



Cite this: *Photochem. Photobiol. Sci.*, 2020, **19**, 604

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

Marjaana Rantala, † Sanna Rantala † and Eva-Mari Aro \*

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.

Received 30th January 2020,

Accepted 27th March 2020

DOI: 10.1039/d0pp00025f

rsc.li/pps

## 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.

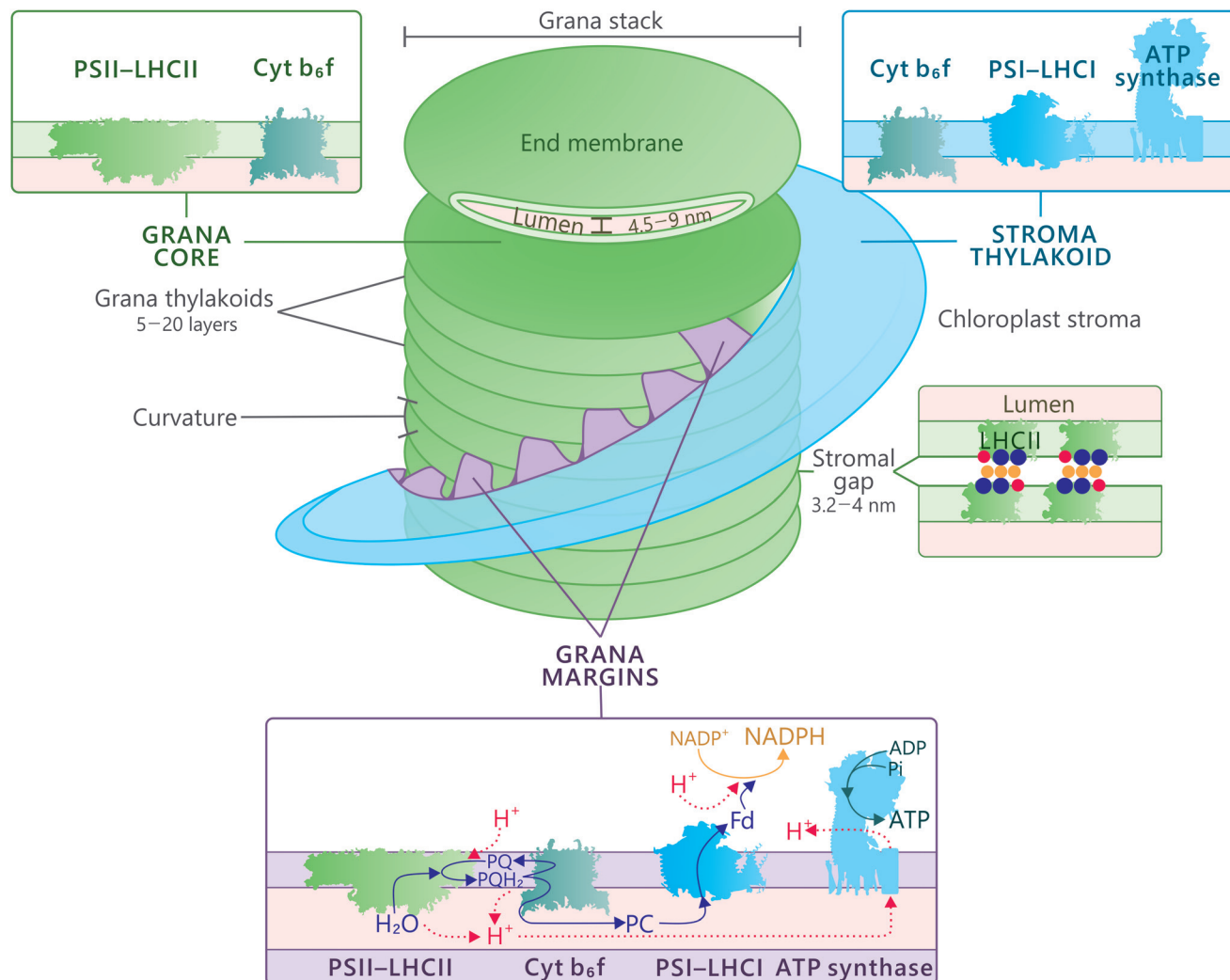
Photosynthetic energy conversion requires highly coordinated interplay between individual thylakoid protein com-

plexes 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.

*Molecular Plant Biology*, Department of Biochemistry, University of Turku, FI-20520 Turku, Finland. E-mail: [evaaro@utu.fi](mailto:evaaro@utu.fi)

† These authors contributed equally to this work.





**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<sub>6</sub>f 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<sup>1</sup> 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.<sup>2,3</sup> 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.<sup>4</sup> The grana stacks account for the majority of the total membrane area and harbor the majority of the total pigment content,<sup>5</sup> but the proportion of grana relative to the stroma thylakoids varies depending on the light condition.<sup>6</sup>

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,<sup>7–10</sup> 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.<sup>5,11</sup> 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.<sup>12,13</sup> Subsequent further optimization of thylakoid fractionation yielded a separation of the curved membrane domain from the rest of the margin domain,<sup>14</sup> 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.<sup>15</sup> Shimoni *et al.*<sup>16</sup> 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,<sup>17</sup> 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,<sup>2,17,18</sup> which according to Anderson *et al.*,<sup>6</sup> 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<sup>6,19</sup> (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.<sup>20</sup> 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,<sup>2,20–22</sup> 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.*<sup>23</sup>). Although the Lhcb1 and Lhcb2 proteins – the most abundant constituents of the grana membranes – are not a prerequisite for the grana formation,<sup>24</sup> but some level of stacking is observed even without the majority of Lhcb proteins,<sup>25</sup> 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.<sup>26,27</sup> 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.<sup>28</sup> Further, CURT1 oligomers located at the curved regions of grana are known to regulate the number and size of the stacks.<sup>10</sup>

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<sup>29</sup> (Fig. 1). PSII-LHCII supercomplexes are densely packed in the appressed grana and cover 80% of the membrane area.<sup>30</sup> 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.<sup>31</sup> Cyt  $b_6f$  is likely located in all thylakoid domains<sup>32,33</sup> and its distribution might be regulated according to light conditions.<sup>34</sup> The grana margins, which should not be confused with the curvature domain in the edge of the grana membranes,<sup>14</sup> are biochemically a fusion of grana and stroma thylakoids, and accommodate both photosystems.<sup>5,35–39</sup>

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

binds half of the chlorophyll pigments in the thylakoid membrane.<sup>40</sup> 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.<sup>41</sup> 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,<sup>42,43</sup> 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,

**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 <sub>A</sub> and F <sub>B</sub> binding, ferredoxin docking
PsaD	PsaD-1 PsaD-2	AT4G02770 AT1G03130	Ferredoxin docking
PsaE	PsaE-1 PsaE-2	AT4G28750 AT2G20260	Ferredoxin docking
PsaF	PsaF	AT1G31330	Plastocyanin docking
PsaG	PsaG	AT1G55670	LHCI stabilization
PsaH	PsaH-1 PsaH-2	AT3G16140 AT1G52230	L-LHCII docking
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

Lhcb1.1–3 are identical, while Lhcb1.4 and Lhcb1.5 show some difference.<sup>44</sup> 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.<sup>44</sup> 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.<sup>44,45</sup> For more information about different Lhcb isoforms and their evolution, see the recent review by Crepin and Caffarri.<sup>43</sup>

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.<sup>46</sup> 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.<sup>41</sup> The PsbA (D1), PsbD (D2) and PsbI proteins as well as the  $\alpha$  (PsbE) and  $\beta$  (PsbF) subunits of the Cyt b<sub>559</sub> complex form the PSII reaction center complex, which is the minimal unit required for primary photochemistry.<sup>47,48</sup> The reaction center is surrounded by two large chlorophyll-binding light harvesting proteins CP47 (PsbB) and CP43 (PsbC) as well as the luminal oxygen evolving complex. The oxygen evolving complex is composed of PsbO, PsbP, PsbQ and PsbR proteins that stabilize the Mn<sub>4</sub>CaO<sub>5</sub> 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.<sup>49</sup> 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 luminal extrinsic PsbTn seems to be important for light acclimation.<sup>50,51</sup> Noteworthy, PsbN is no longer considered as a structural subunit of PSII.<sup>52</sup>

### 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<sup>8,19,53</sup> and interconnects the two photosystems in grana margins<sup>54</sup> (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.<sup>42</sup> Lhcb3 and Lhcb6 are unique to land plants, but intriguingly, are absent from the gymnosperm family Pinaceae.<sup>45,55</sup> 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.<sup>46</sup> The structure of  $C_2S_2M_2$  supercomplex has been determined with cryo-EM at 5.3 Å resolution from *Arabidopsis*<sup>41</sup> and at 2.7–3.2 Å resolution from *Pisum sativum*,<sup>46</sup> 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.<sup>42,56</sup>

In certain conditions, the PSII-LHCII supercomplexes have been reported to form semi-crystalline arrays in grana core.<sup>3,57,58</sup> Besides such lateral re-arrangement, the PSII-LHCII supercomplexes in the array are also vertically associated with complexes across the stromal gap.<sup>46,59</sup> Although the arrays are relatively rarely observed<sup>60</sup> and even suggested to represent only artefacts arising from sample preparation,<sup>61</sup> 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.<sup>62</sup> 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  $b_6/f$  but, concomitantly, restricts the mobility of protein complexes, for instance during the PSII repair cycle (discussed in more detail later).<sup>62</sup> 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.<sup>60,63</sup> Such a dissipative mode of PSII-LHCII com-



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

### PSI-LHCI subunits and protein supercomplexes

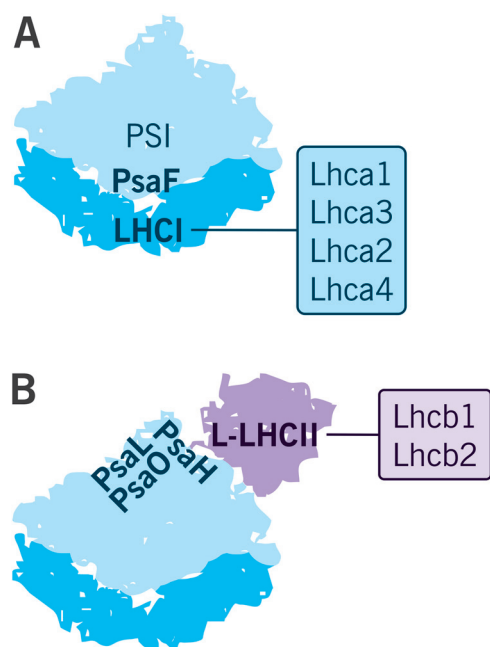
Subunits of PSI-LHCI are presented in Table 2. So far, the structure of plant (*Pisum sativum*) PSI-LHCI has been resolved with X-ray crystallography at 2.6 Å resolution.<sup>68</sup> This structure contains 16 protein subunits and 232 cofactors. PSI reaction center is formed by PsaA/PsaB heterodimer that harbors most of the cofactors involved in the electron transfer reactions *i.e.* 6 chlorophyll *a*, 2 phylloquinones and a Fe<sub>4</sub>S<sub>4</sub> cluster F<sub>X</sub>.<sup>69</sup> 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.<sup>70</sup> PsaC binds the rest of the redox active cofactors *i.e.* Fe<sub>4</sub>S<sub>4</sub> clusters F<sub>A</sub> and F<sub>B</sub> 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.<sup>71</sup>

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.<sup>72</sup> The LHCI comprises of Lhca1–4, which are assembled into dimers Lhca1/Lhca4 and Lhca2/Lhca3.<sup>73,74</sup> 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.<sup>70</sup> 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<sup>75</sup> or by binding additional Lhca proteins on the PsaH<sup>76</sup> or PsaF<sup>77</sup> side of the PSI core. Interestingly, an additional Lhca1/Lhca4 dimer bound to PsaH side was recently found in *Arabidopsis*.<sup>78</sup> (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<sub>6</sub>f in *Chlamydomonas reinhardtii*<sup>81</sup> and recently also in *Arabidopsis*.<sup>82</sup> 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.<sup>82–84</sup> 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<sup>85</sup> (see the next chapters), and the docking has been suggested to be stabilized by PsaI.<sup>86</sup>

### 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.<sup>54,87,88</sup> A megacomplex containing both photosystems and the shared L-LHCII antenna has been suggested to exist based on analysis with native acrylamide gel electrophoresis.<sup>37,87,89</sup> However, the migration of the complex in the native gel electrophoresis system corresponds a mass of several megadaltons<sup>90</sup> 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,<sup>35</sup> 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.<sup>38,39,42,54,85,91,92</sup> We argue that the digitonin-soluble PSII-LHCII-PSI-LHCI cluster originates from such network.<sup>88</sup> 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.



**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.

## 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.<sup>6</sup> Alterations in grana stacking, changes in the width of the lumen and stromal gap and changes in the complex interactions between proteins<sup>93–95</sup> can take place in timescale of minutes.<sup>96</sup> 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.<sup>100</sup> 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

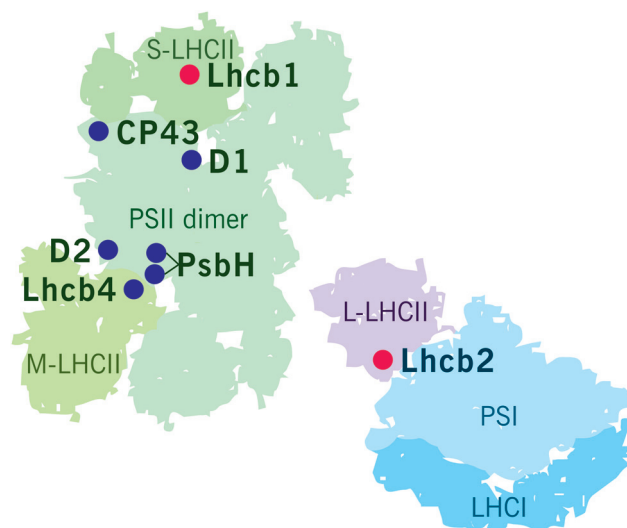
LHCII proteins account for half of the total protein mass of the thylakoid membrane. They are responsible for maximizing the trapping of solar irradiance, delivering equal amount of excitation energy to the two photosystems and thermally dissipating the energy, which exceeds the utilization capacity 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,<sup>101</sup> 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,<sup>102</sup> which is activated by the binding of plastoquinol to the Q<sub>0</sub> site of the Cyt b<sub>6</sub>f complex.<sup>103</sup> The STN7 kinase itself contains three or four phosphosites<sup>100,104</sup> 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.<sup>104</sup> Upon a shift from low to high light, the STN7 kinase becomes inactivated due to changes in stromal redox state.<sup>105</sup> 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).<sup>102,106,107</sup>

The removal of the phosphate group from Lhcb proteins is catalyzed by THYLAKOID-ASSOCIATED PHOSPHATASE OF 38 kDa /PROTEIN PHOSPHATASE1 (TAP38/PPH1).<sup>108,109</sup> The TAP38/PPH1 phosphatase is constitutively expressed and seems to be redox-independent.<sup>108</sup> Nevertheless, the flexible association of the phosphatase with large protein complexes might affect the activity or stability of TAP38/PPH1.<sup>110</sup> Unlike the STN7 kinase, the TAP38 phosphatase selectively targets only the Lhcb1 and Lhcb2 proteins, not the PSII core proteins, by two specific surface cleft sites on the phosphatase.<sup>111</sup>

### 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.<sup>88,90</sup> 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.<sup>42,90,112–114</sup> 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.<sup>90</sup> 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.<sup>85</sup> 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.<sup>87</sup> Conversely, upon low-light-induced LHCII phosphorylation, also the amount of PSII-LHCII-PSI-LHCI cluster increases.<sup>87</sup> As depicted in Fig. 4, the phosphorylation of Lhcb1 takes place mainly in PSII-LHCII supercomplexes,<sup>88,90</sup> mostly in the S-trimer, since the M-trimer contains large amount of Lhcb1.4 isoform, which does not contain a phosphosite.<sup>42</sup> Importantly, the Lhcb1 phosphorylation does not induce the detachment of the LHCII trimer from PSII-LHCII supercomplexes<sup>54,115,116</sup> and the phosphorylated Lhcb1 does not function as an interaction surface between LHCII and PSI-LHCI.<sup>85</sup> Instead, the Lhcb1 phosphorylation seems to indirectly regulate the excitation energy distribution through remodeling of the grana.<sup>113</sup>

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)<sup>14</sup> and the LHCII phosphorylation induces lateral diffusion of PSII-LHCII complexes and L-LHCII to the margins of grana membranes<sup>39</sup> (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,<sup>38</sup> 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.<sup>119</sup> 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.<sup>87,117</sup> In the very core of grana, the Lhcb1 in the PSII-LHCII supercomplexes remains dephosphorylated<sup>90</sup> and the complexes might form semicrystalline arrays,<sup>57,120</sup> 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<sub>6</sub>f 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,<sup>121</sup> leaving their function to be solved. In addition to phosphorylation, ion fluxes are also known to be involved in the regulation of thylakoid ultrastructure<sup>122</sup> 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,<sup>123</sup> 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.<sup>124</sup>

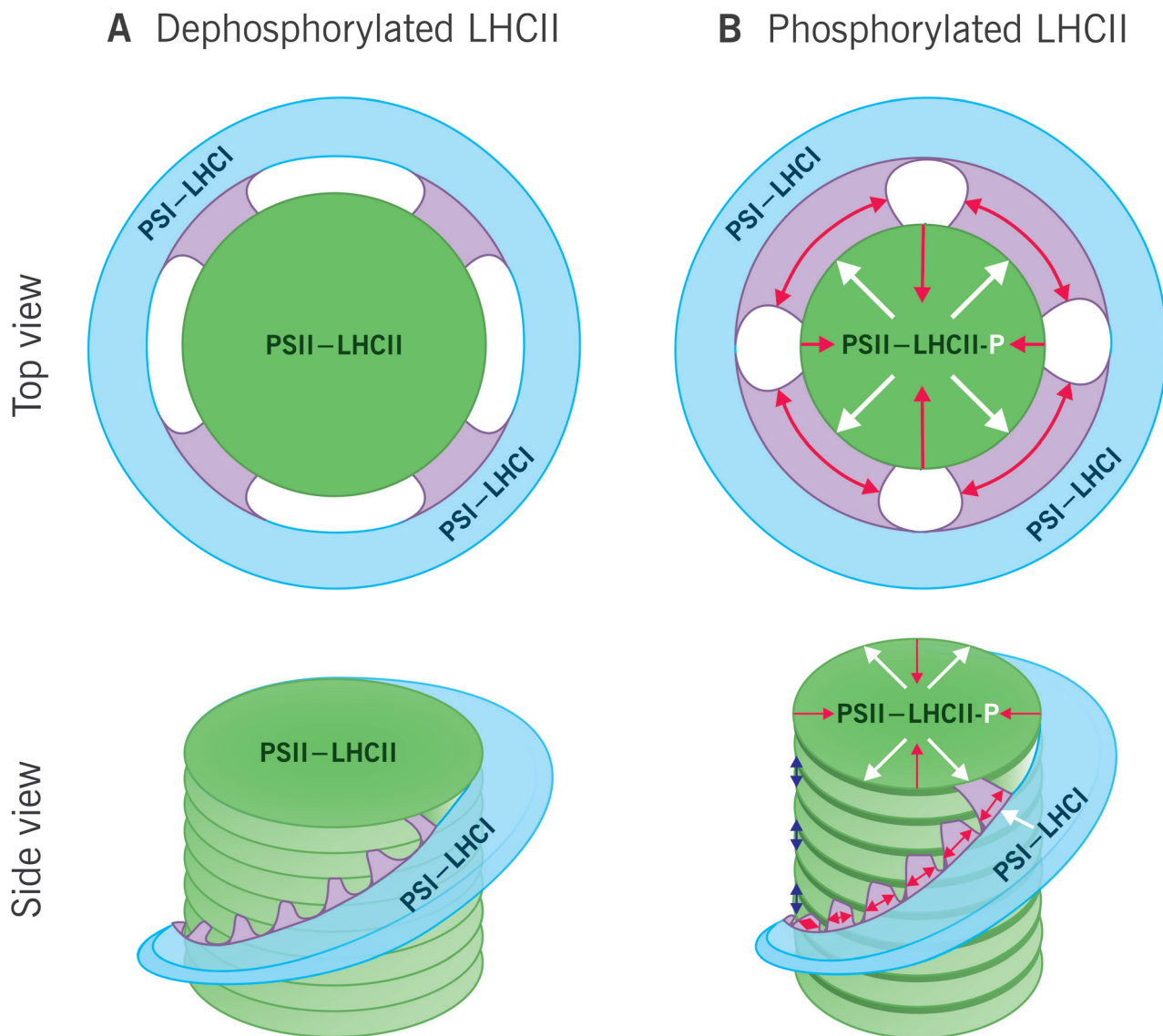
#### PSII core protein phosphorylation – disassembly and repair of PSII complex

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.<sup>125</sup> The degree of light damage is directly proportional to the intensity of light the plants are exposed to,<sup>126</sup> 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.<sup>127–131</sup> 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.<sup>132</sup>

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.<sup>38,133</sup> 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<sup>106,134</sup> 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.<sup>135</sup> 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.<sup>106</sup> Although not essential for the repair *per se*, the PSII core phosphorylation was later shown to facilitate the disassembly of the PSII-LHCII supercomplexes<sup>136,137</sup> and to increase the mobility of the PSII complexes<sup>95,138</sup> under photoinhibitory conditions. Locations

of the PSII core phosphorylation sites at monomer-monomer interface and at the sites of Lhcb association to the core<sup>139</sup> are in support of this suggestion. Opposing to this view, Fristedt *et al.*<sup>140</sup> 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.<sup>10,141</sup> The CURT1 proteins have, in fact, been suggested to facilitate the PSII repair cycle by unstacking the edges of the grana stacks.<sup>142</sup> 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.<sup>14</sup> Moreover, CURT1 proteins might even play a role in the successful connection between LHCII and PSI.<sup>14,143</sup> 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.<sup>144</sup>

## 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.<sup>145</sup> 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.

## Acknowledgements

This work was financially supported by Center of excellence of the Academy of Finland (307335), Jane and Aatos Erkko Foundation and Turku University Foundation. Dr Andrea Trotta is thanked for critically reviewing and commenting the manuscript.

## References

- 1 L. A. Staehelin, Chloroplast structure: from chlorophyll granules to supra-molecular architecture of thylakoid membranes, *Photosynth. Res.*, 2003, **76**, 185–196.
- 2 L. Mustárdy and G. Garab, Granum revisited. A three-dimensional model – where things fall into place, *Trends Plant Sci.*, 2003, **8**, 117–122.
- 3 B. Daum, D. Nicastro, J. Austin, J. R. McIntosh and W. Kühlbrandt, Arrangement of Photosystem II and ATP Synthase in Chloroplast Membranes of Spinach and Pea, *Plant Cell*, 2010, **22**, 1299–1312.
- 4 H. Kirchhoff, C. Hall, M. Wood, M. Herbstová, O. Tsabari, R. Nevo, D. Charuvi, E. Shimoni and Z. Reich, Dynamic control of protein diffusion within the granal thylakoid lumen, *Proc. Natl. Acad. Sci. U. S. A.*, 2011, **108**, 20248–20253.
- 5 P.-Å. Albertsson, A quantitative model of the domain structure of the photosynthetic membrane, *Trends Plant Sci.*, 2001, **6**, 349–354.
- 6 J. M. Anderson, P. Horton, E.-H. Kim and W. S. Chow, Towards elucidation of dynamic structural changes of plant thylakoid architecture, *Philos. Trans. R. Soc., B*, 2012, **367**, 3515–3524.
- 7 D. J. Murphy, The molecular organisation of the photosynthetic membranes of higher plants, *Biochim. Biophys. Acta, Rev. Biomembr.*, 1986, **864**, 33–94.
- 8 J. P. Dekker and E. J. Boekema, Supramolecular organization of thylakoid membrane proteins in green plants, *Biochim. Biophys. Acta, Bioenerg.*, 2005, **1706**, 12–39.
- 9 R. Kouřil, G. T. Oostergetel and E. J. Boekema, Fine structure of granal thylakoid membrane organization using cryo electron tomography, *Biochim. Biophys. Acta, Bioenerg.*, 2011, **1807**, 368–374.
- 10 U. Armbruster, M. Labs, M. Pribil, S. Viola, W. Xu, M. Scharfenberg, A. P. Hertle, U. Rojahn, P. E. Jensen, F. Rappaport, P. Joliot, P. Dörmann, G. Wanner and D. Leister, Arabidopsis CURVATURE THYLAKOID1 Proteins Modify Thylakoid Architecture by Inducing Membrane Curvature, *Plant Cell*, 2013, **25**, 2661–2678.
- 11 R. Danielsson, P.-Å. Albertsson, F. Mamedov and S. Styring, Quantification of photosystem I and II in different parts of the thylakoid membrane from spinach, *Biochim. Biophys. Acta, Bioenerg.*, 2004, **1608**, 53–61.
- 12 S. Puthiyaveetil, O. Tsabari, T. Lowry, S. Lenhert, R. R. Lewis, Z. Reich and H. Kirchhoff, Compartmentalization of the protein repair machinery in



- photosynthetic membranes, *Proc. Natl. Acad. Sci. U. S. A.*, 2014, **111**, 15839–15844.
- 13 H. Koochak, S. Puthiyaveetil, D. L. Mullendore, M. Li and H. Kirchhoff, The structural and functional domains of plant thylakoid membranes, *Plant J.*, 2019, **100**, 213.
  - 14 A. Trotta, A. A. Bajwa, I. Mancini, V. Paakkanen, M. Pribil and E.-M. Aro, The role of phosphorylation dynamics of CURVATURE THYLAKOID 1B in plant thylakoid membranes, *Plant Physiol.*, 2019, **181**, 1615–1631.
  - 15 P.-O. Arvidsson and C. Sundby, A model for the topology of the chloroplast thylakoid membrane, *Funct. Plant Biol.*, 1999, **26**, 687–694.
  - 16 E. Shimoni, O. Rav-Hon, I. Ohad, V. Brumfeld and Z. Reich, Three-Dimensional Organization of Higher-Plant Chloroplast Thylakoid Membranes Revealed by Electron Tomography, *Plant Cell*, 2005, **17**, 2580–2586.
  - 17 D. J. Paolillo, The Three-Dimensional Arrangement of Intergranal Lamellae in Chloroplasts, *J. Cell Sci.*, 1970, **6**, 243–253.
  - 18 J. Brangeon and L. Mustardy, Ontogenetic assembly of intra-chloroplastic lamellae viewed in 3-dimension, *Biol. Cell*, 1979, **36**, 71–80.
  - 19 A. V. Ruban and M. P. Johnson, Visualizing the dynamic structure of the plant photosynthetic membrane, *Nat. Plants*, 2015, **1**, 15161.
  - 20 Y. Bussi, E. Shimoni, A. Weiner, R. Kapon, D. Charuvi, R. Nevo, E. Efrati and Z. Reich, Fundamental helical geometry consolidates the plant photosynthetic membrane, *Proc. Natl. Acad. Sci. U. S. A.*, 2019, **116**, 22366–22375.
  - 21 L. Mustardy, K. Buttle, G. Steinbach and G. Garab, The Three-Dimensional Network of the Thylakoid Membranes in Plants: Quasi-helical Model of the Granum-Stroma Assembly, *Plant Cell*, 2008, **20**, 2552–2557.
  - 22 J. R. Austin and L. A. Staehelin, Three-Dimensional Architecture of Grana and Stroma Thylakoids of Higher Plants as Determined by Electron Tomography, *Plant Physiol.*, 2011, **155**, 1601–1611.
  - 23 W. S. Chow, E.-H. Kim, P. Horton and J. M. Anderson, Grana stacking of thylakoid membranes in higher plant chloroplasts: the physicochemical forces at work and the functional consequences that ensue, *Photochem. Photobiol. Sci.*, 2005, **4**, 1081.
  - 24 J. Andersson, M. Wentworth, R. Walters, C. Howard, A. Ruban, P. Horton and S. Jansson, Absence of the Lhcb1 and Lhcb2 proteins of the light-harvesting complex of photosystem II – effects on photosynthesis, grana stacking and fitness, *Plant J.*, 2003, **35**, 350–361.
  - 25 D. J. Simpson, Freeze-fracture studies on barley plastid membranes. III. Location of the light-harvesting chlorophyll-protein, *Carlsberg Res. Commun.*, 1979, **44**, 305.
  - 26 N. Murata, Control of excitation transfer in photosynthesis. II. Magnesium ion-dependent distribution of excitation energy between two pigment systems in spinach chloroplasts, *Biochim. Biophys. Acta, Bioenerg.*, 1969, **189**, 171–181.
  - 27 J. Barber, An explanation for the relationship between salt-induced thylakoid stacking and the chlorophyll fluorescence changes associated with changes in spillover of energy from photosystem II to photosystem I, *FEBS Lett.*, 1980, **118**, 1–10.
  - 28 J. Standfuss, A. C. T. van Scheltinga, M. Lamborghini and W. Kühlbrandt, Mechanisms of photoprotection and non-photochemical quenching in pea light-harvesting complex at 2.5 Å resolution, *EMBO J.*, 2005, **24**, 919–928.
  - 29 B. Andersson and J. M. Anderson, Lateral heterogeneity in the distribution of chlorophyll-protein complexes of the thylakoid membranes of spinach chloroplasts, *Biochim. Biophys. Acta, Bioenerg.*, 1980, **593**, 427–440.
  - 30 H. Kirchhoff, I. Treggier, W. Haase and U. Kubitschek, Supramolecular Photosystem II Organization in Grana Thylakoid Membranes: Evidence for a Structured Arrangement, *Biochemistry*, 2004, **43**, 9204–9213.
  - 31 R. Nevo, D. Charuvi, O. Tsabari and Z. Reich, Composition, architecture and dynamics of the photosynthetic apparatus in higher plants, *Plant J.*, 2012, **70**, 157–176.
  - 32 J. M. Anderson, The role of chlorophyll-protein complexes in the function and structure of chloroplast thylakoids, *Mol. Cell. Biochem.*, 1982, **46**, 161–172.
  - 33 P.-Å. Albertsson, E. Andreasson, P. Svensson and S.-G. Yu, Localization of cytochrome f in the thylakoid membrane: evidence for multiple domains, *Biochim. Biophys. Acta, Bioenerg.*, 1991, **1098**, 90–94.
  - 34 O. Vallon, L. Bulte, P. Dainese, J. Olive, R. Bassi and F. A. Wollman, Lateral redistribution of cytochrome b6/f complexes along thylakoid membranes upon state transitions, *Proc. Natl. Acad. Sci. U. S. A.*, 1991, **88**, 8262–8266.
  - 35 L. Wollenberger, H. Stefansson, S.-G. Yu and P.-Å. Albertsson, Isolation and characterization of vesicles originating from the chloroplast grana margins, *Biochim. Biophys. Acta, Bioenerg.*, 1994, **1184**, 93–102.
  - 36 R. Danielsson and P.-Å. Albertsson, Fragmentation and separation analysis of the photosynthetic membrane from spinach, *Biochim. Biophys. Acta, Bioenerg.*, 2009, **1787**, 25–36.
  - 37 S. Järvi, M. Suorsa, V. Paakkanen and E.-M. Aro, Optimized native gel systems for separation of thylakoid protein complexes: novel super- and mega-complexes, *Biochem. J.*, 2011, **439**, 207–214.
  - 38 M. Suorsa, M. Rantala, R. Danielsson, S. Järvi, V. Paakkanen, W. P. Schröder, S. Styring, F. Mamedov and E.-M. Aro, Dark-adapted spinach thylakoid protein heterogeneity offers insights into the photosystem II repair cycle, *Biochim. Biophys. Acta, Bioenerg.*, 2014, **1837**, 1463–1471.
  - 39 S. Rantala and M. Tikkanen, Phosphorylation-induced lateral rearrangements of thylakoid protein complexes upon light acclimation, *Plant Direct*, 2018, **2**, 1–12.
  - 40 Z. Liu, H. Yan, K. Wang, T. Kuang, J. Zhang, L. Gui, X. An and W. Chang, Crystal structure of spinach major light-



- harvesting complex at 2.72 Å resolution, *Nature*, 2004, **428**, 287–292.
- 41 L. S. van Bezouwen, S. Caffarri, R. S. Kale, R. Kouřil, A.-M. W. H. Thunnissen, G. T. Oostergetel and E. J. Boekema, Subunit and chlorophyll organization of the plant photosystem II supercomplex, *Nat. Plants*, 2017, **3**, 17080.
  - 42 P. Galka, S. Santabarbara, T. T. H. Khuong, H. Degand, P. Morsomme, R. C. Jennings, E. J. Boekema and S. Caffarri, Functional Analyses of the Plant Photosystem I-Light-Harvesting Complex II Supercomplex Reveal That Light-Harvesting Complex II Loosely Bound to Photosystem II Is a Very Efficient Antenna for Photosystem I in State II, *Plant Cell*, 2012, **24**, 2963–2978.
  - 43 A. Crepin and S. Caffarri, Functions and Evolution of Lhcb Isoforms Composing LHCII, the Major Light Harvesting Complex of Photosystem II of Green Eukaryotic Organisms, *Curr. Protein Pept. Sci.*, 2018, **19**, 699–713.
  - 44 S. Jansson, A guide to the Lhc genes and their relatives in Arabidopsis, *Trends Plant Sci.*, 1999, **4**, 236–240.
  - 45 S. Grebe, A. Trotta, A. A. Bajwa, M. Suorsa, P. J. Gollan, S. Jansson, M. Tikkanen and E.-M. Aro, The unique photosynthetic apparatus of Pinaceae: analysis of photosynthetic complexes in *Picea abies*, *J. Exp. Bot.*, 2019, **70**, 3211–3225.
  - 46 X. Su, J. Ma, X. Wei, P. Cao, D. Zhu, W. Chang, Z. Liu, X. Zhang and M. Li, Structure and assembly mechanism of plant C2S2M2-type PSII-LHCII supercomplex, *Science*, 2017, **357**, 815–820.
  - 47 O. Nanba and K. Satoh, Isolation of a photosystem II reaction center consisting of D-1 and D-2 polypeptides and cytochrome b-559, *Proc. Natl. Acad. Sci. U. S. A.*, 1987, **84**, 109–112.
  - 48 A. N. Webber, L. Packman, D. J. Chapman, J. Barber and J. C. Gray, A fifth chloroplast-encoded polypeptide is present in the photosystem II reaction centre complex, *FEBS Lett.*, 1989, **242**, 259–262.
  - 49 B. Lundin, M. Hansson, B. Schoefs, A. V. Vener and C. Spetea, The Arabidopsis PsbO2 protein regulates dephosphorylation and turnover of the photosystem II reaction centre D1 protein, *Plant J. Cell Mol. Biol.*, 2007, **49**, 528–539.
  - 50 X. Wei, X. Su, P. Cao, X. Liu, W. Chang, M. Li, X. Zhang and Z. Liu, Structure of spinach photosystem II-LHCII supercomplex at 3.2 Å resolution, *Nature*, 2016, **534**, 69–74.
  - 51 Y.-E. Chen, S. Yuan, L. Lezhneva, J. Meurer, S. Schwenkert, F. Mamedov and W. P. Schröder, The Low Molecular Mass Photosystem II Protein PsbTn Is Important for Light Acclimation, *Plant Physiol.*, 2019, **179**, 1739–1753.
  - 52 M. Plöckinger, S. Schwenkert, L. von Sydow, W. P. Schröder and J. Meurer, Functional Update of the Auxiliary Proteins PsbW, PsbY, HCF136, PsbN, TerC and ALB3 in Maintenance and Assembly of PSII, *Front. Plant Sci.*, 2016, **7**, 423.
  - 53 K. Sznee, J. P. Dekker, R. T. Dame, H. van Roon, G. J. L. Wuite and R. N. Frese, Jumping Mode Atomic Force Microscopy on Grana Membranes from Spinach, *J. Biol. Chem.*, 2011, **286**, 39164–39171.
  - 54 M. Grieco, M. Suorsa, A. Jajoo, M. Tikkanen and E.-M. Aro, Light-harvesting II antenna trimers connect energetically the entire photosynthetic machinery—including both photosystems II and I, *Biochim. Biophys. Acta, Bioenerg.*, 2015, **1847**, 607–619.
  - 55 R. Kouřil, L. Nosek, J. Bartoš, E. J. Boekema and P. Ilík, Evolutionary loss of light-harvesting proteins Lhcb6 and Lhcb3 in major land plant groups – break-up of current dogma, *New Phytol.*, 2016, **210**, 808–814.
  - 56 M. M. Koskela, A. Brünje, A. Ivanauskaite, L. S. Lopez, D. Schneider, R. A. DeTar, H.-H. Kunz, I. Finkemeier and P. Mulo, Comparative analysis of thylakoid protein complexes in state transition mutants *nsi* and *stn7*: focus on PSI and LHCII, *Photosynth. Res.*, 2020, DOI: 10.1007/s11120-020-00711-4.
  - 57 H. Kirchhoff, W. Haase, S. Wegner, R. Danielsson, R. Ackermann and P.-A. Albertsson, Low-Light-Induced Formation of Semicrystalline Photosystem II Arrays in Higher Plant Chloroplasts, *Biochemistry*, 2007, **46**, 11169–11176.
  - 58 R. Kouřil, J. P. Dekker and E. J. Boekema, Supramolecular organization of photosystem II in green plants, *Biochim. Biophys. Acta, Bioenerg.*, 2012, **1817**, 2–12.
  - 59 P. Albanese, J. Nield, J. A. M. Tabares, A. Chiodoni, M. Manfredi, F. Gosetti, E. Marengo, G. Saracco, J. Barber and C. Pagliano, Isolation of novel PSII-LHCII megacomplexes from pea plants characterized by a combination of proteomics and electron microscopy, *Photosynth. Res.*, 2016, **130**, 19–31.
  - 60 T. K. Goral, M. P. Johnson, C. D. P. Duffy, A. P. R. Brain, A. V. Ruban and C. W. Mullineaux, Light-harvesting antenna composition controls the macrostructure and dynamics of thylakoid membranes in Arabidopsis, *Plant J.*, 2012, **69**, 289–301.
  - 61 N. M. Tsvetkova, A. P. R. Brain and P. J. Quinn, Structural characteristics of thylakoid membranes of Arabidopsis mutants deficient in lipid fatty acid desaturation, *Biochim. Biophys. Acta, Biomembr.*, 1994, **1192**, 263–271.
  - 62 S. Tietz, S. Puthiyaveetil, H. M. Enlow, R. Yarbrough, M. Wood, D. A. Semchonok, T. Lowry, Z. Li, P. Jahns, E. J. Boekema, S. Lenhert, K. K. Niyogi and H. Kirchhoff, Functional Implications of Photosystem II Crystal Formation in Photosynthetic Membranes, *J. Biol. Chem.*, 2015, **290**, 14091–14106.
  - 63 S. Kereiche, A. Z. Kiss, R. Kouřil, E. J. Boekema and P. Horton, The PsbS protein controls the macro-organisation of photosystem II complexes in the grana membranes of higher plant chloroplasts, *FEBS Lett.*, 2010, **584**, 759–764.





- 64 P. Horton, A. V. Ruban, D. Rees, A. A. Pascal, G. Noctor and A. J. Young, Control of the light-harvesting function of chloroplast membranes by aggregation of the LHCII chlorophyll-protein complex, *FEBS Lett.*, 1991, **292**, 1–4.
- 65 P. Horton, M. Wentworth and A. Ruban, Control of the light harvesting function of chloroplast membranes: The LHCII-aggregation model for non-photochemical quenching, *FEBS Lett.*, 2005, **579**, 4201–4206.
- 66 A. R. Holzwarth, Y. Miloslavina, M. Nilkens and P. Jahns, Identification of two quenching sites active in the regulation of photosynthetic light-harvesting studied by time-resolved fluorescence, *Chem. Phys. Lett.*, 2009, **483**, 262–267.
- 67 N. Betterle, M. Ballottari, S. Zorzan, S. de Bianchi, S. Cazzaniga, L. Dall'Osto, T. Morosinotto and R. Bassi, Light-induced Dissociation of an Antenna Hetero-oligomer Is Needed for Non-photochemical Quenching Induction, *J. Biol. Chem.*, 2009, **284**, 15255–15266.
- 68 Y. Mazar, A. Borovikova, I. Caspy and N. Nelson, Structure of the plant photosystem I supercomplex at 2.6 Å resolution, *Nat. Plants*, 2017, **3**, 17014.
- 69 S. Caffarri, T. Tibiletti, R. Jennings and S. Santabarbara, A comparison between plant photosystem I and photosystem II architecture and functioning, *Curr. Protein Pept. Sci.*, 2014, **15**, 296–331.
- 70 I. Caspy and N. Nelson, Structure of the plant photosystem I, *Biochem. Soc. Trans.*, 2018, **46**, 285–294.
- 71 P. E. Jensen, R. Bassi, E. J. Boekema, J. P. Dekker, S. Jansson, D. Leister, C. Robinson and H. V. Scheller, Structure, function and regulation of plant photosystem I, *Biochim. Biophys. Acta, Bioenerg.*, 2007, **1767**, 335–352.
- 72 E. J. Boekema, P. E. Jensen, E. Schlodder, J. F. L. van Breemen, H. van Roon, H. V. Scheller and J. P. Dekker, Green Plant Photosystem I Binds Light-Harvesting Complex I on One Side of the Complex <sup>†</sup>, *Biochemistry*, 2001, **40**, 1029–1036.
- 73 M. Ballottari, C. Govoni, S. Caffarri and T. Morosinotto, Stoichiometry of LHCI antenna polypeptides and characterization of gap and linker pigments in higher plants Photosystem I, *Eur. J. Biochem.*, 2004, **271**, 4659–4665.
- 74 E. Wientjes and R. Croce, The light-harvesting complexes of higher-plant Photosystem I: Lhca1/4 and Lhca2/3 form two red-emitting heterodimers, *Biochem. J.*, 2011, **433**, 477–485.
- 75 A. Alboresi, C. Le Quiniou, S. K. N. Yadav, M. Scholz, A. Meneghesso, C. Gerotto, D. Simionato, M. Hippler, E. J. Boekema, R. Croce and T. Morosinotto, Conservation of core complex subunits shaped the structure and function of photosystem I in the secondary endosymbiont alga *Nannochloropsis gaditana*, *New Phytol.*, 2017, **213**, 714–726.
- 76 S. Kereiche, R. Kouřil, G. T. Oostergetel, F. Fusetti, E. J. Boekema, A. B. Doust, C. D. van der Weij-de Wit and J. P. Dekker, Association of chlorophyll a/c2 complexes to photosystem I and photosystem II in the cryptophyte *Rhodomonas CS24*, *Biochim. Biophys. Acta, Bioenerg.*, 2008, **1777**, 1122–1128.
- 77 B. Drop, M. Webber-Birungi, F. Fusetti, R. Kouřil, K. E. Redding, E. J. Boekema and R. Croce, Photosystem I of *Chlamydomonas reinhardtii* Contains Nine Light-harvesting Complexes (Lhca) Located on One Side of the Core, *J. Biol. Chem.*, 2011, **286**, 44878–44887.
- 78 A. Crepin, Z. Kučerová, A. Kosta, E. Durand and S. Caffarri, Isolation and characterization of a large photosystem I–light-harvesting complex II supercomplex with an additional Lhca1–a4 dimer in *Arabidopsis*, *Plant J.*, 2019, DOI: 10.1111/tpj.14634.
- 79 E. Boekema and A. Dmitry, in *Light Harvesting in Photosynthesis*, 2018.
- 80 X. Pan, P. Cao, X. Su, Z. Liu and M. Li, Structural analysis and comparison of light-harvesting complexes I and II, *Biochim. Biophys. Acta, Bioenerg.*, 2019, 148038.
- 81 M. Iwai, M. Yokono, N. Inada and J. Minagawa, Live-cell imaging of photosystem II antenna dissociation during state transitions, *Proc. Natl. Acad. Sci. U. S. A.*, 2010, **107**, 2337–2342.
- 82 K. N. S. Yadav, D. A. Semchonok, L. Nosek, R. Kouřil, G. Fucile, E. J. Boekema and L. A. Eichacker, Supercomplexes of plant photosystem I with cytochrome b6f, light-harvesting complex II and NDH, *Biochim. Biophys. Acta, Bioenerg.*, 2017, **1858**, 12–20.
- 83 L. Peng, Y. Fukao, M. Fujiwara, T. Takami and T. Shikanai, Efficient operation of NAD(P)H dehydrogenase requires supercomplex formation with photosystem I via minor LHCI in *Arabidopsis*, *Plant Cell*, 2009, **21**, 3623–3640.
- 84 R. Kouřil, O. Strouhal, L. Nosek, R. Lenobel, I. Chamrád, E. J. Boekema, M. Šebela and P. Ilík, Structural characterization of a plant photosystem I and NAD(P)H dehydrogenase supercomplex, *Plant J.*, 2014, **77**, 568–576.
- 85 X. Pan, J. Ma, X. Su, P. Cao, W. Chang, Z. Liu, X. Zhang and M. Li, Structure of the maize photosystem I supercomplex with light-harvesting complexes I and II, *Science*, 2018, **360**, 1109–1113.
- 86 M. Plöckinger, S. Torabi, M. Rantala, M. Tikkanen, M. Suorsa, P.-E. Jensen, E. M. Aro and J. Meurer, The Low Molecular Weight Protein Psal Stabilizes the Light-Harvesting Complex II Docking Site of Photosystem I, *Plant Physiol.*, 2016, **172**, 450–463.
- 87 M. Suorsa, M. Rantala, F. Mamedov, M. Lespinasse, A. Trotta, M. Grieco, E. Vuorio, M. Tikkanen, S. Järvi and E. Aro, Light acclimation involves dynamic re-organization of the pigment–protein megacomplexes in non-appressed thylakoid domains, *Plant J.*, 2015, **84**, 360–373.
- 88 M. Rantala, M. Tikkanen and E. Aro, Proteomic characterization of hierarchical megacomplex formation in *Arabidopsis* thylakoid membrane, *Plant J.*, 2017, **92**, 951–962.
- 89 M. Yokono, A. Takabayashi, S. Akimoto and A. Tanaka, A megacomplex composed of both photosystem reaction centres in higher plants, *Nat. Commun.*, 2015, **6**, 6675.
- 90 A. Crepin and S. Caffarri, The specific localizations of phosphorylated Lhcb1 and Lhcb2 isoforms reveal the role of Lhcb2 in the formation of the PSI-LHCII supercomplex



- in Arabidopsis during state transitions, *Biochim. Biophys. Acta, Bioenerg.*, 2015, **1847**, 1539–1548.
- 91 P. Svensson and P.-Å. Albertsson, Preparation of highly enriched photosystem II membrane vesicles by a non-detergent method, *Photosynth. Res.*, 1989, **20**, 249–259.
  - 92 S. L. Benson, P. Maheswaran, M. A. Ware, C. N. Hunter, P. Horton, S. Jansson, A. V. Ruban and M. P. Johnson, An intact light harvesting complex I antenna system is required for complete state transitions in *Arabidopsis*, *Nat. Plants*, 2015, **1**, 15176.
  - 93 J. M. Anderson, Photoregulation of the Composition, Function, and Structure of Thylakoid Membranes, *Annu. Rev. Plant Physiol.*, 1986, **37**, 93–136.
  - 94 M. Khatoon, K. Inagawa, P. Pospíšil, A. Yamashita, M. Yoshioka, B. Lundin, J. Horie, N. Morita, A. Jajoo, Y. Yamamoto and Y. Yamamoto, Quality Control of Photosystem II thylakoid unstacking is necessary to avoid further damage to the D1 protein and to facilitate D1 degradation under light stress in spinach thylakoids, *J. Biol. Chem.*, 2009, **284**, 25343–25352.
  - 95 M. Herbstová, S. Tietz, C. Kinzel, M. V. Turkina and H. Kirchhoff, Architectural switch in plant photosynthetic membranes induced by light stress, *Proc. Natl. Acad. Sci. U. S. A.*, 2012, **109**, 20130–20135.
  - 96 P. R. Rozak, R. M. Seiser, W. F. Wacholtz and R. R. Wise, Rapid, reversible alterations in spinach thylakoid appression upon changes in light intensity, *Plant, Cell Environ.*, 2002, **25**, 421–429.
  - 97 M. Tikkanen, M. Grieco, S. Kangasjärvi and E.-M. Aro, Thylakoid Protein Phosphorylation in Higher Plant Chloroplasts Optimizes Electron Transfer under Fluctuating Light, *Plant Physiol.*, 2010, **152**, 723–735.
  - 98 M. Suorsa, S. Järvi, M. Grieco, M. Nurmi, M. Pietrzykowska, M. Rantala, S. Kangasjärvi, V. Paakkarinen, M. Tikkanen, S. Jansson and E.-M. Aro, PROTON GRADIENT REGULATION5 Is Essential for Proper Acclimation of Arabidopsis Photosystem I to Naturally and Artificially Fluctuating Light Conditions, *Plant Cell*, 2012, **24**, 2934–2948.
  - 99 M. Tikkanen, M. Grieco, M. Nurmi, M. Rantala, M. Suorsa and E.-M. Aro, Regulation of the photosynthetic apparatus under fluctuating growth light, *Philos. Trans. R. Soc., B*, 2012, **367**, 3486–3493.
  - 100 S. Reiland, G. Messerli, K. Baerenfaller, B. Gerrits, A. Endler, J. Grossmann, W. Gruissem and S. Baginsky, Large-Scale Arabidopsis Phosphoproteome Profiling Reveals Novel Chloroplast Kinase Substrates and Phosphorylation Networks, *Plant Physiol.*, 2009, **150**, 889–903.
  - 101 J. Bennett, Phosphorylation of chloroplast membrane polypeptides, *Nature*, 1977, **269**, 344–346.
  - 102 S. Bellafiore, F. Barneche, G. Peltier and J.-D. Rochaix, State transitions and light adaptation require chloroplast thylakoid protein kinase STN7, *Nature*, 2005, **433**, 892.
  - 103 A. V. Vener, P. J. M. van Kan, P. R. Rich, I. Ohad and B. Andersson, Plastoquinol at the quinol oxidation site of reduced cytochrome bf mediates signal transduction between light and protein phosphorylation: Thylakoid protein kinase deactivation by a single-turnover flash, *Proc. Natl. Acad. Sci. U. S. A.*, 1997, **94**, 1585–1590.
  - 104 A. Trotta, M. Suorsa, M. Rantala, B. Lundin and E.-M. Aro, Serine and threonine residues of plant STN7 kinase are differentially phosphorylated upon changing light conditions and specifically influence the activity and stability of the kinase, *Plant J.*, 2016, **87**, 484–494.
  - 105 E. Rintamäki, P. Martinsuo, S. Pursiheimo and E.-M. Aro, Cooperative regulation of light-harvesting complex II phosphorylation via the plastoquinol and ferredoxin-thioredoxin system in chloroplasts, *Proc. Natl. Acad. Sci. U. S. A.*, 2000, **97**, 11644–11649.
  - 106 V. Bonardi, P. Pesaresi, T. Becker, E. Schleiff, R. Wagner, T. Pfannschmidt, P. Jahns and D. Leister, Photosystem II core phosphorylation and photosynthetic acclimation require two different protein kinases, *Nature*, 2005, **437**, 1179–1182.
  - 107 M. Tikkanen, M. Piippo, M. Suorsa, S. Sirpiö, M. Mulo, J. Vainonen, A. Vener, Y. Allahverdiyeva and E.-M. Aro, State transitions revisited—a buffering system for dynamic low light acclimation of Arabidopsis, *Plant Mol. Biol.*, 2006, **62**, 779.
  - 108 M. Pribil, P. Pesaresi, A. Hertle, R. Barbato and D. Leister, Role of plastid protein phosphatase TAP38 in LHCII dephosphorylation and thylakoid electron flow, *PLoS Biol.*, 2010, **8**, e1000288.
  - 109 A. Shapiguzov, B. Ingelsson, I. Samol, C. Andres, F. Kessler, J.-D. Rochaix, A. V. Vener and M. Goldschmidt-Clermont, The PPH1 phosphatase is specifically involved in LHCII dephosphorylation and state transitions in Arabidopsis, *Proc. Natl. Acad. Sci. U. S. A.*, 2010, **107**, 4782–4787.
  - 110 M. Rantala, N. Lehtimäki, E.-M. Aro and M. Suorsa, Downregulation of TAP38/PPH1 enables LHCII hyperphosphorylation in Arabidopsis mutant lacking LHCII docking site in PSI, *FEBS Lett.*, 2016, **590**, 787–794.
  - 111 X. Wei, J. Guo, M. Li and Z. Liu, Structural Mechanism Underlying the Specific Recognition between the Arabidopsis State-Transition Phosphatase TAP38/PPH1 and Phosphorylated Light-Harvesting Complex Protein Lhcb1, *Plant Cell*, 2015, **27**, 1113–1127.
  - 112 C. Leoni, M. Pietrzykowska, A. Z. Kiss, M. Suorsa, L. R. Ceci, E.-M. Aro and S. Jansson, Very rapid phosphorylation kinetics suggest a unique role for Lhcb2 during state transitions in Arabidopsis, *Plant J.*, 2013, **76**, 236–246.
  - 113 M. Pietrzykowska, M. Suorsa, D. A. Semchonok, M. Tikkanen, E. J. Boekema, E.-M. Aro and S. Jansson, The Light-Harvesting Chlorophyll a/b Binding Proteins Lhcb1 and Lhcb2 Play Complementary Roles during State Transitions in Arabidopsis, *Plant Cell*, 2014, **26**, 3646–3660.
  - 114 P. Longoni, D. Douchi, F. Cariti, G. Fucile and M. Goldschmidt-Clermont, Phosphorylation of the Light-



- Harvesting Complex II Isoform Lhcb2 Is Central to State Transitions, *Plant Physiol.*, 2015, **169**, 2874–2883.
- 115 E. Wientjes, H. van Amerongen and R. Croce, LHCII is an antenna of both photosystems after long-term acclimation, *Biochim. Biophys. Acta, Bioenerg.*, 2013, **1827**, 420–426.
  - 116 M. Giovanardi, L. Pantaleoni, L. Ferroni, C. Pagliano, P. Albanese, C. Baldissierotto and S. Pancaldi, In pea stipules a functional photosynthetic electron flow occurs despite a reduced dynamicity of LHCII association with photosystems, *Biochim. Biophys. Acta, Bioenerg.*, 2018, **1859**, 1025–1038.
  - 117 N. R. Mekala, M. Suorsa, M. Rantala, E.-M. Aro and M. Tikkanen, Plants actively avoid state transitions upon changes in light intensity: Role of Light-Harvesting Complex II protein dephosphorylation in high light, *Plant Physiol.*, 2015, **168**, 721–734.
  - 118 P. Pesaresi, A. Hertle, M. Pribil, T. Kleine, R. Wagner, H. Strissel, A. Ihnatowicz, V. Bonardi, M. Scharfenberg, A. Schneider, T. Pfannschmidt and D. Leister, Arabidopsis STN7 Kinase provides a link between short- and long-term photosynthetic acclimation, *Plant Cell*, 2009, **21**, 2402–2423.
  - 119 W. H. Wood, S. F. H. Barnett, S. Flannery, C. N. Hunter and M. P. Johnson, Dynamic thylakoid stacking is regulated by LHCII phosphorylation but not its interaction with photosystem I, *Plant Physiol.*, 2019, **180**, 2152–2166.
  - 120 R. Kouřil, E. Wientjes, J. B. Bultema, R. Croce and E. J. Boekema, High-light vs. low-light: Effect of light acclimation on photosystem II composition and organization in *Arabidopsis thaliana*, *Biochim. Biophys. Acta, Bioenerg.*, 2013, **1827**, 411–419.
  - 121 B. Ingelsson and A. V. Vener, Phosphoproteomics of *Arabidopsis* chloroplasts reveals involvement of the STN7 kinase in phosphorylation of nucleoid protein pTAC16, *FEBS Lett.*, 2012, **586**, 1265–1271.
  - 122 C. Spetea, A. Herdean, G. Alloreant, L. Carraretto, G. Finazzi and I. Szabo, An update on the regulation of photosynthesis by thylakoid ion channels and transporters in *Arabidopsis*, *Physiol. Plant.*, 2017, **161**, 16–27.
  - 123 M. M. Koskela, A. Brünje, A. Ivanauskaite, M. Grabsztunowicz, I. Lassowskat, U. Neumann, T. V. Dinh, J. Sindlinger, D. Schwarzer, M. Wirtz, E. Tyystjärvi, I. Finkemeier and P. Mulo, Chloroplast acetyltransferase NSI is required for state transitions in *Arabidopsis thaliana*, *Plant Cell*, 2018, **30**, 1695–1709.
  - 124 P. Albanese, S. Tamara, G. Saracco, R. A. Scheltema and C. Pagliano, How paired PSII-LHCII supercomplexes mediate the stacking of plant thylakoid membranes unveiled by structural mass-spectrometry, *Nat. Commun.*, 2020, **11**, 1–14.
  - 125 B. Demmig-Adams and W. Adams, Photoprotection and other responses of plants to high light stress, *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 1992, **43**, 599–626.
  - 126 E. Tyystjärvi and E. M. Aro, The rate constant of photoinhibition, measured in lincomycin-treated leaves, is directly proportional to light intensity, *Proc. Natl. Acad. Sci. U. S. A.*, 1996, **93**, 2213–2218.
  - 127 E.-M. Aro, I. Virgin and B. Andersson, Photoinhibition of Photosystem II. Inactivation, protein damage and turnover, *Biochim. Biophys. Acta, Bioenerg.*, 1993, **1143**, 113–134.
  - 128 M. A. Gururani, J. Venkatesh and L. S. P. Tran, Regulation of photosynthesis during abiotic stress-induced photoinhibition, *Mol. Plant*, 2015, **8**, 1304–1320.
  - 129 S. Järvi, M. Suorsa and E.-M. Aro, Photosystem II repair in plant chloroplasts—Regulation, assisting proteins and shared components with photosystem II biogenesis, *Biochim. Biophys. Acta, Bioenerg.*, 2015, **1847**, 900–909.
  - 130 Y. Yamamoto, Quality Control of Photosystem II: The mechanisms for avoidance and tolerance of light and heat stresses are closely linked to membrane fluidity of the thylakoids, *Front. Plant Sci.*, 2016, **7**, 1136.
  - 131 E. Baena-González, R. Barbato and E.-M. Aro, Role of phosphorylation in the repair cycle and oligomeric structure of photosystem II, *Planta*, 1999, **208**, 196–204.
  - 132 M. Tikkanen, N. R. Mekala and E.-M. Aro, Photosystem II photoinhibition-repair cycle protects Photosystem I from irreversible damage, *Biochim. Biophys. Acta, Bioenerg.*, 2014, **1837**, 210–215.
  - 133 K. Nishimura, Y. Kato and W. Sakamoto, Essentials of Proteolytic Machineries in Chloroplasts, *Mol. Plant*, 2017, **10**, 4–19.
  - 134 J. P. Vainonen, M. Hansson and A. V. Vener, STN8 protein kinase in *Arabidopsis thaliana* is specific in phosphorylation of Photosystem II core proteins, *J. Biol. Chem.*, 2005, **280**, 33679–33686.
  - 135 I. Samol, A. Shapiguzov, B. Ingelsson, G. Fucile, M. Crèvecoeur, A. V. Vener, J.-D. Rochaix and M. Goldschmidt-Clermont, Identification of a Photosystem II phosphatase involved in light acclimation in *Arabidopsis*, *Plant Cell*, 2012, **24**, 2596–2609.
  - 136 M. Tikkanen, M. Nurmi, S. Kangasjärvi and E.-M. Aro, Core protein phosphorylation facilitates the repair of photodamaged photosystem II at high light, *Biochim. Biophys. Acta, Bioenerg.*, 2008, **1777**, 1432–1437.
  - 137 R. Fristedt, A. Willig, P. Granath, M. Crèvecoeur, J.-D. Rochaix and A. V. Vener, Phosphorylation of Photosystem II Controls Functional Macroscopic Folding of Photosynthetic Membranes in *Arabidopsis*, *Plant Cell*, 2009, **21**, 3950–3964.
  - 138 T. K. Goral, M. P. Johnson, A. P. R. Brain, H. Kirchhoff, A. V. Ruban and C. W. Mullineaux, Visualizing the mobility and distribution of chlorophyll proteins in higher plant thylakoid membranes: effects of photoinhibition and protein phosphorylation, *Plant J.*, 2010, **62**, 948–959.
  - 139 S. Puthiyaveetil and H. Kirchhoff, A phosphorylation map of the photosystem II supercomplex C2S2M2, *Front. Plant Sci.*, 2013, **4**, 459.
  - 140 R. Fristedt, P. Granath and A. V. Vener, A Protein Phosphorylation Threshold for Functional Stacking of



- Plant Photosynthetic Membranes, *PLoS One*, 2010, **5**, e10963.
- 141 M. Pribil, O. Sandoval-Ibáñez, W. Xu, A. Sharma, M. Labs, Q. Liu, C. Galgenmüller, T. Schneider, M. Wessels, S. Matsubara, S. Jansson, G. Wanner and D. Leister, Fine-Tuning of Photosynthesis Requires CURVATURE THYLAKOID1-Mediated Thylakoid Plasticity, *Plant Physiol.*, 2018, **176**, 2351–2364.
  - 142 Y. Yamamoto, H. Hori, S. Kai, T. Ishikawa, A. Ohnishi, N. Tsumura and N. Morita, Quality control of Photosystem II: reversible and irreversible protein aggregation decides the fate of Photosystem II under excessive illumination, *Front. Plant Sci.*, 2013, **4**, 433.
  - 143 Q.-B. Yu, G. Li, G. Wang, J.-C. Sun, P.-C. Wang, C. Wang, H.-L. Mi, W.-M. Ma, J. Cui, Y.-L. Cui, K. Chong, Y.-X. Li, Y.-H. Li, Z. Zhao, T.-L. Shi and Z.-N. Yang, Construction of a chloroplast protein interaction network and functional mining of photosynthetic proteins in *Arabidopsis thaliana*, *Cell Res.*, 2008, **18**, 1007–1019.
  - 144 R. Yokoyama, H. Yamamoto, M. Kondo, S. Takeda, K. Ifuku, Y. Fukao, Y. Kamei, M. Nishimura and T. Shikanai, Grana-Localized Proteins, RIQ1 and RIQ2, Affect the Organization of Light-Harvesting Complex II and Grana Stacking in *Arabidopsis*, *Plant Cell*, 2016, **28**, 2261–2275.
  - 145 S. Järvi, M. Rantala and E.-M. Aro, in *Photosynthesis and Bioenergetics*, World Scientific, 2017, pp. 243–263.

