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Composition, phosphorylation and dynamic organization of photosynthetic protein complexes in plant thylakoid membrane

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The photosystems (PS), catalyzing the photosynthetic reactions of higher plants, are unevenly distributed in the thylakoid membrane: PSII, together with its light harvesting complex (LHC)II, is enriched in the appressed grana stacks, while PSI–LHCI resides in the non-appressed stroma thylakoids, which wind around the grana stacks. The two photosystems interact in a third membrane domain, the grana margins, which connect the grana and stroma thylakoids and allow the loosely bound LHCII to serve as an additional antenna for PSI. The light harvesting is balanced by reversible phosphorylation of LHCII proteins. Nevertheless, light energy also damages PSII and the repair process is regulated by reversible phosphorylation of PSII core proteins. Here, we discuss the detailed composition and organization of PSII–LHCII and PSI–LHCI (super)complexes in the thylakoid membrane of angiosperm chloroplasts and address the role of thylakoid protein phosphorylation in dynamics of the entire protein complex network of the photosynthetic membrane. Finally, we scrutinize the phosphorylation-dependent dynamics of the protein complexes in context of thylakoid ultrastructure and present a model on the reorganization of the entire thylakoid network in response to changes in thylakoid protein phosphorylation.

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Introduction

The chloroplast enclosed thylakoid system of photosynthetic organisms is a unique membrane network that hosts the energy conversion machinery responsible for producing the chemical energy that fuels life on Earth. The photosynthetic machinery, composed of photosystem (PS) II, cytochrome (Cyt) b_6f, PSI and ATP synthase mediates the first phase of photosynthesis, the light reactions. In the linear electron transfer chain, the two photosystems are interconnected by the Cyt b_6f complex and the two mobile electron carriers, plastoquinone in the lipid bilayer and plastocyanin in the thylakoid lumen (Fig. 1). The energy of solar irradiance is first captured by PS-bound light harvesting complexes (LHC) and then converted and stabilized as chemical energy by electron and proton transfer reactions mediated by the main thylakoid protein complexes. The light energy is stored in NADPH and ATP molecules, which are used in subsequent energy-demanding reactions such as carbon assimilation.

Photosynthetic energy conversion requires highly coordinated interplay between individual thylakoid protein complexes and therefore depends on the molecular architecture of the photosynthetic machinery. While the basic electron and proton transport reactions as well as the involved enzyme complexes have, in principle, remained fundamentally similar in all oxygenic photosynthetic organisms throughout the evolution, the arrangement of the photosynthetic machinery and the organization of the thylakoid membrane varies radically between different organisms. A unique landmark of plant thylakoid membrane is the formation of appressed membrane discs and the uneven lateral distribution of the photosynthetic protein complexes along the membrane plane. This membrane system is capable of adjusting its function upon the most subtle changes in environmental conditions by making use of a multitude of regulatory processes. Reversible protein phosphorylation, *i.e.* the addition of negatively charged phosphate group (P) from ATP to a sidechain of certain amino acids, is among the most important post-translational modifications of proteins in all eukaryotic cells. In plant thylakoid membrane, the protein phosphorylation regulates the energy conversion reactions and the repair of damaged photosynthetic machinery through remodeling the protein complex arrangement and thylakoid ultrastructure. Here, we provide an overview of the composition and protein phosphorylation-dependent dynamic organization of the photosynthetic machinery in angiosperms.

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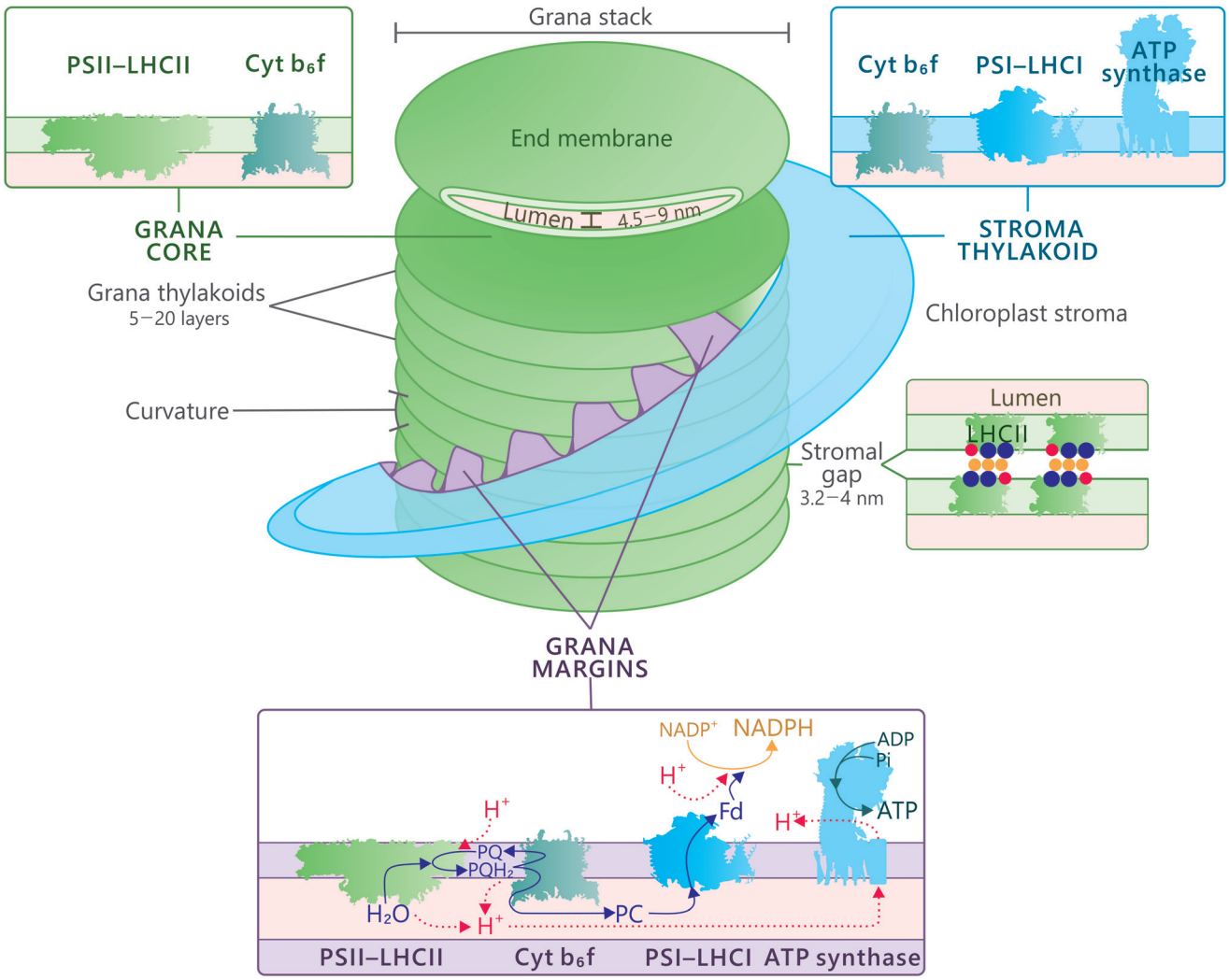

Fig. 1 The helical fretwork model of thylakoid membrane and the putative location of photosynthetic protein complexes involved in linear electron transfer. The different domains of the thylakoid system are presented along with their characteristic protein complex composition, emphasising the results from our laboratory. The thylakoid membranes of higher plants are folded into layers of appressed grana stacks (green). The top and the bottom layer of each grana stack is called an end membrane and the bended edges of the grana thylakoids are termed the curvature area. The space between the adjacent thylakoid layers in grana is designated as stromal gap, in which the negatively and positively charged amino acid residues (blue and red dots, respectively) of the neighboring LHCII trimers interact and where cations, most importantly Mg^{2+} (orange dots), are screened between the negatively charged stromal loops of adjacent LHCII trimers. The separate grana stacks are helically connected by four to six non-appressed stroma thylakoids (blue). The interface between the appressed and non-appressed membranes is called grana margins (purple), hosting both photosystems. Linear electron flow (blue arrows) between the photosystems produces NADPH, and simultaneously protons are pumped from the chloroplast stroma into the thylakoid lumen (dashed red arrows). The consequent proton motive force powers the synthesis of ATP. The thylakoid system is surrounded by aqueous chloroplast stroma and the network itself encloses another aqueous space called the lumen, connecting the different thylakoid domains internally. The protein complexes are unevenly distributed in the thylakoid membrane: the flat PSII–LHCII supercomplexes are mainly found in the grana, while the large PSI–LHCI and ATP synthase reside in the non-appressed stroma thylakoids and grana margins. Cyt b_6f is likely found in all domains.

Thylakoid membrane ultrastructure and distribution of thylakoid protein complexes

In plants and algae, the photosynthetic energy transduction occurs in chloroplast-enclosed flattened vesicles, the thylakoids. As illustrated in Fig. 1, the thylakoid network in higher plants is differentiated into two morphologically distinct domains: the cylindrical grana appressions and the interconnecting and non-appressed stroma thylakoids. Each chloroplast contains approximately 40–60 grana stacks[1] that are formed usually by 5–20 folded thylakoid layers and have a diameter ranging from 300 to 600 nm, depending on the plant species and the light condition.[2,3] The width of the thylakoid enclosed lumen varies between 4.5 nm in darkness up to 9 nm

in light, and that of the stromal gap between neighboring grana membranes from 3.2 to 4.0 nm.[4] The grana stacks account for the majority of the total membrane area and harbor the majority of the total pigment content,[5] but the proportion of grana relative to the stroma thylakoids varies depending on the light condition.[6]

The interface between grana and stroma thylakoids, denoted as grana margins, is a biochemically distinct thylakoid fraction, but the exact composition and location of this domain is under debate. Based on structural analysis, it was proposed that grana margins consist of the highly curved regions of the grana stacks and is protein-free,[7–10] whereas another interpretation suggests that grana margin is represented by a larger annular ring at the periphery of the grana stack and is enriched in photosynthetic protein complexes.[5,11] A recently characterized thylakoid fraction, obtained by digitonin solubilisation and subsequent centrifugation steps, was reported to be a combination of a highly curved grana margins, straight membranes at the interphase between grana and stroma as well as of the fret-like protrusions (the slits) between grana and stroma membranes.[12,13] Subsequent further optimization of thylakoid fractionation yielded a separation of the curved membrane domain from the rest of the margin domain,[14] and accordingly, the nomenclature adopted in Fig. 1 includes a distinction between the grana curvature and grana margin domains.

Different structural models exist on the exact three-dimensional configuration of the thylakoid membrane. The overall structure is similar in all models, but the uncertainty arises from different interpretations on how the stroma thylakoids are joined to the grana appressions. Folded fork model postulates that a granum represents a bifurcation of the stroma membranes and is formed of repeated units each containing three grana stacks.[15] Shimoni *et al.*[16] proposed that a granum is a bifurcation of a lamellar stroma membrane sheet and the neighboring grana units, each composed of paired layers that are further connected by membrane bridges. Helical fretwork model, originally proposed by Paolillo,[17] suggests that the stroma thylakoids wind around the grana stacks as right-handed helices and are interconnected to the grana by several narrow membrane slits,[2,17,18] which according to Anderson *et al.*,[6] might represent the grana margins. The membrane slits guarantee the continuum of the lipid and lumenal phases of the thylakoid membrane and allow both functional and structural interaction between the photosynthetic protein complexes[6,19] (Fig. 1). Recently, Bussi *et al.* combined different electron tomography techniques and demonstrated that, in addition to the four to six right handed helices winding around grana, the membrane consists of several left-handed helical junctions that connect adjacent lamellar sheets or helices and consolidate the geometry of the membrane system by minimizing the surface and bending energies.[20] Since the helical fretwork model is the most prevalent of the above mentioned models and supported by data obtained with different electron microscopy techniques as well as modelling,[2,20–22] we scrutinize the thylakoid protein complex dynamics in the frame of this model.

The functional relevance and the exact mechanism of the grana stacking are still under debate (comprehensively reviewed by Chow *et al.*[23]). Although the Lhcb1 and Lhcb2 proteins – the most abundant constituents of the grana membranes – are not a prerequisite for the grana formation,[24] but some level of stacking is observed even without the majority of Lhcb proteins,[25] they are generally considered to play an essential role in stabilizing the stacking. The negatively charged stromal loops of Lhcb proteins form salt bridges with cations, most importantly with divalent Mg^{2+}, which are screened between adjacent membrane layers.[26,27] Although this electrostatic screening is considered as the most important stabilizing force of the membrane appressions, the interaction between the positively charged N-terminus of one Lhcb protein with the negatively charged stromal loop of another Lhcb protein on the opposite membrane bilayer might also be involved in grana formation.[28] Further, CURT1 oligomers located at the curved regions of grana are known to regulate the number and size of the stacks.[10]

The photosynthetic protein complexes embedded in the thylakoid membrane account for more than 70% of the membrane area and are unevenly distributed in the thylakoid membrane[29] (Fig. 1). PSII–LHCII supercomplexes are densely packed in the appressed grana and cover 80% of the membrane area.[30] PSI–LHCI and ATP synthase with their 5 nm and 16 nm stromal protrusion are unable to fit to the stromal gap between adjacent grana layers and are enriched in the stroma-exposed thylakoids.[31] Cyt b_6f is likely located in all thylakoid domains[32,33] and its distribution might be regulated according to light conditions.[34] The grana margins, which should not be confused with the curvature domain in the edge of the grana membranes,[14] are biochemically a fusion of grana and stroma thylakoids, and accommodate both photosystems.[5,35–39]

Composition and LHCII-mediated supramolecular arrangement of the photosystems

The photosynthetic machinery is composed of large protein complexes, each comprising several nucleus and chloroplast-encoded protein subunits (Table 1 for PSII–LHCII and Table 2 for PSI–LHCI) and prosthetic groups. The first event of photosynthetic light reactions is the absorption of solar irradiation by the pigment-binding light harvesting complex (LHC) antennas. The LHC proteins associate peripherally with both photosystems and are mainly responsible for collecting the solar energy and for transferring the excitation energy to the reaction centers located in the photosystem core complexes. The LHC proteins typically contain three transmembrane α-helices (A, B, C) and two lumenal helices (D, E).

LHCII and PSII core subunits

The light harvesting complex II (LHCII), the antenna system of photosystem II, consists of six different Lhcb proteins and

Table 1 Encoding gene, AGI identifier and the function of the PSII–LHCII subunits in Arabidopsis. Arabidopsis genome initiative (AGI) locus identifiers for each gene were obtained from the Arabidopsis information resource (TAIR)

Protein	Gene	AGI identifier	Function
Lhcb1	*Lhcb1.1*	AT1G29920	Light harvesting in S-, M- and L-LHCII
	Lhcb1.2	AT1G29910	
	Lhcb1.3	AT1G29930	
	Lhcb1.4	AT2G34430	
	Lhcb1.5	AT2G34420	
Lhcb2	*Lhcb2.1*	AT2G05100	Light harvesting in S- and L-LHCII
	Lhcb2.2	AT2G05070	
	Lhcb2.3	AT3G27690	
	Lhcb2.4	AT3G27690	
Lhcb3	*Lhcb3*	AT5G54270	Light harvesting in M-LHCII
CP29	*Lhcb4.1*	AT5G01530	Light harvesting, M-LHCII binding
	Lhcb4.2	AT3G08940	
	Lhcb4.3	AT2G40100	
CP26	*Lhcb5*	AT4G10340	Light harvesting, S-LHCII binding
CP24	*Lhcb6*	AT1G15820	Light harvesting, M-LHCII binding
D1	*PsbA*	ATCG00020	Reaction center
CP47	*PsbB*	ATCG00680	Light harvesting
CP43	*PsbC*	ATCG00280	Light harvesting
D2	*PsbD*	ATCG00270	Reaction center
Cyt b559 α	*PsbE*	ATCG00580	Reaction center
Cyt b559 β	*PsbF*	ATCG00570	Reaction center
PsbH	*PsbH*	ATCG00710	Antenna association
PsbI	*PsbI*	ATCG00080	Reaction center
PsbJ	*PsbJ*	ATCG00550	Core stabilization
PsbK	*PsbK*	ATCG00070	Core stabilization
PsbL	*PsbL*	ATCG00560	PSII dimerization
PsbM	*PsbM*	ATCG00220	PSII dimerization
PsbTc	*PsbTc*	ATCG00690	PSII dimerization
PsbTn	*PsbTn*	AT3G21055	Light acclimation
PsbZ	*PsbZ*	ATCG00300	Antenna association
PsbO	*PsbO-1*	AT5G66570	
	PsbO-2	AT3G50820	Regulation of D1 dephosphorylation and turnover
PsbP	*PsbP-1*	AT1G06680	Stabilization of Mn_4CaO_5 complex
	PsbP-2	AT2G30790	Stabilization of Mn_4CaO_5 complex
PsbQ	*PsbQ-1*	AT4G21280	Stabilization of Mn_4CaO_5 complex
	PsbQ-2	AT4G05180	Stabilization of Mn_4CaO_5 complex
PsbR	*PsbR*	AT1G79040	Stabilization of Mn_4CaO_5 complex
PsbS	*PsbS*	AT1G44575	Thermal dissipation
PsbW	*PsbW*	AT2G30570	Core stabilization, antenna association
PsbX	*PsbX*	AT2G06520	Core stabilization

Table 2 Encoding gene, AGI identifier and function of PSI–LHCI subunits in Arabidopsis. Arabidopsis genome initiative (AGI) locus identifiers for each gene were obtained from the Arabidopsis information resource (TAIR)

Protein	Gene	AGI identifier	Function
Lhca1	Lhca1	AT3G54890	Light harvesting
Lhca2	Lhca2	AT3G61470	Light harvesting
Lhca3	Lhca3	AT1G61520	Light harvesting
Lhca4	Lhca4	AT3G47470	Light harvesting
Lhca5	Lhca5	AT1G45474	NDH-1 docking
Lhca6	Lhca6	AT1G19150	NDH-1 docking
PsaA	PsaA	ATCG00350	Reaction center
PsaB	PsaB	ATCG00340	Reaction center
PsaC	PsaC	ATCG01060	F_A and F_B binding, ferredoxin docking
PsaD	PsaD-1	AT4G02770	Ferredoxin docking
	PsaD-2	AT1G03130	
PsaE	PsaE-1	AT4G28750	Ferredoxin docking
	PsaE-2	AT2G20260	
PsaF	PsaF	AT1G31330	Plastocyanin docking
PsaG	PsaG	AT1G55670	LHCI stabilization
PsaH	PsaH-1	AT3G16140	L-LHCII docking
	PsaH-2	AT1G52230	
PsaI	PsaI	ATCG00510	Stabilization of LHCII docking
PsaJ	PsaJ	ATCG00630	PsaF stabilization
PsaK	PsaK	AT1G30380	LHCI stabilization
PsaL	PsaL	AT4G12800	L-LHCII docking
PsaN	PsaN	AT5G64040	Plastocyanin docking
PsaO	PsaO	AT1G08380	L-LHCII docking

binds half of the chlorophyll pigments in the thylakoid membrane.[40] The three major Lhcb proteins, Lhcb1, Lhcb2 and Lhcb3, are assembled into trimers that are bound to PSII core *via* Lhcb4 (CP29), Lhcb5 (CP26) and Lhcb6 (CP24) proteins (Table 1). Each of the major Lhcb monomers bind eight chlorophyll *a* and six chlorophyll *b* pigments as well as four carotenoids.[41] The three isoforms of the major Lhcb proteins are present in different molar ratios. In *Arabidopsis thaliana* (Arabidopsis), the Lhcb1:2 : 3 ratio has been estimated to be 7 : 3 : 1 based on proteomic data,[42,43] but the ratio depends on the light condition. Each of the major Lhcb isoforms is present as several subisoforms. In Arabidopsis, five subisoforms are found (encoded by *Lhcb1.1–5*), and of these, Lhcb1.1–3 are identical, while Lhcb1.4 and Lhcb1.5 show some difference.[44] Of the Lhcb2 subisoforms (encoded by *Lhcb2.1–4*), on the other hand, Lhcb2.1, Lhcb2.2 and Lhcb2.3 are identical and Lhcb2.4 diverges by one amino acid.[44] Moreover, instead of a separate gene, *Lhcb2.3* appears to represent one of the alleles of *Lhcb2.1*. From the three Lhcb4 subunits (encoded by *Lhcb4.1–3*), Lhcb4.3 diverges significantly from Lhcb4.1 and Lhcb4.2 by lacking a fraction of the C-terminal part located in the lumen.[44,45] For more information about different Lhcb isoforms and their evolution, see the recent review by Crepin and Caffarri.[43]

The PSII core complex functions as a dimer and is surrounded by peripheral LHC antenna system. Thus far, the structure of plant (*Pisum sativum*) PSII–LHCII supercomplex has been resolved by cryo-electron microscopy at 2.7 Å resolution.[46] One PSII core monomer consists of 20–23 protein subunits, depending on the organism, and harbors several pigments, including 35 chlorophylls, two pheophytins and 10 carotenoids as well as several other co-factors such as quinones.[41] The PsbA (D1), PsbD (D2) and PsbI proteins as well as the α (PsbE) and β (PsbF) subunits of the Cyt b_{559} complex form the PSII reaction center complex, which is the minimal unit required for primary photochemistry.[47,48] The reaction center is surrounded by two large chlorophyll-binding light harvesting proteins CP47 (PsbB) and CP43 (PsbC) as well as the lumenal oxygen evolving complex. The oxygen evolving complex is composed of PsbO, PsbP, PsbQ and PsbR proteins that stabilize the Mn_4CaO_5 cluster. PsbO-Q are encoded by two genes, and for PsbO, the two isoforms have been suggested to

possess different functions: the major isoform PsbO-1 stabilizes PSII activity, while the minor isoform PsbO-2 has been suggested to regulate the dephosphorylation and turnover of D1.[49] In addition, the core complex includes several so-called low molecular mass (<10 kDa) subunits: PsbH-M, PsbTc, PsbTn, PsbW, PsbX and PsbZ.

PsbTc and PsbTn that were previously considered as plastidial and nucleic paralogues of PsbT but are now recognized as functionally separate subunits. The transmembrane protein PsbTc has been suggested to contribute to PSII dimerization, whereas the lumenal extrinsic PsbTn seems to be important for light acclimation.[50,51] Noteworthy, PsbN is no longer considered as a structural subunit of PSII.[52]

PSII–LHCII supercomplexes

PSII–LHCII supercomplexes are comprised of PSII core dimers and the peripheral LHCII antenna complexes. In the most abundant form of PSII–LHCII supercomplexes, $C_2S_2M_2$, both core monomers (C) are associated with a strongly bound S-LHCII trimer and a moderately bound M-LHCII trimer, which are associated to the PSII core *via* minor antenna proteins Lhcb5 and Lhcb4/6, respectively (Fig. 2A). In addition to the S- and M-trimers, a large pool of LHCII trimers, denoted as L-LHCII (loosely-bound), exists in the thylakoid membrane and likely occupies the spaces between individual PSII–LHCII supercomplexes in the grana core[8,19,53] and interconnects the two photosystems in grana margins[54] (discussed in more detail later) (Fig. 2B).

The distinct LHCII trimers of the major Lhcb proteins play different roles in the structure of the photosynthetic apparatus. In the model plant Arabidopsis, the S-LHCII trimer is composed of Lhcb1 and Lhcb2 proteins, whereas the M-trimer consists of Lhcb1 (especially Lhcb1.4) and Lhcb3 proteins.[42] Lhcb3 and Lhcb6 are unique to land plants, but intriguingly, are absent from the gymnosperm family Pinaceae.[45,55] In land plants, particular structural features in the N-terminus and in the AC- and BC-loops of the Lhcb3 protein facilitate its interaction with Lhcb6 and determine the binding orientation of the M-trimer to the core complex.[46] The structure of $C_2S_2M_2$ supercomplex has been determined with cryo-EM at 5.3 Å resolution from Arabidopsis[41] and at 2.7–3.2 Å resolution from *Pisum sativum*,[46] and, based on the location of individual pigments, these structures suggest potential energy transfer routes from LHCII antenna to the core. The composition of L-trimers is not yet well defined, but they probably consist mainly of Lhcb1 (especially Lhcb1.5) and Lhcb2 (Lhcb2.2) proteins.[42,56]

In certain conditions, the PSII–LHCII supercomplexes have been reported to form semi-crystalline arrays in grana core.[3,57,58] Besides such lateral re-arrangement, the PSII–LHCII supercomplexes in the array are also vertically associated with complexes across the stromal gap.[46,59] Although the arrays are relatively rarely observed[60] and even suggested to represent only artefacts arising from sample preparation,[61] they might also serve a function in changing light conditions: switching of the supramolecular organization of PSII–LHCII complexes

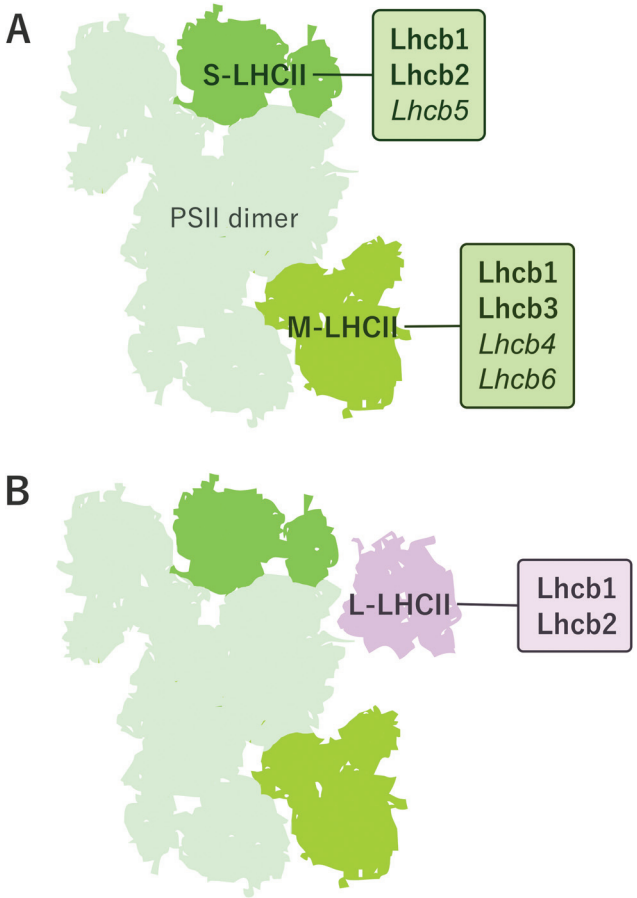

Fig. 2 The composition and arrangement of peripheral light harvesting antenna complexes of PSII in angiosperms. (A) The S-LHCII trimer contains mostly Lhcb1 and Lhcb2 proteins and is strongly bound to PSII core by Lhcb5 protein. The M-trimer in Arabidopsis is composed of Lhcb1 and Lhcb3 proteins and it is bound to the PSII core by the Lhcb4 and Lhcb6 proteins. The Lhcb monomers connecting the trimers are italicized. (B) PSII-LHCII supercomplex may bind additional L-LHCII trimers, which are loosely (L) associated to PSII core and mainly composed of Lhcb1 and Lhcb2 proteins, but their exact location is still elusive.

from disordered to well-arranged semi-crystalline arrays is assumed to be important in regulation of the diffusion efficiency of plastoquinol as well as for photoprotection and protein repair.[62] The semi-crystalline PSII–LHCII arrays are the most abundant in low light-acclimated plants. The highly ordered organization of the $C_2S_2M_2$ supercomplexes in grana probably allows faster energy transfer between the complexes and more fluent lateral diffusion of lipophilic plastoquinol between PSII and Cyt b_6f but, concomitantly, restricts the mobility of protein complexes, for instance during the PSII repair cycle (discussed in more detail later).[62] Under prolonged high light conditions, on the other hand, the frequency of semi-crystalline arrays decreases, facilitating the lateral mobility of membrane protein complexes and enabling thermal dissipation from LHCII in a process involving PsbS and zeaxanthin.[60,63] Such a dissipative mode of PSII–LHCII com-

plexes has been suggested to represent an LHCII aggregate,[64,65] a dissociated LHCII trimer[66] or a dissociated pentamer LHCII-CP24-CP29 *i.e.* M-LHCII.[67]

PSI–LHCI subunits and protein supercomplexes

Subunits of PSI–LHCI are presented in Table 2. So far, the structure of plant (*Pisum sativum*) PSI–LHCI has been resolved with X-ray crystallography at 2.6 Å resolution.[68] This structure contains 16 protein subunits and 232 cofactors. PSI reaction center is formed by PsaA/PsaB heterodimer that harbors most of the cofactors involved in the electron transfer reactions *i.e.* 6 chlorophyll a, 2 phylloquinones and a Fe_4S_4 cluster F_X.[69] The reaction center is surrounded by PsaC-PsaL, PsaN and PsaO, subunits of which the PsaH, PsaG and PsaO are unique to plants and algae.[70] PsaC binds the rest of the redox active cofactors *i.e.* Fe_4S_4 clusters F_A and F_B and is involved, together with PsaD and PsaE, in docking ferredoxin on the stromal side of PSI. PsaF, stabilized by PsaJ, docks plastocyanin together with PsaN and mediates the binding of LHCI antenna, whereas PsaG and PsaK are involved in stabilization of the LHCI antenna.[71]

As illustrated in Fig. 3A, the PSI core forms a stable supercomplex with the LHCI antenna, which is associated to the PsaF side of the core complex.[72] The LHCI comprises of Lhca1–4, which are assembled into dimers Lhca1/Lhca4 and Lhca2/Lhca3.[73,74] The fact that the Lhca proteins form dimers while the Lhcb proteins preferentially oligomerize as trimers likely derives from the slightly different orientation of their N-terminus.[70] It is important to note that, despite the highly conserved PSI–LHCI complex with four Lhca proteins, some species diverge from this model by dividing the Lhca proteins between the PsaF and the PsaH side[75] or by binding additional Lhca proteins on the PsaH[76] or PsaF[77] side of the PSI core. Interestingly, an additional Lhca1/Lhca4 dimer bound to PsaH side was recently found in Arabidopsis.[78] (For a recent review on the supercomplex diversity, see ref. 79 and 80.) PSI–LHCI has also been reported to physically interact with Cyt b_6f in *Chlamydomonas reinhardtii*[81] and recently also in Arabidopsis.[82] Moreover, up to six PSI–LHCI complexes have been found attached to type I NADH dehydrogenase (NDH-1) complex *via* LHCI proteins Lhca5 and Lhca6.[82–84] Importantly, PSI–LHCI is also able to bind L-LHCII. As depicted in Fig. 3B, PsaL, PsaH and PsaO form the docking site for L-LHCII[85] (see the next chapters), and the docking has been suggested to be stabilized by PsaI.[86]

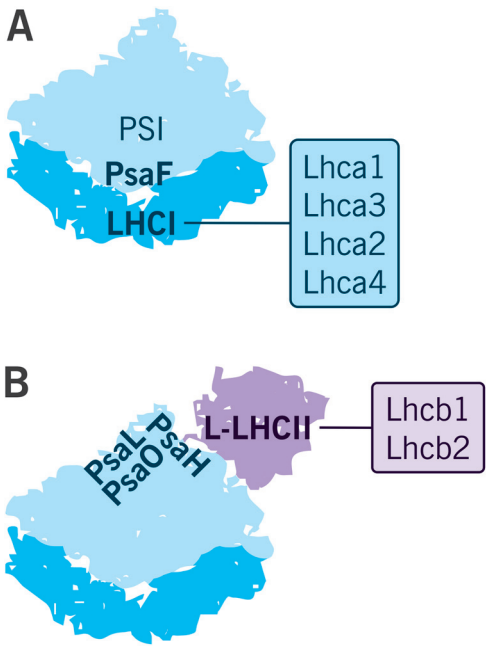

Fig. 3 The composition and arrangement of peripheral light harvesting antenna complexes of PSI in angiosperms. (A) The LHCI antenna is composed of four major LHCI proteins Lhca1–4, which bind to the PsaF side of PSI. (B) L-LHCII can also associate with PSI-LHCI under certain environmental conditions. This interaction is mediated by PsaH, PsaL and PsaO proteins.

L-LHCII connecting PSII and PSI

Although both photosystems have their own light harvesting antennas, the majority of the light energy is harvested and transferred to both PSII and PSI reaction centers by mutual L-LHCII system that likely resides in the grana margins.[54,87,88] A megacomplex containing both photosystems and the shared L-LHCII antenna has been suggested to exist based on analysis with native acrylamide gel electrophoresis.[37,87,89] However, the migration of the complex in the native gel electrophoresis system corresponds a mass of several megadaltons[90] and therefore likely represents a larger cluster of proteins (here assigned as PSII–LHCII–PSI–LHCI cluster) rather than a defined complex. Nevertheless, since the mechanical fractionation of the thylakoid membrane revealed both PSII and PSI in the grana margin fraction,[35] the evidence has accumulated at accelerating rate to show that the two photosystems are enriched in grana margins and become interconnected *via* mutual LHCII antenna system.[38,39,42,54,85,91,92] We argue that the digitonin-soluble PSII–LHCII–PSI–LHCI cluster originates from such network.[88] Nevertheless, such a cluster has not been successfully confirmed by electron microscopy, apparently due to a weak interaction *via* L-LHCII, and for this reason, more detailed biophysical, biochemical and structural analyses are needed.

Protein phosphorylation – flexible organization of protein complexes and dynamics of the entire thylakoid ultrastructure upon changing light conditions ensure fluent photosynthesis

As discussed above, a high-level organization of thylakoid protein complexes is essential for the key reactions of photosynthesis, yet the protein–protein interactions are highly

dynamic and dependent on environmental cues, particularly on changes in light conditions. The amount and spectral properties of sunlight entering a plant leaf undergo seasonal and cyclic variations, but the sun flecks, caused by brief movements of the canopy or clouds in the sky, introduce rapid changes in the input of irradiation taking place in timescale of seconds or minutes. These changes come along with substantial impact on the photosynthetic machinery, as an equilibrium between optimal light utilization and avoidance of destructive consequences of excess light energy requires subtle fine-tuning of the entire network of photosynthetic membrane system. The variations in environmental cues ensue structural changes of the extraordinary plastic thylakoid membrane system.[6] Alterations in grana stacking, changes in the width of the lumen and stromal gap and changes in the complex interactions between proteins[93–95] can take place in timescale of minutes.[96] Subsequent systematic research on the interaction, activation and relaxation of different regulatory mechanisms of photosynthetic light reactions, operating efficiently in fluctuating light conditions (*e.g.* ref. 97–99), have paved the way towards unifying models of the structural and functional dynamics of the thylakoid membrane.

Post-translational protein phosphorylation constitutes one of the most important and extensively scrutinized short-term regulatory mechanisms that affects the thylakoid ultrastructure and the enzymatic activity, affinity and stability of the proteins, and contributes to many other aspects in cellular regulation in all eukaryotes. Addition of a phosphate group to serine, threonine (Thr) or tyrosine residue of the client proteins is catalyzed by specific protein kinases, while the removal of the phosphate group is mediated by counteracting protein phosphatases. Almost 200 phosphoproteins have been found in the chloroplast of the model plant Arabidopsis and at least 28 of them are located in the thylakoid membrane.[100] Here, the reversible phosphorylation of PSII–LHCII proteins and subsequent changes in protein complex interactions and thylakoid structural dynamics are discussed.

Reversible LHCII antenna phosphorylation

LHCII proteins account for half of the total protein mass of the thylakoid membrane. They are responsible for maximizing the trapping of solar irradiance, delivering equal amount of excitation energy to the two photosystems and thermally dissipating the energy, which exceeds the utilization capacity of the photosynthetic machinery. Several light harvesting proteins are reversibly phosphorylated by specific protein kinases and phosphatases. The phosphorylation of the N-terminal Thr residues of Lhcb1 and Lhcb2 proteins, first described and linked to the regulation of light harvesting already decades ago,[101] has an important role in short-term light acclimation. The addition of a phosphate group to the Lhcb proteins is catalyzed by redox-dependent STN7 kinase,[102] which is activated by the binding of plastoquinol to the Q_0 site of the Cyt b_6f complex.[103] The STN7 kinase itself contains three or four phosphosites[100,104] and the activity and turn-over of the kinase is likely regulated by phosphorylation of the Ser and two of the Thr residues of the STN7 kinase.[104] Upon a shift from low to high light, the STN7 kinase becomes inactivated due to changes in stromal redox state.[105] In addition to Lhcb1 and Lhcb2, STN7 can phosphorylate to a minor extent also the PSII core proteins D1, D2 and CP43 in specific conditions as well as the monomeric antenna protein Lhcb4 (CP29).[102,106,107]

The removal of the phosphate group from Lhcb proteins is catalyzed by THYLAKOID-ASSOCIATED PHOSPHATASE OF 38 kDa /PROTEIN PHOSPHATASE1 (TAP38/PPH1).[108,109] The TAP38/PPH1 phosphatase is constitutively expressed and seems to be redox-independent.[108] Nevertheless, the flexible association of the phosphatase with large protein complexes might affect the activity or stability of TAP38/PPH1.[110] Unlike the STN7 kinase, the TAP38 phosphatase selectively targets only the Lhcb1 and Lchb2 proteins, not the PSII core proteins, by two specific surface cleft sites on the phosphatase.[111]

LHCII antenna phosphorylation and reorganization of protein complexes in grana margins allow fluent electron flow between the two photosystems

The reversible LHCII phosphorylation is an important regulatory mechanism that ensures balanced excitation energy distribution between PSII and PSI. The two photosystems work in series and must obtain similar amount of excitation energy in order to maintain ETC in balance despite prompt short-term changes in light quantity and quality. The STN7 kinase phosphorylates Lhcb1 and Lhcb2 proteins in different pools of LHCII trimers (S, M and L) (Fig. 4). The phosphorylation of Lhcb2 occurs almost exclusively in the pool of loosely bound L-LHCII trimers.[88,90] When phosphorylated, the affinity of L-LHCII trimer to the PSI–LHCI complex increases and it attaches to the LHCII docking site in PSI, thus forming an isolatable LHCII–PSI–LHCI complex.[42,90,112–114]Remarkably, only

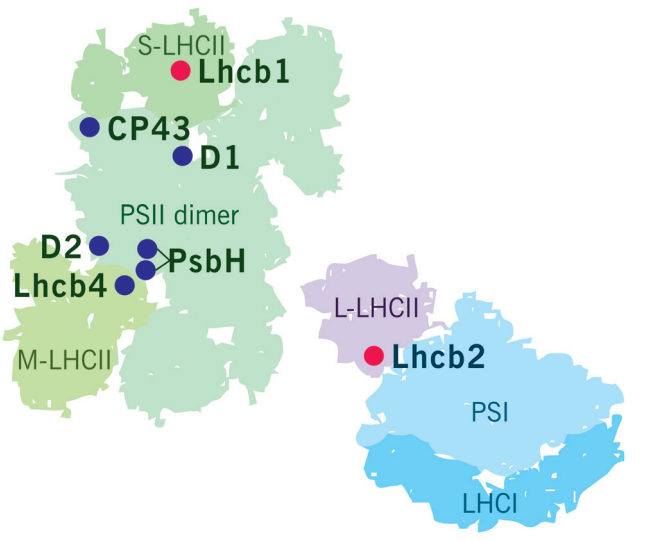

Fig. 4 STN7 and STN8 dependent phosphosites in PSII–LHCII supercomplex and in PSI–LHCI–LHCII supercomplex. Main targets of the STN7 kinase are marked with red dots and those of the STN8 kinase with blue dots.

one phosphorylated Lhcb2 protein is required for the association of LHCII trimer to PSI.[90] Recent cryo-EM structure of the PSI–LHCII complex demonstrated that the phosphorylated Thr-3 of the Lhcb2 protein directly interacts with PsaL, whereas the two preceding arginine residues, Arg-1 and Arg-2, bind to PsaH, PsaL and PsaO residues.[85] In addition to the formation of an isolatable PSI–LHCII complex, the accumulation of the aforementioned PSII–LHCII–PSI–LHCI cluster (demonstrated by native-PAGE) is largely dependent on LHCII phosphorylation: upon complete LHCII dephosphorylation, as demonstrated by the *stn7* mutant, the amount of the PSII–LHCII–PSI–LHCI cluster is clearly reduced, and the amount of large PSI–LHCI supercomplexes concomitantly increased.[87] Conversely, upon low-light-induced LHCII phosphorylation, also the amount of PSII–LHCII–PSI–LHCI cluster increases.[87] As depicted in Fig. 4, the phosphorylation of Lhcb1 takes place mainly in PSII–LHCII supercomplexes,[88,90] mostly in the S-trimer, since the M-trimer contains large amount of Lhcb1.4 isoform, which does not contain a phosphosite.[42] Importantly, the Lhcb1 phosphorylation does not induce the detachment of the LHCII trimer from PSII–LHCII supercomplexes[54,115,116] and the phosphorylated Lhcb1 does not function as an interaction surface between LHCII and PSI–LHCI.[85] Instead, the Lhcb1 phosphorylation seems to indirectly regulate the excitation energy distribution through remodeling of the grana.[113]

To merge the biochemical data that has been obtained on phosphorylation-dependent dynamics of thylakoid protein complexes and supercomplexes (*e.g.* ref. 39, 42, 54, 87, 89, 90, 113, 115, 117 and 118) with the dynamics of the entire thylakoid ultrastructure, we suggest a hypothetical model illustrated in Fig. 5. The model assumes that the biochemically distinct grana margin domains are located in the junctional slits between the grana and stroma membranes. Yet, further research is necessary for validation of the structural domains and, in particular, to reach a consensus about the 3D structure of the thylakoid membrane network. As depicted in Fig. 5A, when Lhcb1 and Lhcb2 proteins are dephosphorylated, as generally is the case in the dark, strong lateral heterogeneity between the two photosystems exist: PSII–LHCII complexes are tightly packed in grana, whereas PSI–LHCI is located in the stroma thylakoids. In light (Fig. 5B), the Lhcb1 proteins in the PSII–LHCII supercomplexes and the Lhcb2 proteins in the L-LHCII trimers become phosphorylated, the stromal gap get wider (blue arrows in Fig. 5B)[14] and the LHCII phosphorylation induces lateral diffusion of PSII–LHCII complexes and L-LHCII to the margins of grana membranes[39] (white arrows in Fig. 5B). Concomitantly, the phosphorylated PSII–LHCII complexes from the grana core and the PSI–LHCI complexes from stroma thylakoids get partially mixed,[38] thus increasing the relative area of the margin domain and decreasing that of the grana (red arrows in Fig. 5B). Indeed, the decreasing of the grana core area in response to LHCII phosphorylation was recently demonstrated with 3D structured illumination microscopy.[119] The lateral movement of PSII–LHCII complexes towards grana margins, where also PSI–LHCI complexes are present, locally decreases the lateral heterogeneity and allows the contact of the two photosystems, thus directing excitation energy from L-LHCII towards PSI–LHCI in this domain.[87,117] In the very core of grana, the Lhcb1 in the PSII–LHCII supercomplexes remains dephosphorylated[90] and the complexes might form semicrystalline arrays,[57,120] where excitation energy transfers fast from one complex to another and where also the diffusion of plastoquinol is fast in the lipid channels between the arrays thus rapidly reaching the Cyt b_6f complex.

Better understanding of the extraordinary complexity of the Lhcb1 and Lhcb2 phosphorylation and their role in modulation of the thylakoid membrane ultrastructure requires further research. Intriguingly, both Lhcb1 and Lhcb2 proteins contain several additional phosphosites that do not seem to be phosphorylated by STN7 or STN8,[121] leaving their function to be solved. In addition to phosphorylation, ion fluxes are also known to be involved in the regulation of thylakoid ultrastructure[122] but their interaction with phosphorylation of Lhcb proteins is likewise still elusive. Further, recent research has shown that thylakoid protein acetylation is also required for the regulation of excitation energy balancing between the two photosystems,[123] introducing a whole new aspect of post-translational protein modification for regulation of photosynthesis. In addition, acetylation of N-terminal regions of PSII–LHCII proteins was recently shown to be extensive, likely indicating an important role of acetylation in grana stacking.[124]

PSII core protein phosphorylation – disassembly and repair of PSII complex

Light energy drives the photosynthetic reactions, but the energy beyond that utilized by photochemistry can lead to oxidative damage of the photosynthetic apparatus, and consequently result in reduction of the photosynthetic capacity. The strong oxidative chemistry performed by the water splitting PSII makes the enzyme complex particularly vulnerable to oxidative damage in light. In fact, even low light induces damage to the PSII reaction center protein D1 leading to irreversible photoinactivation of the entire reaction centre.[125] The degree of light damage is directly proportional to the intensity of light the plants are exposed to,[126] but the photoinhibitory phenotype arises only when the rate of the damage exceeds the rate of repair. The restoration of PSII function occurs *via* a complex repair cycle in which the injured PSII complexes migrate from the grana core to the non-appressed membrane regions, where the damaged D1 protein is degraded by specific proteases and replaced with *de novo* synthesized protein.[127–131] As the damage is targeted to the D1 protein, PSII photoinhibition may be considered as evolutionary strategy to protect the rest of the PSII subunits and even PSI.[132]

The majority of the auxiliary proteins involved in the PSII repair cycle, such as the Deg and FtsH proteases, are located in the non-appressed thylakoids.[38,133] The PSII core protein phosphorylation appears to affect PSII repair cycle, although the exact role is poorly understood. As depicted in Fig. 4, the N-terminal Thr residues of D1, D2, CP43 and Thr-4 in PsbH are phosphorylated by the STN8 kinase upon exposure to high light[106,134] and, conversely, dephosphorylated by PSII CORE

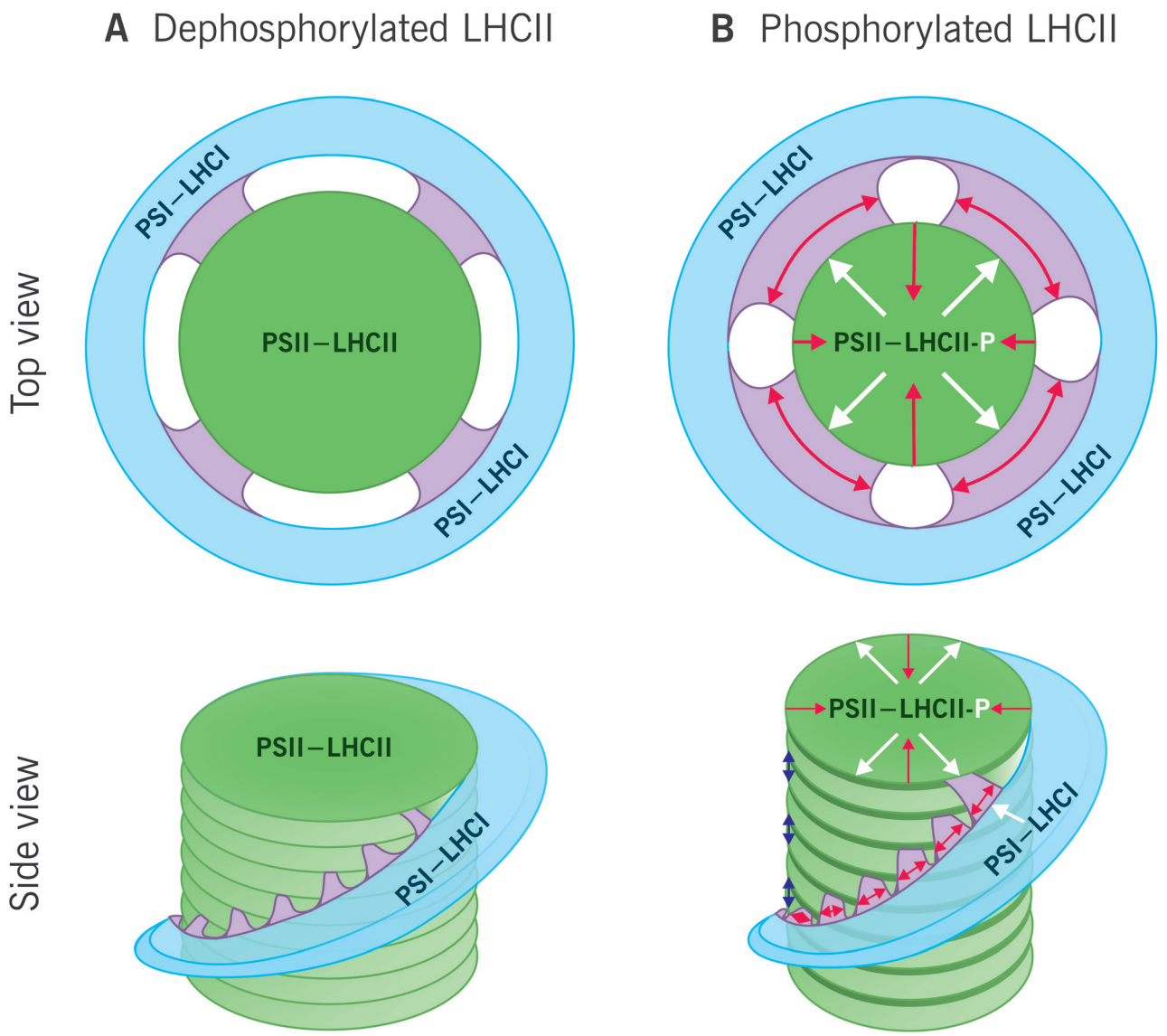

Fig. 5 The effect of LHCII protein phosphorylation on reorganization of the thylakoid ultrastructure. (A) In the dark, the LHCII proteins mainly exist in dephosphorylated form and strong lateral heterogeneity prevails: the PSII–LHCII complexes are tightly packed in the appressed grana stacks (green), whereas PSI–LHCI is located in the non-appressed stroma thylakoids (blue). The grana margins (purple), hosting both photosystems, are relatively narrow. (B) Light conditions favouring the phosphorylation (white P) of Lhcb1 in PSII–LHCII supercomplexes and Lhcb2 in L-LHCII trimers, induce an increase in the stromal gap (blue arrows) and lateral movement of PSII–LHCII as well as phosphorylated L-LHCII towards the grana margins (white arrows). Consequently, the relative area of the margins increases, and the width of the grana stacks decreases (red arrows). As the lateral heterogeneity of the protein complexes locally decreases, the phosphorylated L-LHCII can direct its excitation energy also towards PSI.

PHOSPHATASE (PBCP) when plants are transferred to low light or darkness.[135] Initially, the phosphorylation was considered to mark the D1 protein for degradation, but later investigations of the *stn8* and *stn7snt8* mutants demonstrated that the mutants are not more susceptible to light damage and made the authors to conclude that the PSII protein phosphorylation is not crucial for PSII repair.[106] Although not essential for the repair *per se*, the PSII core phosphorylation was later shown to facilitate the disassembly of the PSII–LHCII supercomplexes[136,137] and to increase the mobility of the PSII complexes[95,138] under photoinhibitory conditions. Locations of the PSII core phosphorylation sites at monomer–monomer interface and at the sites of Lhcb association to the core[139] are in support of this suggestion. Opposing to this view, Fristedt *et al.*[140] argued that the PSII core phosphorylation instead induces macroscopic rearrangements to the thylakoid membrane and allow the PSII repair cycle by decreasing the membrane cohesion. The different hypotheses on the roles of PSII core protein phosphorylation are not necessarily mutually exclusive.

Beside the PSII core protein phosphorylation, dynamic thylakoid rearrangements under high light exposure might also

arise from changes in the oligomerization state of the CURVATURE THYLAKOID1 proteins CURT1A-D.[10,141] The CURT1 proteins have, in fact, been suggested to facilitate the PSII repair cycle by unstacking the edges of the grana stacks.[142] This regulation likely involves phosphorylation, since CURT1B was recently shown to be phosphorylated by STN8 and to follow the phosphorylation dynamics of PSII core proteins D1 and D2.[14] Moreover, CURT1 proteins might even play a role in the successful connection between LHCII and PSI.[14,143] On the contrary, Reduced Induction of Non-Photochemical Quenching (RIQ) proteins RIQ1 and RIQ2 seem to act oppositely to the CURT1 proteins with respect to grana stacking, yet both the RIQ and CURT1 proteins seem to be essential for sufficient thermal dissipation from LHCII upon exposure of plants to high light.[144]

Concluding remarks

Discussion above focuses on protein phosphorylation-dependent rearrangements of the photosynthetic machinery in the thylakoid membrane of angiosperms. More generally speaking, the photosynthetic light reactions are regulated by a plethora of mechanisms, with distinct evolutionary diversity between cyanobacteria, algae and land plants, and such differences are closely related to the ultrastructure of the thylakoid membrane network.[145] Phosphorylation of thylakoid proteins and consequent modifications in thylakoid ultrastructure make a specific example of short-term dynamic regulation of light reactions with concomitant rearrangements in thylakoid architecture, in order to allow rapid acclimation of the photosynthetic apparatus to changing environmental cues. Yet, different land plants, and to a minor extent also different angiosperms, show modifications in phosphorylation target proteins of both the LHCII and PSII core proteins as well as in the number and location of phosphosites in each protein. Thus, we still miss a wealth of information related to the exact roles and mechanisms of thylakoid protein phosphorylation involved in response to environmental cues of a wide range of different plant species. Although the phosphorylation dynamics of LHCII and PSII core proteins are the most extensively scrutinized, and the only phosphoproteins in our model of thylakoid reorganization (Fig. 5), they likely represent only a tip of an iceberg of the factors that regulate the thylakoid ultrastructure and optimize photosynthetic reactions. As discussed above, also the CURT1B protein, which are phosphorylated by the same kinase as the PSII core proteins, have an important role in regulation of thylakoid rearrangements. Moreover, other post-translational protein modifications, such as lysine acetylation, seem to bring a completely new layer of regulatory mechanisms for thylakoid protein phosphorylation.

Conflicts of interest

There are no conflicts to declare.

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