Mol. Biol. 34 (1997) 181–189.
[86] S. Baginsky, K. Tiller, T. Pfannschmidt, G. Link, PTK, the chloroplast RNA polymerase-associated protein kinase from mustard (*Sinapis alba*), mediates redox control of plastid in vitro transcription, Plant Mol. Biol. 39 (1999) 1013–1023.
[87] K. Ogrzewalla, M. Piotrowski, S. Reinbothe, G. Link, The plastid transcription kinase from mustard (*Sinapis alba* L.). A nuclear-encoded CK2-type chloroplast enzyme with redox-sensitive function, Eur. J. Biochem. 269 (2002) 3329–3337.
[88] E. Kanervo, M. Suorsa, E.M. Aro, Functional flexibility and acclimation of the thylakoid membrane, Photochem. Photobiol. Sci. 4 (2005) 1072–1080.
[89] M. Rogner, E.J. Boekema, J. Barber, How does photosystem 2 split water? The structural basis of efficient energy conversion, Trends Biochem. Sci. 21 (1996) 44–49.
[90] A. Rokka, M. Suorsa, A. Saleem, N. Battchikova, E.M. Aro, Synthesis and assembly of thylakoid protein complexes: multiple assembly steps of photosystem II, Biochem. J. 388 (2005) 159–168.
[91] P.F. Huesgen, H. Schuhmann, I. Adamska, The family of Deg proteases in cyanobacteria and chloroplasts of higher plants, Physiol. Plant. 123 (2005) 413–420.
[92] M. Lindahl, C. Spetea, T. Hundal, A.B. Oppenheim, Z. Adam, B. Andersson, The thylakoid FtsH protease plays a role in the light-induced turnover of the photosystem II D1 protein, Plant Cell 12 (2000) 419–431.
[93] M. Frenkel, S. Bellafiore, J.D. Rochaix, S. Jansson, Hierarchy amongst photosynthetic acclimation responses for plant fitness, Physiol. Plant. 129 (2007) 455–459.
[94] T.K. Goral, M.P. Johnson, A.P. Brain, H. Kirchhoff, A.V. Ruban, C.W. Mullineaux, Visualising the mobility and distribution of chlorophyll-proteins in higher plant thylakoid membranes: effects of photoinhibition and protein phosphorylation, Plant J. 62 (2010) 948–959.