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Review

Environmentally modulated phosphorylation and dynamics of proteins in photosynthetic membranes

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Abstract

Recent advances in vectorial proteomics of protein domains exposed to the surface of photosynthetic thylakoid membranes of plants and the green alga *Chlamydomonas reinhardtii* allowed mapping of in vivo phosphorylation sites in integral and peripheral membrane proteins. In plants, significant changes of thylakoid protein phosphorylation are observed in response to stress, particularly in photosystem II under high light or high temperature stress. Thylakoid protein phosphorylation in the algae is much more responsive to the ambient redox and light conditions, as well as to CO_2 availability. The light-dependent multiple and differential phosphorylation of CP29 linker protein in the green algae is suggested to control photosynthetic state transitions and uncoupling of light harvesting proteins from photosystem II under high light. The similar role for regulation of the dynamic distribution of light harvesting proteins in plants is proposed for the TSP9 protein, which together with other recently discovered peripheral proteins undergoes specific environment- and redox-dependent phosphorylation at the thylakoid surface. This review focuses on the environmentally modulated reversible phosphorylation of thylakoid proteins related to their membrane dynamics and affinity towards particular photosynthetic protein complexes.

Keywords: Light harvesting; Photosynthesis; Photosystem II; Protein phosphorylation; Stress responses; Thylakoid membrane

1. Introduction

The light-induced phosphorylation of several proteins in thylakoid photosynthetic membranes of plant chloroplasts was discovered by J. Bennett in 1977 [1]. The major phosphorylated proteins have been identified as D1, D2, CP43 and PsbH subunits of photosystem II (PSII) as well as a few polypeptides belonging to the light harvesting complex (LHCII) of this photosystem. Reversible phosphorylation of these membrane proteins was found to be light- and redox-regulated in chloroplasts or thylakoids isolated from different eukaryotic species performing oxygenic photosynthesis [2–4]. Application of biochemical techniques detecting in vivo phosphorylated proteins has also revealed significant and differential environment-dependent changes in phosphorylation of the photosynthetic proteins, particularly in stressful conditions. Pronounced phosphorylation of PSII polypeptides has been found in plants and green algae subjected to high light stress [5–8], as well as in plants exposed to drought stress [9]. The dark-sustained phosphorylation of the PSII reaction centre proteins in response to combined magnesium and sulphur deficiency [10], subfreezing temperatures [11], or high light stress [12] has also been documented. On the contrary, extremely fast dephosphorylation of the D1, D2 and CP43 proteins was identified in plant leaves as an immediate response to abrupt elevation of temperature [13,14]. Additionally, circadian oscillation of D1 protein phosphorylation was revealed and the phase of this oscillation was resettled by light [15]. Specific phosphorylation of CP29, the minor light-harvesting protein of PSII, has been discovered in plants subjected to cold stress [16,17] and combined high light and cold treatment [18]. Rapid phosphorylation of a few proteins at the surface of thylakoid membranes has recently been found in the cells of green alga exposed to environment with a limiting carbon dioxide [19]. These differential changes in thylakoid protein phosphorylation under variable environment are implicated in a number of regulatory and adaptive responses of the photosynthetic apparatus [20], however the

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molecular mechanisms of these regulatory events remain largely unknown.

In a few recent years we have witnessed dramatic increase in the knowledge of the structural and molecular details of photosynthetic machinery and its functioning. Efficient applications of forward and reverse genetics revealed numerous previously unknown components of photosynthetic protein complexes and regulatory elements involved in their biogenesis and regulation. The field of protein phosphorylation has been significantly advanced by the discovery and characterization of several protein kinases involved in phosphorylation of thylakoid proteins [21–27]. The novel detection techniques, particularly vectorial proteomics [28], have lifted the understanding of protein phosphorylation to the real molecular level of the exact amino acid residues undergoing modifications in the proteins. Only during the recent months the number of identified in vivo phosphorylation sites in the proteins from thylakoid membranes has doubled [8,19,29,30]. The combination of in vivo protein phosphorylation mapping with the advanced techniques for isolation and characterization of photosynthetic protein complexes from the cells or leaf tissues exposed to distinct environmental conditions allows unraveling of molecular details of photosynthetic regulation and adaptation. In this paper I analyze the progress in the field of thylakoid protein phosphorylation in the frame of the currently emerging paradigm connecting environmentally induced differential and reversible phosphorylation with protein dynamics and changing affinities towards the various membrane protein complexes.

2. Vectorial proteomics of thylakoid membranes

Vectorial proteomics is a methodology for the differential identification and characterization of proteins and their domains exposed to the opposite sides of biological membranes [28]. This approach was originally introduced for characterization of protein phosphorylation in thylakoid membranes of *Arabidopsis thaliana* [14]. Phosphorylation is an intrinsically hydrophilic process restricted to the surface-exposed domains of membrane proteins and to peripheral proteins attached to the membrane surface (Fig. 1). To surpass the notoriously difficult task of membrane protein purification prior to their biochemical characterization, vectorial proteomics uses proteolytic shaving of the hydrophilic domains exposed to the surface of uniformly oriented membrane vesicles (Fig. 1). Particularly, the treatment of isolated thylakoids with trypsin removes from the membrane phosphorylated peptides of all major phosphorylated proteins, as revealed by Western blotting with anti-phosphothreonine antibodies [8,14,31]. The soluble peptides are collected in the supernatant after centrifugation and usually enriched for the phosphorylated peptides by chromatographic techniques [32]. As a final step mass spectrometric sequencing of the phosphorylated peptides identifies the exact amino acid residues which are phosphorylated. Vectorial proteomics has also revealed a number of processing sites in the thylakoid membrane proteins as well as their modifications by acetylation and deamidation [8,14,33–35]. The use of one protease, trypsin, has limited current applications of vectorial proteomics to the peptides cleavable from the membrane proteins by this enzyme. Nevertheless, application of this methodology allowed mapping of the most of the presently known in vivo protein phosphorylation sites in the photosynthetic membranes, as summarized below.

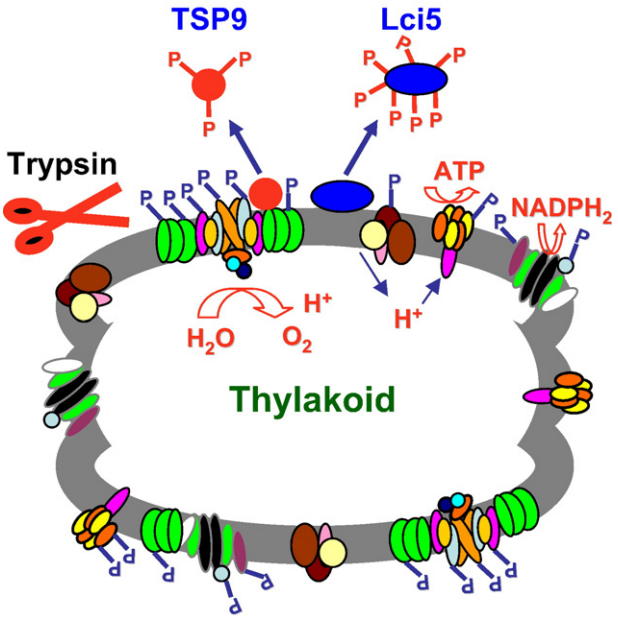

Fig. 1. Schematic outline of vectorial protomics [28] applied for characterization of protein phosphorylation in thylakoid membranes. Thylakoids are isolated from plant leaves or algal cells in the presence of phosphatase inhibitors protecting in vivo phosphorylated proteins. The peripheral phosphorylated proteins, like TSP9 [34] or Lci5 [19] are removed from the membranes by high salt wash and digested with trypsin. The surface exposed phosphorylated parts of the membranes proteins are cleaved by trypsin and removed from the membranes by centrifugation [8,14,33]. The phosphorylated peptides from either extrinsic or membrane proteins are enriched and sequenced using mass spectrometry, which identifies the proteins that were phosphorylated in vivo and the exact sites of phosphorylation.

3. Phosphorylation mapping in thylakoid proteins

Phosphorylation of thylakoid proteins has been studied by many different techniques, a number of which did not unambiguously prove phosphorylation of the exact protein, as recently reviewed in [20]. The ultimate proof of phosphorylation is in detection of phosphorylated amino acid residue in the protein sequence, usually obtained by the mapping of protein phosphorylation sites [20]. Table 1 presents the latest update on the phosphorylation sites identified in the proteins of plant thylakoids. Remarkably, these data show that phosphorylation occurs not only in PSII and LHCII subunits but also in all the other major protein complexes of the photosynthetic membranes: photosystem I (PSI), cytochrome bf complex and ATP synthase (CF1). In PSI the extrinsic stroma-exposed subunit PsaD was first found phosphorylated when vectorial proteomics was applied for characterization of thylakoids isolated from light-exposed leaves of *Arabidopsis* [33]. The same work has also identified a previously unknown membrane phosphoprotein named TMP14 [33], which recently has been characterized

Table 1
Phosphorylation sites identified in thylakoid proteins from plants exposed to different environmental conditions

Protein	Species	Phosphorylated residue	Environmental conditions	Reference
D1	Spinach	Thr-2(1)	Darkness/light	[90]
	Arabidopsis	Thr-2(1)	Darkness/light	[14]
D2	Spinach	Thr-2(1)	Darkness/light	[90]
	Arabidopsis	Thr-2(1)	Darkness/light	[14]
CP43	Spinach	Thr-15(1)	Darkness/light	[90]
	Spinach	Thr-20(6)	Light	[29]
	Spinach	Thr-22(8)	Light	[29]
	Spinach	Thr-346(332)	Light	[29]
	Arabidopsis	Thr-15(1)	Darkness/light	[14]
PsbH	Spinach	Thr-3(2)	Darkness/light	[38]
	Arabidopsis	Thr-3(2)	Darkness/light	[14]
	Arabidopsis	Thr-5(4)	Light	[14]
LHCII	Spinach	Thr-38(3)	Light	[91]
	Spinach	Thr-40(3)	Light	[91]
	Spinach	Ser-(3)	Light	[91]
	Spinach	Thr-44	Light	[29]
	Spinach	Ser-49	Light	[29]
	Arabidopsis	Thr-38(3)	Light	[14]
	Arabidopsis	Thr-41(3)	Light	[25]
CP29	Maize	Thr-112(83)	Cold stress	[37]
	Arabidopsis	Thr-37(6)	Light	[33]
PsaD	Arabidopsis	Thr-48	Darkness/light	[33]
PSI-P	Arabidopsis	Thr-66	Light	[33, 36]
Rieske Fe–S	Spinach	Thr-70(2)	Light	[29]
protein	Spinach	Ser-71(3)	Light	[29]
CF1 [a]	Barley	Ser-79/Thr-82		[30]
β subunit	Barley	Thr-252/255		[30]
	Barley	Thr-454		[30]
	Barley	Thr-489		[30]
TSP9	Spinach	Thr 66(46)	Light	[34]
	Spinach	Thr-73(53)	Light	[34]
	Spinach	Thr-80(60)	Light	[34]

The phosphorylated amino acid residues are numbered according to their positions in the initial translation products of the proteins (the numbers in brackets correspond to their positions in the mature proteins).

[a] The exact sites of phosphorylation have not been determined by sequencing in the phosphorylated peptides from the β subunit of CF1 (ATP synthase).

as a novel subunit of plant PSI and renamed as PSI-P [36]. Vectorial proteomics of stroma membranes from spinach thylakoids revealed N-terminal phosphorylations of Rieske Fe–S protein of cytochrome bf complex, and, interestingly, previously unknown multiple phosphorylation of CP43 subunit of PSII [29]. Conventional proteomics applied for characterization of ATP synthase from barley uncovered multiple phosphorylation of its β-subunit, while the exact phosphorylated residues were not revealed by the sequencing of the detected phosphorylated peptides [30]. The earlier identified site of phosphorylation in maize CP29 protein has not been confirmed by sequencing, but deduced from the peptide mapping analysis [37]. All other phosphorylation sites (Table 1) in plant thylakoid proteins were identified by mass spectrometric sequencing, with exception of Thr-2 in PsbH that was detected by N-terminal sequencing based on Edman degradation [38]. Phosphorylation of majority of the thylakoid proteins listed in Table 1 is light-dependent and was found in plant leaves harvested during the daytime. Phosphorylation of a few proteins, particularly the D1, D2, CP43 and PsbH subunits of PSII core, persists in darkness [14,20] (Table 1). However, it does not mean that the latter proteins are phosphorylated constitutively, indeed, their dephosphorylation could occur in the other conditions, like it happens in plant leaves exposed to elevated temperatures [13,14].

The real progress has recently been made in the mapping of protein phosphorylation sites in thylakoids from the green alga *Chlamydomonas reinhardtii* [8,19] (Table 2), while several years ago only a couple of the phosphorylation sites were known from this alga [39,40]. The proteins phosphorylated in vivo include five subunits of PSII core: D1, D2, CP43, PsbH and PsbR; five polypeptides of LHCII (Lhcbm proteins) and two minor light harvesting proteins CP29 and CP26 (Table 2). Besides these, four other previously unknown phosphorylated proteins (Lci5, UEP and unknown proteins A and B) have been discovered [8,19]. Remarkably, only three sites (two in CP29 and one in the unknown protein A) of more than 20 identified (Table 2) were found phosphorylated in all studied environmental conditions. All the other phosphorylation events occurred in the algal cells exposed to specific environment, as it indicated in Table 2. These distinct environment-dependent modifications in the alga differ quite significantly from those in plants (Table 1), which, for instance in case of the core subunits of plant PSII, could change just slightly in the leaves exposed to light or darkness [14,25]. Accordingly, the green alga *C.*

Table 2
Phosphorylation sites identified in thylakoid proteins from green alga *Chlamydomonas reinhardtii* exposed to different environmental conditions

Protein	Phosphorylated residue	Environmental conditions	Reference
D1	Thr-2	Light	[8]
D2	Thr-2	State2/light	[8]
CP43	Thr-3	Light	[8]
PsbH	Thr-3		[39]
PsbR	Ser-43	State2/light	[8]
Lhcbm1	Thr-27	State2/light	[8]
Lhcbm4	Thr-19 and Thr-23	High light	[8]
Lhcbm6	Thr-18 and Thr-22	High light	[8]
Lhcbm9	Thr-19 and Thr-23	High light	[8]
Lhcbm10	Thr-26	State2/light	[8]
CP26 (Lhcb5)	Thr-10	High light	[8]
CP29 (Lhcb4)	Thr-7	Darkness/light	[8]
	Thr-17	State 2/light	[8]
	Thr-33	Darkness/light	[8]
	Ser-103	State 2/light	[8]
	Thr-11	High light	[8]
	Thr-18	High light	[8]
Lci5	Thr-116, Thr-176, Thr-237	Low CO_2	[19]
	Ser-136 and Ser-137	Low CO_2	[19]
	Ser-196 and Ser-197	Low CO_2	[19]
UEP [a]	AAAGADsADEEAEAR	Low CO_2	[19]
Unknown protein A [a]	VFEsEAGEPEAK	Darkness/light	[8]
Unknown protein A [a]	DVDsEEAR	Light	[8]
Unknown protein B [a]	GEIEEADsDDEAR	State2/light	[8]

The phosphorylated amino acid residues are numbered according to their positions in the initial translation products of the proteins.

[a] The sequences of phosphorylated peptides from the proteins that were not annotated are shown in single amino acid code with a low case s designated phosphorylated serine residues.

reinhardtii provides a better experimental model for the functional analyses of particular protein phosphorylation reactions in relation to protein dynamics and complex formation in the thylakoid membranes of the cells exposed to a particular environment.

4. Protein phosphorylation and dynamics of light harvesting proteins

Phosphorylation and dynamics of LHCII have been in focus of investigations from the early 1980s in relation to the photosynthetic state transitions [41–43]. State transitions depend on light and redox conditions and consist of a lateral migration of a mobile LHCII in the thylakoid membranes between PSII and PSI: LHCII is bound to PSII in State 1, while part of LHCII migrates to PSI in State 2. Coupling of the mobile LHCII with the particular photosystem increases its photosynthetic efficiency. State 1 corresponds to the oxidised and State 2 to the reduced state of plastoquinone in the thylakoid membranes, which correlates with non-phosphorylated and phosphorylated status of LHCII, respectively. It has long been accepted that non-phosphorylated LHCII binds to PSII, while phosphorylated LHCII migrates to PSI [2,43,44]. This model has recently been challenged because the PSI-bound LHCII was found non-phosphorylated [22,45] and, on contrary, the phosphorylated LHCII was not binding to PSI in particular plant mutants [46,47]. Nevertheless, the direct involvement of protein phosphorylation in the state transitions has been proved in the studies of Stt7 and STN7 protein kinase mutants in *C. reinhardtii* and *Arabidopsis*, respectively, because lack of these protein kinases resulted in disappearance of state transitions [23,24]. Phosphorylation of several LHCII subunits was drastically reduced in the kinase mutants, while it was not proven that Stt7 and STN7 directly phosphorylated LHCII [23,24]. The substrates of these two protein kinases, as well as the proteins phosphorylation of which causes photosynthetic State 1 to State 2 transition remain to be determined. Notably, *Arabidopsis* mutants lacking STN7 were found defective in phosphorylation of not only LHCII [24], but also of the minor light harvesting protein CP29 [27].

Looking for the structural basis of state transitions the group of J. Barber has isolated and characterized the complexes of PSI from the cells of *C. reinhardtii* exposed to either State 1 or State 2 [48]. The additional protein density found by electron microscopy in PSI from the State 2 cells has been identified as a phosphorylated CP29, a minor light harvesting protein of PSII [48]. Biochemical analyses performed by the other research group have also found phosphorylated CP29 in the PSI preparations from the algal cells exposed to State 2 [49]. Mass spectrometric analyses revealed that CP29 was doubly phosphorylated in State 1 and quadruple phosphorylated in State 2 exposed cells [48] (see Table 2). Therefore, it has been postulated that redox-dependent multiple phosphorylation of CP29 in the green algae is an integral part of state transitions: the phosphorylation-induced structural changes of CP29 determine the affinity of LHCII for either photosystem [48]. CP29 is a well-characterized linker protein required for binding

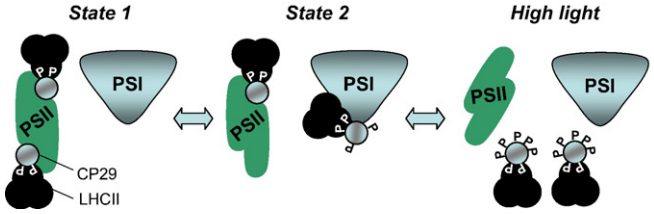

Fig. 2. The mechanism suggested [8,48] for adaptive regulation of light harvesting in green alga via differential environmentally-modulated phosphorylation of CP29 linker protein. CP29 is doubly phosphorylated in oxidising conditions and darkness (State 1) and it links LHCII to PSII. Quadruple phosphorylation of CP29 in reducing conditions (State 2) leads to its dissociation from PSII and binding to PSI, linking the mobile LHCII to PSI. Phosphorylation of CP29 at seven different sites under high light stress uncouples this protein together with LHCII from the photosystems, which leads to the thermal energy dissipation.

of LHCII to PSII [50]. Accordingly, the novel emerging paradigm suggests that doubly phosphorylated CP29 links LHCII to PSII, while the quadruple phosphorylation of this linker protein uncouples it from PSII and leads to migration and binding of CP29–LHCII complex to PSI, as it schematically outlined in Fig. 2.

CP29 has been found specifically phosphorylated in several plants under cold and high light stress [16,17,51]. Isolation of pigment-containing phosphorylated and non-phosphorylated forms of CP29 and their spectroscopic and biochemical analyses revealed reversible conformational changes in this protein upon phosphorylation [52]. The phosphorylation-induced conformational changes in CP29 could significantly affect the affinity of this linker protein to PSII or PSI. Moreover, the hyper-phosphorylation of CP29 at seven distinct sites in the algal cells exposed to high light (Table 2) may uncouple this protein together with LHCII from both photosystems (Fig. 2). Under high light the excess energy absorbed by the thylakoid membrane is dissipated via nonradiative de-excitation in order to protect against photodamage. The mechanism for increase of the thermal energy dissipation under high light stress may consist in phosphorylation-dependent uncoupling of LHCII from the photosystems (Fig. 2). Notably, besides the multiple phosphorylation of CP29, high light induces in green alga phosphorylation of the other PSII–LHCII linker protein, CP26 [8], which also may contribute to dissociation of LHCII from PSII. Such a mechanism is in agreement with genetic analyses of green algae mutants that demonstrated crucial importance of Lhcbm1, the most abundant LHCII protein in *C. reinhardtii*, for thermal energy dissipation under high light stress [53]. Moreover, the differential light-dependent phosphorylation of the LHCII proteins themselves (Tables 1 and 2) may be involved in control of light harvesting by dynamic re-structuring of LHCII complexes and their capability to regulate the flow of absorbed energy [54,55].

5. Dynamics of PSII turnover and phosphorylation

PSII is the engine of oxygenic photosynthesis performing light-driven extraction of electrons from water with a concomitant production of oxygen. During functioning PSII

can also undergo photoinactivation, which is proportional to light intensity [56]. The steady work of this photosystem depends on the balance between the rates of light-induced damage and repair of PSII, the process including partial disassembly of inactivated PSII, proteolytic degradation of the photo-damaged reaction centre protein D1 and co-translational insertion of newly synthesised D1 protein in PSII [57–60]. Therefore, D1 protein has the fastest turnover rate among the thylakoid proteins of plants, algae and cyanobacteria [60–62]. The thylakoid membranes of plants are heterogeneously organised in the appressed to non-appressed regions, called grana and stroma membrane domains, respectively. In contrast to cyanobacteria, the repair cycle of PSII in plants includes the lateral migration of PSII units between the grana and stroma membrane regions [58,59,63], as it is illustrated in Fig. 3. PSII is localized in the grana and its subunits become heavily phosphorylated when the plant leaves are exposed to high light [6,7,64,65]. The detailed characterization of subunit composition and of their phosphorylation in PSII isolated from different domains of plant thylakoid membrane [7] revealed dissociation of PSII dimers and their gradual dephosphorylation during migration from the grana to stroma (Fig. 3). The lateral migration and partial disassembly of PSII occurred with sequential dephosphorylation of CP43, D2 and D1 polypeptides of PSII [7]. These data along with the others summarized in [4,20,58,66] suggested that reversible phosphorylation of PSII subunits allows for coordinated disassembly of PSII complex during its lateral membrane migration and for coordinated biodegradation and biosynthesis of the D1 protein [4,7]. One recent publication had claimed that "reversible protein phosphorylation is not essential for PSII repair" because "PSII activity under high-intensity light is affected only slightly in *stn8* mutants" [26]. This claim was based on the fact that the authors have not observed phosphorylation of PSII subunits in the *Arabidopsis* mutants lacking STN8 protein kinase. However, in vivo phosphorylation of D1, D2, CP43 and PsbH subunits of PSII has been found and quantified in the same *stn8* mutants in another laboratory [25].

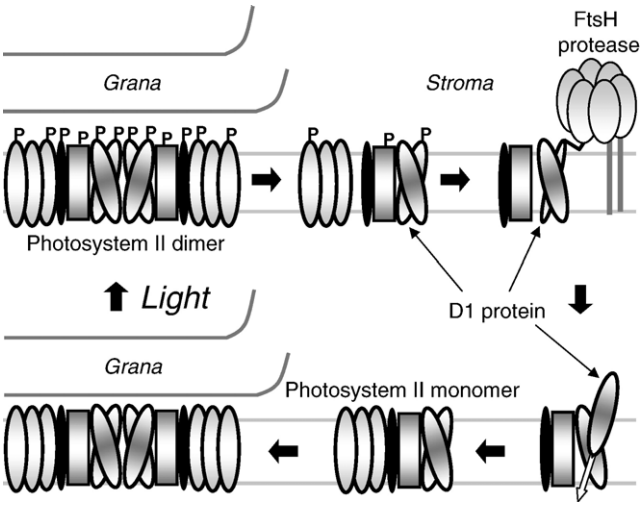

Fig. 3. Schematic model illustrating multiple light-induced protein phosphorylations of PSII proteins in the grana of thylakoids [6,7,64,65], dephosphorylation of the photo-damaged PSII and its partial disassembly during migration to the stroma [7,66], degradation of the damaged D1 protein by FtsH protease [69], insertion of the newly synthesised D1 in the remaining PSII complex [57] and final assembly of the photosystem in the thylakoid grana [63,66].

Turnover of the D1 protein includes degradation of the photo-damaged polypeptide and co-translational insertion of the newly synthesized protein in the partially disassembled PSII complexes as a crucial part of the PSII repair cycle [59]. Two thylakoid proteases, Deg P2 [67] and FtsH [68], have been implicated in proteolytic degradation of the photo-damaged D1. However, the recent works suggest a model in which FtsH proteases alone can be responsible for the removal of the damaged D1 in cyanobacteria and plants [69,70]. FtsH is a membrane protease consisting of six subunits (see Fig. 3). This protease complex digests membrane proteins in the ATP-dependent manner by translocation of the protein terminus through a central pore within the hexameric FtsH enzyme and subsequent degradation [69]. The degradation of damaged D1 is suggested to proceed from its N-terminus [69], exposed to the chloroplast stroma (Fig. 3). Such a mechanism for proteolytic digestion of the photo-damaged D1 protein may explain why the N-terminal phosphorylation of D1 in plant PSII (Table 1) protects this protein from the degradation [69], as has been documented experimentally in several studies [6,71–74]. Phosphorylated N-terminus of D1 may have a very low affinity towards the active site pore of FtsH, which justifies the requirement of D1 dephosphorylation prior to its proteolysis [6,73,74]. Accordingly, reversible phosphorylation of the subunits of plant PSII is not only tightly coupled to its membrane dynamics and disassembly (Fig. 3) but also could be involved in biodegradation of these polypeptides, because at least three of them are N-terminally phosphorylated (Table 1).

The reaction centre D1 protein of PSII is vulnerable not only to light, but also to high temperature. Subjection of spinach leaves to a moderate heat stress at 40 °C induces fast degradation of D1 by FtsH [75]. This fast proteolysis is in a good correlation with a very rapid D1 dephosphorylation at elevated temperatures. If phosphorylation level of D1 is rather stable in plants grown at standard light and temperature conditions [14], the almost complete D1 dephosphorylation occurs in a time scale of minutes in the leaves exposed to 42 °C [13]. The heat-shock-induced dephosphorylation of N-terminal threonine residue in D1, as well as in D2 and CP43 is catalysed by a cyclophilin-regulated PP2A-like protein phosphatase intrinsic to thylakoid membranes [76]. This protein phosphatase is specific in dephosphorylation of N-terminal threonine residues of PSII subunits [76] and is likely involved in the quality control of PSII under the stress conditions via dephosphorylation of partially disassembled PSII complexes migrated in the stroma domains of thylakoids (Fig. 3).

6. Environmentally induced phosphorylation of peripheral thylakoid proteins

Most of the proteins found phosphorylated in plant and algal thylakoids are intrinsic membrane proteins, except four

phosphoproteins: TSP9 (Thylakoid Soluble Phosphoprotein of 9 kDa) and PsaD in plants (Table 1); and Lci5 (Low CO_2 inducible protein 5) and UEP (Unknown Expressed protein) in the green alga (Table 2), which do not contain any transmembrane domains. PsaD is one of the peripheral subunits of PSI involved in the docking of the electron carrier ferredoxin at the stromal side of the photosynthetic membrane [77]. Together with the PsaC subunit PsaD is involved in the proper electron transfer form the iron–sulphur centres of PSI to ferredoxin [78]. Thus, it has been speculated that phosphorylation of PsaD could regulate the electron transfer from PSI to the electron acceptors in the chloroplast stroma [33]. While phosphorylation of PsaD has been identified in the thylakoids isolated from the light-exposed leaves of *Arabidopsis* [33], it is not presently known how the phosphorylation status of this protein depends on the environmental clues. On the other hand, phosphorylation of the other peripheral thylakoid proteins occurs in the very distinct ambient conditions: light is required for TSP9 phosphorylation [34], while Lci5 and UEP become phosphorylated in alga cells only under limitation in environmental CO_2 [19]. At the same time phosphorylation of these three proteins also requires reduction of electron carriers in the thylakoid membranes [19,34]. The functions of TSP9, Lci5 and UEP are yet unknown, however, these proteins envisage previously unknown class of basic proteins undergoing environmentally-induced redox-dependent phosphorylation at the surface of oxygenic photosynthetic membranes.

The light-induced phosphorylation of TSP9 causes its partial release from the thylakoids into the chloroplast stroma [34]. This phosphorylation-dependent relocation was the basis for the proposed involvement of TSP9 in plant cell signalling from the surface of the photosynthetic membranes [34]. Our recent results (Hansson, M., Vener, A.V., and Carlberg, I., unpublished data) on localization of TSP9 in thylakoid membranes and within the thylakoid protein complexes reveal close association of this protein with LHCII and cross-linking of TSP9 with LHCII subunits and with peripheral subunits of PSII (CP29, CP26 and PsbS). Localization of TSP9 at the interface between PSII and LHCII suggests possible involvement of this protein in regulation of light harvesting. It should be stressed that such a regulation has to be restricted to plants since TSP9 is a plant-specific protein: the genes for homologous proteins could be found in more than 40 plant species but not in algae or cyanobacteria. Plants do differ from green algae in the magnitude of state transitions: only 15–20% of LHCII migrates between the photosystems in plants, while 80% of LHCII is mobile in green algae [23,24,79]. There are also differences between plants and algae in regulation of thermal dissipation of the excessive light energy absorbed by LHCII [53,80]. The mechanism proposed in Fig. 2 for the regulation of the algal light harvesting should be different in plants due to the fact that 7 distinct sites could be phosphorylated in the algal CP29 (Table 2) and only 2 phosphorylation sites are found in plant CP29 (Table 1). Importantly, the single N-terminal phosphorylation of CP29 in *Arabidopsis* is catalysed by STN7 kinase essential for state transitions [27]. The multiple light-induced phosphorylation of plant-specific TSP9, which like CP29 is located at the interface between PSII and LHCII, may be involved in the light-dependent dissociation of LHCII from PSII in plants. This working hypothesis has to be experimentally scrutinized, while the current results in my laboratory confirm the model of TSP9 involvement in the light-dependent control of plant light harvesting.

Recently discovered phosphorylation of two peripheral proteins, Lci5 and UEP, specifically occurring at the surface of thylakoids during acclimation of the green alga to low CO_2 could be the early cellular response to limitation in environmental inorganic carbon [19]. Aquatic algae meet constrains in supply of CO_2 and acclimate to this limitation by induction of a special cellular response called CO_2-concentrating mechanism [81,82]. The cellular signals triggering induction of the CO_2-concentrating mechanism are presently unknown, while protein phosphorylation has been suggested as a link connecting sensing of CO_2 with the activation of this process [81,83,84]. Notably, induction of CO_2-concentrating mechanism in algae also depends on reduction of electron carriers in the thylakoid membrane [85,86], which favours possible involvement of Lci5 and UEP in this induction. Like the plant specific TSP9, Lci5 could be multiply phosphorylated (at 3 threonine and 4 serine residues, see Table 2) by one of the redox-dependent thylakoid protein kinases. It is also a very basic protein that could be released from the membranes by high ionic strength [19]. The properties of these recently identified peripheral proteins are well suited for the potential signal transducers and/or mobile regulators that may be on/off-switched via phosphorylation induced at the thylakoid surface in response to the changing environment.

7. Conclusions and future perspectives

The recent progress in molecular characterization of protein phosphorylation in the thylakoid membranes of plants and green algae revealed many novel phosphoproteins, as well as multiple phosphorylations of several membrane and peripheral proteins. Most of these phosphorylation events were found dependent on the environmental conditions, which prompt consideration of their involvement in the processes of adaptation and acclimation of photosynthetic machinery. It has been established that protein kinases Stt7 and STN7 are essential for photosynthetic state transitions in *C. reinhardtii* and *Arabidopsis*, respectively. It is proposed that differential redox- and light-dependent phosphorylation of CP29 linker protein in green algae and of TSP9 protein in plants could be responsible for regulation of dynamic distribution of LHCII from PSII to PSI. Hyper-phosphorylation of algal CP29 under high light stress is suggested as a mechanism for uncoupling of LHCII from both photosystems. Reversible phosphorylation of plant PSII proteins occurs during the repair cycle and lateral membrane migration of this photosystem upon its damage, disassembly and repair. Reversible phosphorylation of the D1 reaction centre protein correlates with the distinct stages of its turnover under high light or high temperature stress indicating involvement in the quality control of PSII. The recently discovered environmentally-induced phosphorylation of

peripheral thylakoid proteins in plants and algae has characteristics of cellular signalling and regulatory responses to light and limitation in environmental carbon and awaits further molecular characterizations.

Current knowledge demonstrates that reversible protein phosphorylation in thylakoid membranes is closely related to protein dynamics in the course of adaptive adjustments of photosynthetic machinery, like regulation of light harvesting and protein turnover. We can expect that the further methodological developments will result in comprehensive identification of most phosphorylation events in the photosynthetic proteins and allow quantitative analyses of these modifications in vivo under changing environment. The combined application of quantitative proteomics, reverse genetics and site directed mutagenesis, isolations of membrane protein complexes and measurements of photosynthetic and enzymatic activities should provide the answers to the long standing following questions. How many and which protein kinases and phosphatases are involved in reversible phosphorylation of thylakoid proteins? What are the mechanisms for redox control of protein kinases by plastoquinone and cytochrome bf complex [87–89]? Do the thylakoid protein kinases work in a phosphorylation-dependent cascade [22,23]? What are the functions of all known and newly discovered phosphoproteins? How important are individual protein modifications for the particular adaptive responses in thylakoid membranes? What are the roles of complex and distinct protein phosphorylation patterns in relation to changing affinities of individual proteins and dynamic distribution of protein complexes in thylakoid membranes? Addressing of these questions will obviously advance our understanding of the molecular mechanisms for regulation of oxygenic photosynthesis.

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