Physiologia Plantarum
An International Journal for Plant Biology

Physiologia Plantarum 161: 28–44. 2017 ISSN 0031-9317

MINIREVIEW

Why we need to know the structure of phosphorylated chloroplast light-harvesting complex II

John F. Allen*

Research Department of Genetics, Evolution and Environment, Darwin Building, University College London, Gower Street, London, WC1E 6BT, UK

Correspondence
*Corresponding author,
e-mail: j.f.allen@ucl.ac.uk

Received 27 November 2016;
revised 27 February 2017

doi:10.1111/ppl.12577

In oxygenic photosynthesis there are two 'light states' – adaptations of the photosynthetic apparatus to spectral composition that otherwise favours either photosystem I or photosystem II. In chloroplasts of green plants the transition to light state 2 depends on phosphorylation of apoproteins of a membrane-intrinsic antenna, the chlorophyll-*a*/*b*-binding, light-harvesting complex II (LHC II), and on the resulting redistribution of absorbed excitation energy from photosystem II to photosystem I. The transition to light state 1 reverses these events and requires a phospho-LHC II phosphatase. Current structures of LHC II reveal little about possible steric effects of phosphorylation. The surface-exposed N-terminal domain of an LHC II polypeptide contains its phosphorylation site and is disordered in its unphosphorylated form. A molecular recognition hypothesis proposes that state transitions are a consequence of movement of LHC II between binding sites on photosystems I and II. In state 1, LHC II forms part of the antenna of photosystem II. In state 2, a unique but as yet unidentified 3-D structure of phospho-LHC II may attach it instead to photosystem I. One possibility is that the LHC II N-terminus becomes ordered upon phosphorylation, adopting a local alpha-helical secondary structure that initiates changes in LHC II tertiary and quaternary structure that sever contact with photosystem II while securing contact with photosystem I. In order to understand redistribution of absorbed excitation energy in photosynthesis we need to know the structure of LHC II in its phosphorylated form, and in its complex with photosystem I.

A brief history of chloroplast protein phosphorylation

Bennett (1977) reported that chloroplast membrane polypeptides become labelled with ^{32}P, either from ^{32}P-orthophosphate supplied to a suspension of isolated, intact pea chloroplasts, or from [$\gamma-^{32}P$]ATP present in a suspension of pea thylakoid membranes. The polypeptides retained the radioactive label after separation by sodium dodecyl sulfate-polyacrylaminde gel electrophoresis (SDS-PAGE), indicating post-translational, covalent modification by phosphorylation. The most conspicuous phosphoproteins had apparent molecular masses of 26 and 9 kDa. Bennett identified two phosphoprotein bands at about 26 kDa as polypeptide components of the 'chlorophyll-*a*/*b*-binding protein' of a green pigment–protein complex (Thornber 1975), also known as the chloroplast light-harvesting pigment–protein complex of photosystem II, LHCP, or LHC II (Bennett 1979a). LHC polypeptides are encoded in the cell nucleus, synthesized on cytosolic ribosomes, and imported into the chloroplast as precursors. The 9 kDa phosphoprotein was eventually identified as the product of the chloroplast *psbH* gene, and as a single transmembrane-helix component of photosystem II.

Phosphorylation of thylakoid membrane proteins was seen to occur in the light and not in the dark, so the membrane-bound LHC II kinase is light-dependent (Bennett 1979b). The phospho-LHC II phosphatase (Bennett 1980) is light-independent and sensitive to inhibition by sodium fluoride.

Excitation energy re-distribution

Phosphorylation of LHC II correlates with an ATP-induced decrease in chlorophyll fluorescence yield of thylakoids at room temperature (Bennett et al. 1980b). Variation in room temperature fluorescence yield is a property of photosystem II, so LHC II phosphorylation was assumed to divert absorbed excitation energy away from photosystem II. Phosphorylation of LHC II is also accompanied by changes in low-temperature (77 K) fluorescence emission spectra that indicate re-distribution of absorbed excitation energy to photosystem I at the expense of photosystem II (Bennett et al. 1980b).

Control by the redox state of the plastoquinone pool

The light-dependency of LHC II phosphorylation was suggested to be a consequence of photosynthetic electron transport, and a reasonable expectation at the time was that the LHC II kinase is activated, like other chloroplast components, by thioredoxin and reduced ferredoxin, accepting electrons from photosystem I (Bennett 1979b). However, experiments designed to test this idea suggested that the site of redox control of LHC II kinase activity lies, instead, between photosystem I and photosystem II, most likely at the level of the plastoquinone pool (Bennett et al. 1980a, Allen et al. 1981). The room-temperature fluorescence changes and low-temperature spectra reporting on redistribution of excitation energy appeared to be regulated in the same way – by plastoquinone redox state, with phosphorylation and its effects being activated when plastoquinone is in its reduced form, plastoquinol (Allen et al. 1981). It was proposed that control of distribution of absorbed excitation energy by redox state of plastoquinone accounted for the phenomenon of state transitions, that is, the physiological redistribution of absorbed excitation energy seen in algal cell cultures and leaves of higher plants (Bennett et al. 1980a, Horton and Black 1980, Allen et al. 1981). In light at wavelengths absorbed preferentially by photosystem II, 'light 2', photosystem II transports electrons faster than photosystem I, plastoquinone becomes reduced, the LHC II kinase is activated, and phospho-LHC II supplies absorbed excitation to the reaction centre of photosystem I as if to correct the wavelength-dependent imbalance in energy distribution between photosystem I and photosystem II. Conversely, in 'light 1', photosystem I runs faster than photosystem II, plastoquinone becomes oxidized, the kinase is inactive, and the light-independent phospho-LHC II phosphatase activity predominates, returning a proportion of absorbed excitation to photosystem II at the expense of photosystem I. This general mechanism, outlined in Fig. 1, is consistent with redox effects on the ATP-induced fluorescence quenching and ^{32}P-labelling of LHC II (Allen and Horton 1981, Allen et al. 1981, Horton et al. 1981). The mechanism is also supported by induction of phosphorylation by light 2 and of dephosphorylation by light 1; by inhibition of the state 1 transition by the phosphoprotein phosphatase inhibitor sodium fluoride; and by a close match between the kinetics of phosphorylation and dephosphorylation with fluorescence changes that monitor the transitions to state 2 and to state 1, respectively, in thylakoids isolated from pea chloroplasts (Telfer et al. 1983) and *Chlorella* cells (Saito et al. 1983).

The protein kinase acting on LHC II was identified by Depege et al. (2003) from chlorophyll fluorescence imaging to screen for state transition-deficient mutants of *Chlamydomonas*. The LHC II kinase gene is in the cell nucleus, as are lhc genes, formerly known as cab (chlorophyll *a-b* protein) genes, encoding LHC II polypeptides. The *Chlamydomonas* LHC II kinase, identified in a 'state transition thylakoid' mutant, was termed Stt7 (Depege et al. 2003), while its 'state transition nucleus' homologue in *Arabidopsis* was termed Stn7 (Bellafiore et al. 2005). Related kinases act on reaction centre polypeptides and are termed Stn8 (Depege et al. 2003, Bellafiore et al. 2005, Bonardi et al. 2005, Vainonen et al. 2005, Reiland et al. 2011). LHC II kinase activity requires an intact cytochrome b_6-f complex (Bennett et al. 1988) with which the kinase interacts (Shapiguzov et al. 2016). The phospho-LHC II phosphatase was also identified by screening for chlorophyll fluorescence mutants (Pribil et al. 2010, Shapiguzov et al. 2010). Activation of the kinase Stn7 requires occupancy of the plastoquinol binding site of the cytochrome b_6-f complex (Vener et al. 1997, Zito et al. 1999). The kinase is inactivated by reduced thioredoxin (Rintamaki et al. 2000, Puthiyaveetil 2011), essentially turning off state transitions at higher light intensities, when increased quantum yield of primary photochemistry is not required, and better suppressed (Brinkert et al. 2016).

State transitions are changes in optical absorption cross-section

Prior to the discovery of the role of LHC II phosphorylation, regulation of excitation energy distribution was widely thought to involve 'spillover' to photosystem I of excitation energy surplus to the capacity of photosystem II to use it in reaction centre photochemistry, that is, in charge separation. Two sets of observations counted against this model, and suggested instead that the optical absorption cross-section of photosystem I increased as that of photosystem II

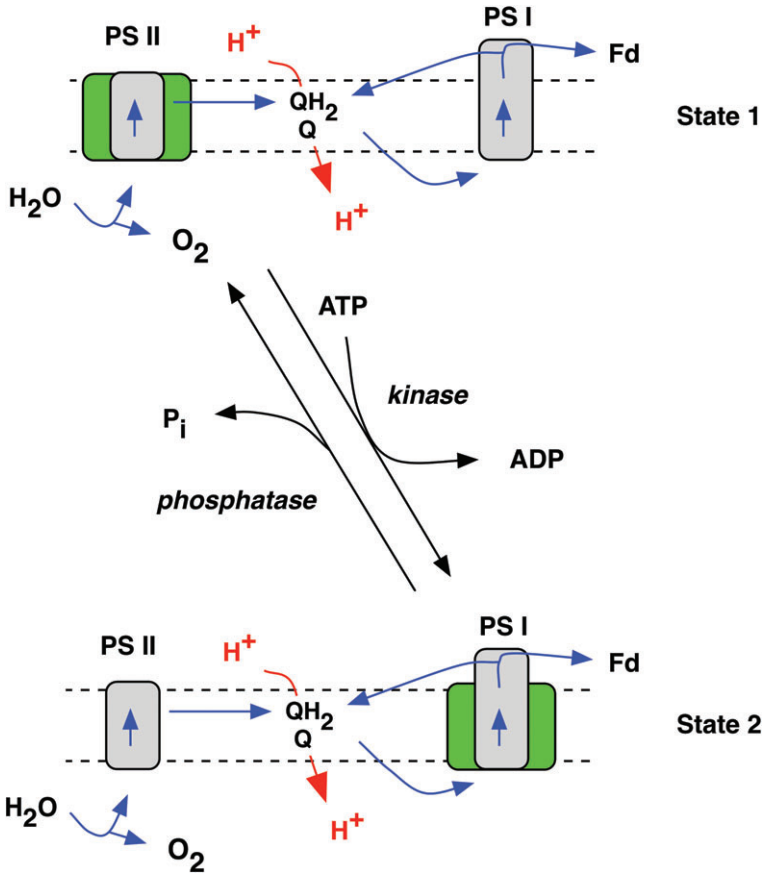

Fig. 1. Depiction of complementary changes in absorption cross-section of photosystems (PS) I and II during state transitions in chloroplasts. Electron transport (blue) is coupled to proton (H^+) translocation (red) across the thylakoid membrane. PS I and PS II are connected in series by plastoquinone. The reduced quinone pool, QH_2, activates a protein kinase that causes a chlorophyll-binding light harvesting protein (green) to become phosphorylated and to move from photosystem II to photosystem I. When the quinone pool is oxidized, Q, the kinase is inactive, and a phosphoprotein phosphatase activity returns the light-harvesting protein from photosystem I to photosystem II.

decreased. The first was lateral migration of phosphorylated LHC II. ^{32}P-labelled proteins moved between thylakoid domains, from photosystem II-rich grana stacks to unstacked domains originating from stroma lamellae or grana margins (Andersson et al. 1982). At the time it was unclear whether phospho-LHC II moved in its complex with photosystem II; however, mounting evidence for lateral separation of photosystem I and photosystem II in thylakoid membranes made it simpler to suppose that LHC II moved, physically, between the two photosystems. Lateral movement of LHC II after its phosphorylation was also a conclusion from freeze-fracture electron microscopy (Simpson 1983) and from electron microscopy of immuno-gold-labelled chloroplasts (Bassi et al. 1988). Lateral movement of phospho-LHC II to unappressed thylakoids is also observed by single-particle tracking (Consoli et al. 2005). An independent set of observations indicating complementary changes in the absorption cross-section of the two photosystems arose from re-interpretation of fluorescence induction transients and 77 K fluorescence emission spectra (Horton 1983). A simplified scheme of reversible plastoquinone redox-controlled protein phosphorylation in chloroplast state transitions is depicted in Fig. 1.

The idea of 'spillover' of excitation energy from photosystem II to photosystem I in light-state 2 persisted for red algae and cyanobacteria (Biggins et al. 1984), and the proposal of lateral movement of the light-harvesting, membrane-extrinsic phycobilisome (Allen et al. 1985), analogous but unrelated to LHC II, was not readily accepted. Decreased energy transfer to photosystem II in cyanobacterial cells in state 2, when plastoquinone became reduced, was demonstrated by fluorescence experiments on cyanobacterial cell suspensions (Mullineaux et al. 1986). The complementary increase in energy transfer to photosystem I was suggested by 77 K fluorescence emission spectra (Allen

et al. 1985), and demonstrated directly by increased yield of photosystem I photochemistry at limiting intensities of phycobilisome-absorbed light (Tsinoremas et al. 1989, Mullineaux 1992). The lateral mobility of phycobilisomes is also supported by fluorescence lifetime studies (Mullineaux and Holzwarth 1991) and fluorescence microscopy (Mullineaux 2008). There seems to be no agreed mechanism for reallocation of light-harvesting phycobiliproteins between photosystem I and photosystem II during cyanobacterial and red algal state transitions. Freeze-fracture particles adopt linear arrays in state 1 (Olive et al. 1986) that are seen also for cyanobacterial photosystem II in crystals of a 'native-like' superstructure (Hellmich et al. 2014), and similar changes accompany state transitions also in phycocyanin-less cyanobacterial mutants (Vernotte et al. 1990). While mechanisms may differ, the overall process of excitation energy redistribution between photosystems is functionally the same in phycobilisome-containing organisms as in green plants and algae (Williams and Allen 1987, Allen 1992b), as outlined in Fig. 2. In state 2 excitation energy otherwise lost to heat in a photoprotective mechanism (Ruban et al. 2007, Cogdell and Gardiner 2015, Harris et al. 2016, Kirilovsky and Kerfeld 2016) is conserved by photosystem I photochemistry, and the result is an overall gain in quantum efficiency. In all cases, however, plastoquinone redox control allows state transitions eventually to move light-harvesting pigment molecules between the two photosystems in proportion to the capacity of the two reactions centres to use the absorbed excitation energy in photochemistry. It now seems to be generally agreed for algae and green plants (Wollman 2001, Allen 2003b, Goldschmidt-Clermont and Bassi 2015, Nawrocki et al. 2016) that light-states 1 and 2 are states of physiological adaptation that maximize quantum yield of photosynthesis at limiting light intensity (Bonaventura and Myers 1969, Murata 1969, Myers 1971).

Multiple thylakoid phosphoproteins

Mass spectrometry identifies a number of chloroplast phosphoproteins and their phosphorylation sites in *Chlamydomonas* and higher plants (Vener 2007, Baginsky 2016). A general rule seems to be that phosphorylation occurs within N-terminal, surface-exposed domains, as suggested originally by results from trypsin proteolysis of thylakoid membranes and mass spectrometry of the products (Michel et al. 1988). A map of phosphorylation sites in relation to structure of a photosystem II supercomplex suggests roles in supercomplex disassembly and in the D1 repair cycle (Puthiyaveetil and Kirchhoff 2013).

Autoradiography of ^{32}P-labelled SDS gels of pea thylakoids reveals at least 13 phosphorylated polypeptides (Silverstein et al. 1993b). By potentiometric redox titration the midpoint potential, E_m, of the extent of phosphorylation at pH 7 is +38 mV, n = 1. This value is consistent with control being exerted by the redox state of PQ/PQ$^{\bullet-}$, either at the acceptor side of the bicarbonate-depleted photosystem II reaction centre (Brinkert et al. 2016) or within the cytochrome b_6-f complex. Most of the polypeptides resolved, including LHC II and psbH, become more phosphorylated at lower potentials than E_m, while for two, at 46 and 63 kDa, the sign is reversed so that phosphorylation increases with increasing potential, when plastoquinone is oxidized (Silverstein et al. 1993b). For all thylakoid phosphoproteins, dephosphorylation in the dark is independent of redox potential (Silverstein et al. 1993a), confirming the assumption (Allen et al. 1981) that redox regulatory control applies to the protein kinase and not to the phosphoprotein phosphatase.

The 9 kDa phosphoprotein, psbH

Protein sequencing identified the 9 kDa phosphoprotein reported by Bennett (1977) as the product of the chloroplast *psbH* gene (Farchaus and Dilley 1986). Its phosphorylation, including at Threonine-2 (Michel and Bennett 1987), is light- and redox-dependent, as is that of LHC II, while dark dephosphorylation of the psbH protein is slower than that of LHC II (Allen and Findlay 1986, Reiland et al. 2011), suggesting either the action of different phosphoprotein phosphatases or else a phosphorylation sites with different accessibilities to a single enzyme. The latter possibility can easily be envisaged if phospho-psbH remains with grana stacks while phospho-LHC II does not. The psbH protein was predicted from amino acid composition to be a single transmembrane helix, chlorophyll-binding protein with sequence similarity to the first of the three transmembrane helices together with the phosphorylated N-terminal domain of LHC II (Allen and Findlay 1986). Unlike LHC II, psbH is present in cyanobacteria, where is seems not to be a phosphoprotein. Photosystem II structures, for example those of Wei et al. (2016) for chloroplasts and Umena et al. (2011) for a cyanobacterium, show psbH within the core antenna system where it is possible to imagine its phosphorylation in chloroplasts playing a role in regulation of excitation energy transfer to the reaction centre.

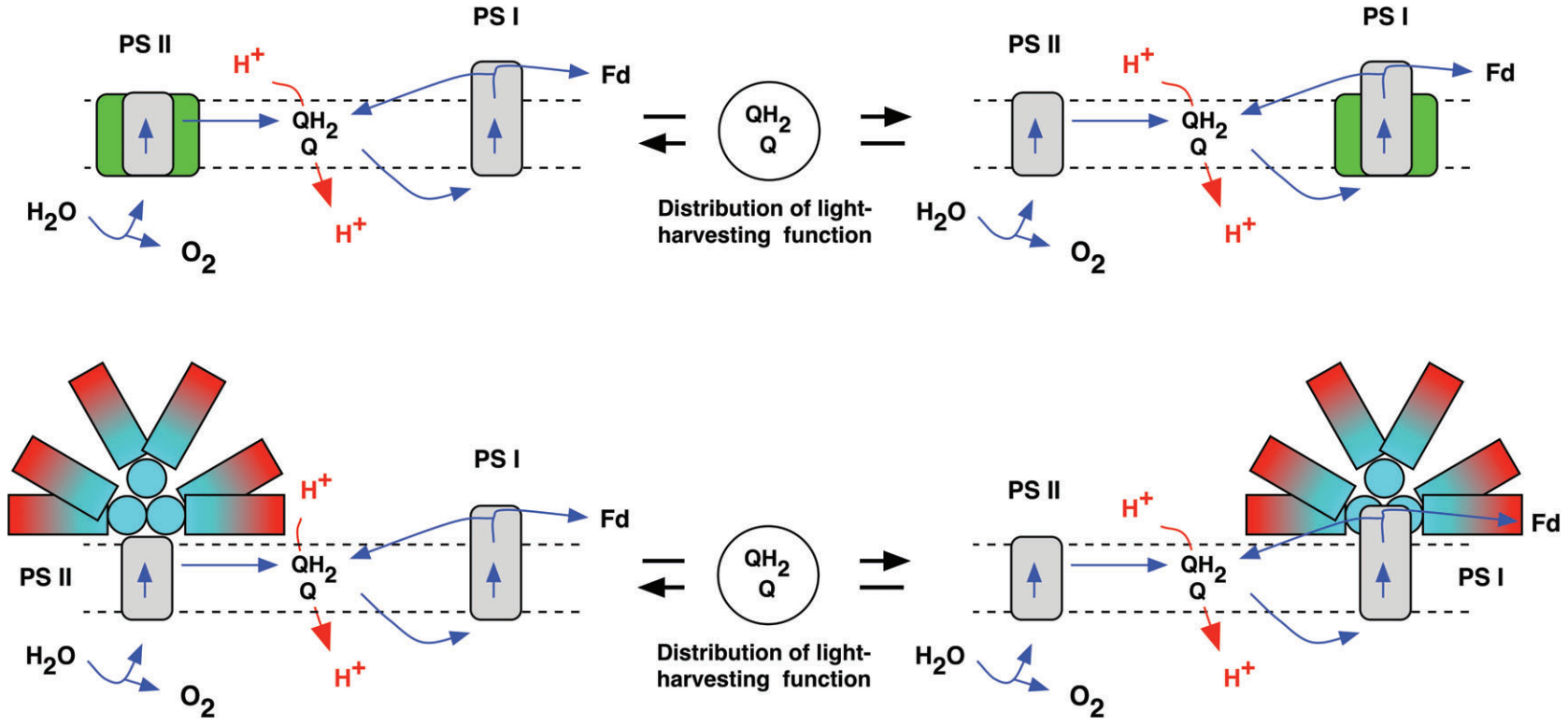

Fig. 2. Comparison of complementary changes in absorption cross-section of photosystems (PS) I and II during state transitions in chloroplasts (upper figure) and in cyanobacteria or red algae (lower figure). In the latter the mobile, membrane-intrinsic light-harvesting chlorophyll-protein is functionally replaced by a membrane-extrinsic light harvesting phycobilisome (Allen et al. 1985).

Photosystem II reaction centre phosphoproteins

The photosystem II apoproteins D1 and D2 at 32 and 34 kDa are products of chloroplast genes *psbA* and *psbD*, and both are phosphorylated at threonine-2 (Reiland et al. 2011) in a light- and redox-dependent reaction. The effect of reaction centre core phosphorylation is to maintain the rate of photosystem II electron transport at light intensities that would otherwise result in its photoinhibition (Harrison and Allen 1991). While the mechanism of this effect is unknown, it is interesting to consider the possibility that phosphorylation on the acceptor side of the photosystem II reaction centre favours forward electron transport from Q_A to the plastoquinone pool over the back reaction to pheophytin or to the donor side, analogous to the effect of weak bicarbonate binding in the vicinity of the iron atom between Q_A and Q_B (Brinkert et al. 2016). Phosphorylation of photosystem II reaction centre core polypeptides also plays a key role in the photosystem II repair cycle by which the D1 polypeptide is replaced. There is evidence for a role of photosystem II core phosphorylation in facilitating the degradation of photodamaged D1 (Baena-González et al. 1999, Tikkanen et al. 2008, Theis and Schroda 2016).

Inner antenna proteins

The inner antenna of photosystem II is formed of two chlorophyll-proteins, CP47 and CP43, products of the chloroplast genes psbB and psbC, respectively. Each has six transmembrane helices and their amino acid sequences provide additional indication of homology with segments of the larger reaction centre apoproteins of photosystem I. CP 43 is phosphorylated at threonine-15 (Reiland et al. 2011).

CP29 is a chlorophyll-binding protein and product of the nuclear gene *Lhcb4*. It has multiple phosphorylation sites, some of which are phosphorylated only in state 2 (Kargul et al. 2005, Fristedt and Vener 2011). As with the other phosphoproteins of photosystem II, CP29 is phosphorylated near its N-terminus, on the stromal-exposed (N-phase) of the thylakoid. While the molecular mechanism is unknown, it is clear from experiments with *Chlamydomonas* thylakoid fractions that CP29 moves upon phosphorylation from photosystem II in grana stacks to photosystem I in unappressed thylakoids (Kargul et al. 2005, Takahashi et al. 2014).

The photosystem II supercomplex

This complex is seen in blue-native gels and is formed upon phosphorylation of photosystem II core subunits including CP43 (Dietzel et al. 2011). Supercomplex generation requires the lumenal subunit Psb27 and occurs even in *Arabidopsis stn7* mutants incapable of state transitions (Dietzel et al. 2011). Nevertheless state transitions occur in a *psb27* mutant incapable of supercomplex formation, and state transition kinetics are then increased (Dietzel et al. 2011). A map of phosphorylation sites in the photosystem II supercomplex is consistent with their separate functional effects (Puthiyaveetil and Kirchhoff 2013) and structural reorganization of photosystem II chlorophyll-binding proteins is described (Kargul and Barber 2008). A proportion of LHC II serves as the antenna of photosystem I in *Arabidopsis* chloroplasts adapted to increased light intensity (Wientjes et al. 2013b). In addition, individual LHC II trimers, termed S and M, remain connected to photosystem II upon phosphorylation, indicating that specific trimers migrate to photosystem I (Wientjes et al. 2013a, Crepin and Caffarri 2015). Trimers may be composed of different individual polypeptide chains, giving homotrimers and heterotrimers with specific mobilities and kinetics of phosphorylation (Leoni et al. 2013, Pietrzykowska et al. 2014, Crepin and Caffarri 2015). In *Arabidopsis*, trimers termed M and S remain bound the photosystem II supercomplex even when phosphorylated on chains Lhcb-1 and Lhcb-2 (Crepin and Caffarri 2015).

Remodelling of supercomplexes has received special attention in the green alga *Chlamydomonas reinhardtii* (Minagawa 2011) where a large increase in photosystem I antenna size accompanies incubation of cells in darkness and under anaerobic conditions, consistent with the idea that photosystem I cyclic photophosphorylation is favoured in state 2 (Minagawa 2013). Nevertheless, comparison of wild-type with LHC II kinase- and plastocyanin-deficient mutants suggests clearly that state transitions in *Chlamydomonas* induce complementary changes in the antenna size of the two photosystems, thus serving to redistribute excitation energy and to enhance quantum yield (Nawrocki et al. 2016).

What does phosphorylation do to LHC II?

Structures of LHC II in its unphosphorylated state have been obtained by cryo-EM of 2-D crystals (Kühlbrandt 1994) and by X-ray diffraction by 3-D crystals from spinach and pea (Liu et al. 2004, Standfuss et al. 2005). As shown in Figs 3 and 4, the complex is a trimer of polypeptides, each monomer (Fig. 3) having three membrane-spanning helices and non-covalently binding carotenoids and 14 chlorophyll molecules; 8 of chlorophyll *a* and 6 of chlorophyll *b*. The X-ray structure from spinach is presented within a complete photosystem II supercomplex obtained by single-particle cryo-EM,

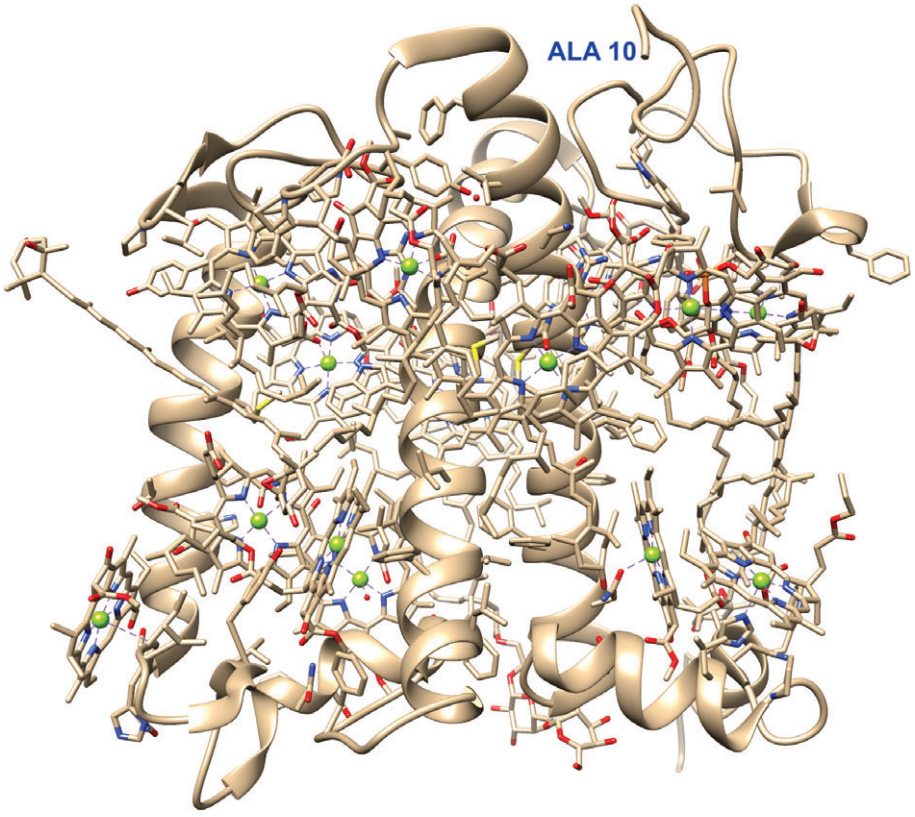

Fig. 3. The monomer (chain A) of pea LHC II as obtained by Standfuss et al. (2005) and rendered using Chimera (Huang et al., 1996) from the PDB file 2BHW. The view is parallel to and within the thylakoid membrane with the stromal side (N-phase) at the top. From right to left in the graphic the three transmembrane helices are A, B, and C and the lumenal, amphipathic helices are D and E. The residue ALA 10 is the N-terminal limit of the resolved structure.

where additional, monomeric, forms of LHC II are also resolved (Wei et al. 2016).

Whatever its oligomeric state, a proportion of LHC II is clearly an exception to the rule that each photosystem has its own, specific complement of light-harvesting pigments and pigment-proteins. LHC II becomes post-translationally modified, by phosphorylation of one or more threonine side chains close to the amino terminus. Upon phosphorylation, a subpopulation of LHC II migrates away from photosystem II and functions instead as part of the light-harvesting antenna of photosystem I.

Molecular recognition and protein–protein interactions

Following the precedent of the resolved structural change upon phosphorylation of serine-14 in a soluble enzyme, rabbit glycogen phosphorylase (Barford et al. 1991, Johnson and Barford 1993), it was proposed that phosphorylation of LHC II induces a local change in secondary structure around its surface-exposed, N-terminal phosphorylation site, as the phosphate group shields fixed, positively charged side chains that otherwise prohibit helix formation (Allen 1990, 1992b, 1992a). This model stands up well to nuclear magnetic resonance spectroscopy (NMR) and Fourier transfer infra-red spectroscopy (FTIR) investigations on synthetic peptides and the native protein (Allen and Nilsson 1997, Nilsson et al. 1997, Allen and Forsberg 2001). Support for a conformational change of this kind has been obtained from electron paramagnetic resonance (EPR) spectroscopy (Jeschke et al. 2005). The basic side-chains flanking the phosphorylation site are crucial for this interpretation and are predicted to be required for the conformational change, and thus for the state 2 transition. Recognition of the phosphorylation site by the LHC II kinase also requires two basic adjacent, N-terminal residues (Liu et al. 2016). Site-directed replacement of RK with RR favours substrate recognition and increases the rate of phosphorylation (Liu et al. 2016). 3-D structural studies can be expected to distinguish between kinase recognition and the predicted conformational change in interpreting effects of site-directed mutagenesis on the state 2 transition. In addition, it will be important to

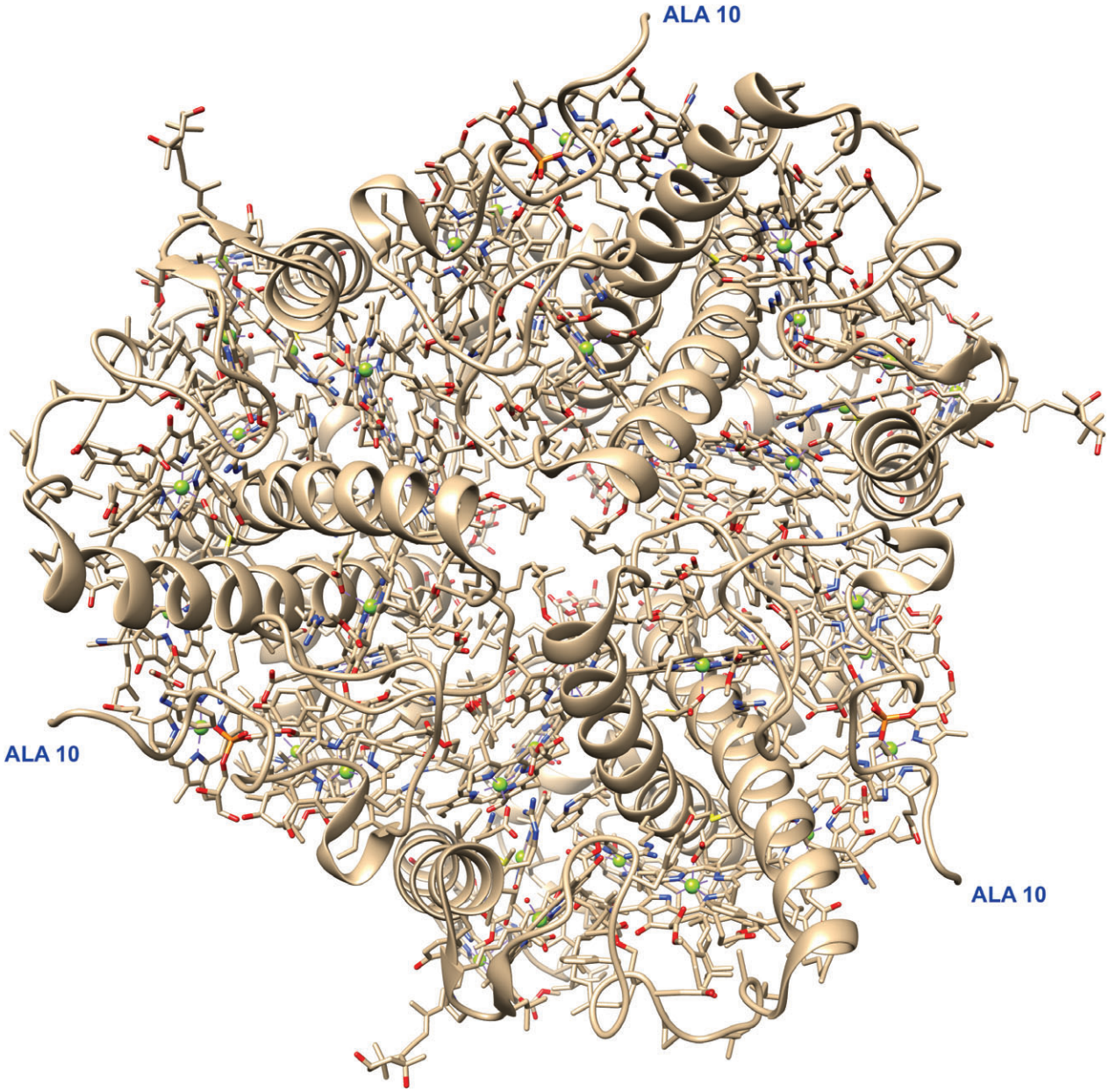

Fig. 4. The trimeric structure of pea LHC II as obtained by Standfuss et al. (2005) and rendered using Chimera (Huang et al., 1996) from the PDB file 2BHW. The view is from the stromal side of the thylakoid membrane (N-phase). The residue ALA 10 is the N-terminal limit of the resolved structure.

determine which of the basic side chains flanking the phosphorylation site might be screened electrostatically from each other by the negative charge of the phosphate group. In pea these side chains are arginine-1, lysine-2, lysine-7 and lysine-8. These are at −4, −3, +2 and +3 from the phosphothreonine, Thr-5. All except lysine-7 would be approximately one alpha-helix-turn away from Thr-5, and close to the phosphate group in phospho-LHC II. Glycogen phosphorylase has basic side chains at −2, −3, −4 and +2 from the phosphorylation site, serine-14. The analogous positions of basic side chains adjacent to their phosphorylation sites are consistent with a phosphorylation-induced local alpha-helix in LHC II as seen in X-ray structures of the two forms of glycogen phosphorylase. In contrast, a completely different mechanism of regulation by phosphorylation is resolved in the enzyme isocitrate dehydrogenase from *Escherichia coli*, where the protein shows no global

structural change, and is inactivated by the phosphate group on an internal serine, Ser-114, presenting an electrostatic and steric obstacle to substrate binding (Hurley et al. 1990).

Replacement of its phosphorylated threonine or serine residue with glutamate or aspartate is predicted to lock LHC II into its phosphorylated, state 2 conformation, though this will not necessarily be the result if the phosphoryl group of phospho-LHC II itself forms salt bridges that underlie the conformation change. Furthermore, if phospho-LHC II resembles glycogen phosphorylase *a* (Barford et al. 1991), then the phosphate will be required for salt bridge interactions with basic side chains of another polypeptide chain, perhaps a neighbouring LHC II in the same trimer, in a different LHC II trimer, or in another protein. It will be important to know whether inter-chain interactions involve LHC II or other polypeptides, including psaH, L, and I and LHC I of photosystem I, and whether phosphorylation serves to recruit additional regulatory co-factors, in the way that glycogen phosphorylase *a* requires AMP for activity (Barford et al. 1991).

Evidence for phosphorylation inducing the LHC II conformational change

2-D NMR data on the synthetic phosphopeptide (Nilsson et al. 1997) are interpreted as evidence for the small, compact structure shown in Fig. 5. At the N-terminus of an LHC II in the photosystem II or I supercomplex, intra-peptide salt bridge interactions are likely to be replaced by interactions with other, adjacent side chains. Alternatively, a compact N-terminus such as that seen in the free phosphopeptide (Fig. 5) could present an obstacle either to LHC II trimer formation or to the trimer binding to the photosystem II core antenna system. It furthermore remains to be seen whether such a small, local, secondary structure functions as a new recognition surface or as a 'plug-in' module for insertion and reattachment of LHC II to photosystem I. Induction of a global conformational change could create a docking surface and a variety of contact points for functional interaction with photosystem I.

The structural effect of phosphorylation of pea LHC II is the subject of molecular dynamics simulations (Ding et al. 2014). In this study, the 9 N-terminal amino acid residues are added, by homology modelling, to the crystal structure of Standfuss et al. (2005), and three forms of LHC II are simulated: the unphosphorylated trimer; the trimer phosphorylated at Thr-5 on all three chains; and the trimer phosphorylated at both Ser-3 and Thr-5 on all three chains. The two phosphorylated forms cover possibilities suggested by synthetic peptides as model substrates (Michel and Bennett 1989). The results of the simulation indicate decreased H-bond contacts suggesting a global conformational change in the structure of the LHC II monomer upon phosphorylation (Ding et al. 2014). The largest effects are seen at the N-terminus around the phosphorylation site itself, while surface-exposed loops on both sides of the membrane also contain residues with altered interaction both within and between trimers (Ding et al. 2014). In contrast, the membrane-spanning helices are relatively unaffected, supporting the idea of a rigid core with flexible termini, as concluded from EPR studies (Dockter et al. 2012). Ding et al. (2014) also report eventual reorientation of the transmembrane helices and transfer of the phosphorylation-induced conformational change from the stromal N-terminus to lumenal loops and the two small lumenal amphipathic helices.

The structure of pea LHC II as obtained by Standfuss et al. (2005) is depicted in Figs 3 and 4. In the PDB file 2BHW all three chains A, B, and C are traced with an N-terminus at Alanine-10, and so no information is available for the disordered N-terminal domain containing the phosphorylation site and otherwise corresponding to the synthetic phosphopeptide shown in Fig. 5. In the comparable structure from spinach, 1RWT, the N-terminus is disordered beyond Serine-14.

Resolving molecular recognition between LHC II and photosystems I and II

The structure described for photosystem I from pea chloroplasts (Amunts et al. 2010, Mazor et al. 2015) reveals plant photosystem I to be monomeric, unlike the trimeric photosystem I of cyanobacteria (Jordan et al. 2001). Trimer formation in plant photosystem I may be prohibited by the presence of a single-helix subunit not found in cyanobacteria, PsaH, mutational analysis of which indicates that it is a requirement for binding of phospho-LHC II (Lunde et al. 2000, Haldrup et al. 2001). LHC II binding requires additional small photosystem I subunits, including a 4 kDa protein termed psaI that is thought to provide an LHC II docking site together with psaH (Lunde et al. 2000, Haldrup et al. 2001) and psaL (Plöchinger et al. 2016). The four monomeric LHC I subunits of photosystem I may also be required (Wang and Grimm 2016). The binding affinity of PsaI/H/L for phospho-LHC II may be greatest on the lumen-exposed side of the membrane although the phosphorylation site itself is on the stromal side. In this event, the transmembrane structural changes suggested by LHC II molecular dynamics simulations (Ding et al. 2014) will be an essential feature of guided molecular recognition. The

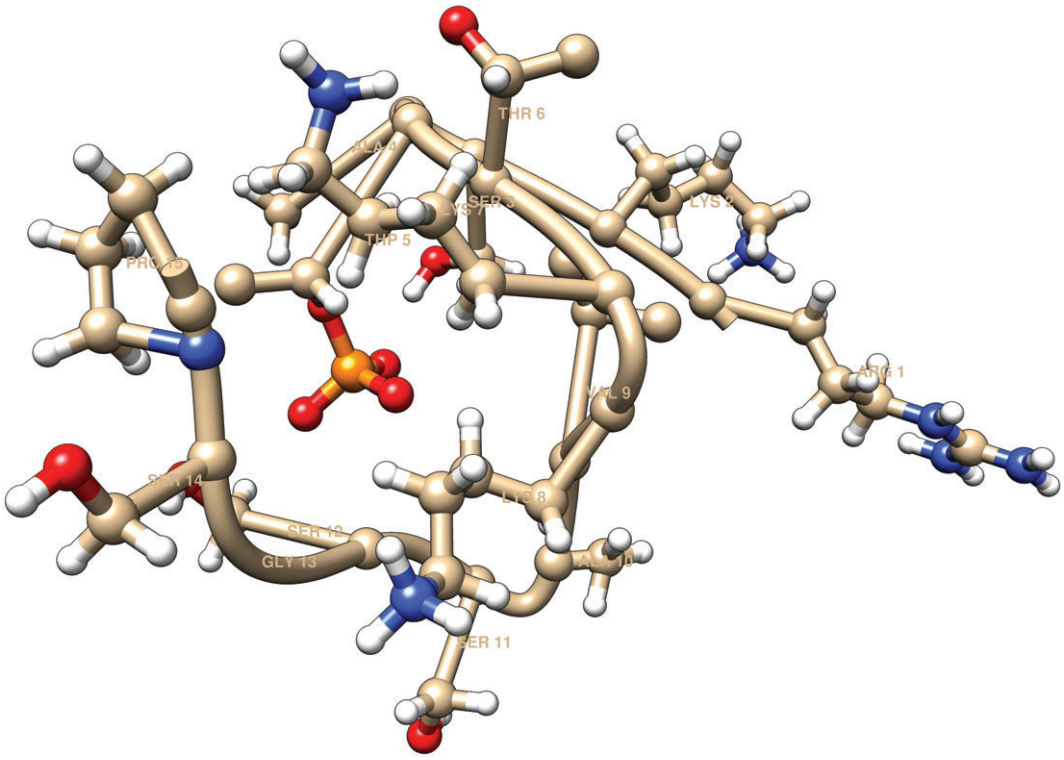

Fig. 5. Structure derived from NMR 2-D spectra of the synthetic phosphopeptide RKSATpTKKVASSGSP (Nilsson et al. 1997) corresponding to the surface-exposed N-terminus of one chain of pea LHC II. Tp is phospho-threonine in position 5. The structure is rendered using Chimera (Huang et al. 1996). In contrast, the unphosphorylated peptide is disordered and unresolved (Nilsson et al. 1997). For the PDB file see Appendix S1.

state 2 transition may also be favoured by an intact light-harvesting complex I antenna (Benson et al. 2015, Bressan et al. 2016).

Each plant photosystem I carries four monomeric LHC I proteins, homologues of LHC II, arranged in a 'cescent moon shape' around the opposite side of the complex to PsaH, when viewed normal to the membrane plane (Amunts et al. 2010, Mazor et al. 2015, Qin et al. 2015). Around psaH is a space almost symmetrical with the 4× LHC I 'crescent moon'. Were this space to be filled with 4× monomeric phospho-LHC II molecules, the twofold, non-crystallographic symmetry of plant PS I would be completed, and $4 \times 14 = 56$ chlorophyll molecules (32 Chl a and 24 Chl b) would enlarge the light-harvesting antenna by $56/167 = 33\%$, which is larger than functional estimates of the increase in the optical absorption cross-section of photosystem I upon complete phosphorylation of LHC II during the transition to light-state 2 (Allen 1992b). In contrast, addition of a single trimer of LHC II to photosystem I in state 2 is suggested by EM of *Arabidopsis* digitonin-solubilized material, giving a resolution of about 16 Å (Dekker and Boekema 2005, Kouril et al. 2005). This would add $3 \times 14 = 42$ chlorophylls, increasing photosystem I absorption cross-section by 25%. Comparable work with the green alga *Chlamydomonas* shows that a monomeric antenna protein attaches to PS I in state 2 (Kargul et al. 2005, Takahashi et al. 2006). Also in *Chlamydomonas*, there is a report that six to seven additional LHC I proteins are added to PS I in state 2, with no apparent participation of LHC II (Subramanyam et al. 2006). There is clear conflict and controversy in these observations and in predictions of the composition of photosystem I in state 2. It is an open question whether LHC II is added to the reaction centre of photosystem I as a monomer, as a trimer, or as some combination of the two. Direct interaction between LHC II and photosystem I in Triton-solubilized material has been observed by freeze-fracture EM, and accompanies energy transfer from chlorophyll *b*, contributing to photosystem I fluorescence emission at 77 K (Williams et al. 1987, Galka et al. 2012).

In *Arabidopsis*, the LHC II L-trimer ('L' for 'loosely-bound' to photosystem II) contains Lhcb-1 and Lhcb-2 chains and forms part of the antenna of photosystem I when Lhcb-2 is phosphorylated (Crepin and Caffarri 2015). Crepin and Caffarri (2015) conclude that phosphorylation of only one polypeptide chain of the L-trimer is sufficient for formation of the LHC II-PS

I complex; that this chain is Lhcb2; and that its binding site with photosystem I involves a direct interaction of the phosphothreonine with PsaH.

One approach to resolving the structure of phospho-LHC II and its binding site would be to isolate, purify and crystallize the phospho-LHC II-PS I complex from phosphorylated thylakoids according to a method, for photosystem I alone, such as that of Ben-Shem et al. (2003a, 2003b)). Attachment of four phospho-LHC II monomers to PS I would be expected to increase its total mass from 525 to about 670 kDa. In contrast, attachment of one trimer (Kouril et al. 2005) should increase total mass to about 634 kDa. The exact location of the N-terminal phosphorylation site and the polypeptide composition of LHC II trimers both differ between pea, spinach, *Arabidopsis* and *Chlamydomonas*. It is therefore possible that the composition of the phospho-LHC II-photosystem I supercomplex is species-specific.

Spatial scales of resolution of interactions – cellular, supramolecular, molecular and atomic

Structures of *Arabidopsis* chloroplast photosystem 1 in state 2 have been obtained by cryo-EM and show electron densities that correspond to those of the LHC II trimers resolved by X-ray crystallography. The LHC II trimer appears to be attached to the periphery of photosystem I at a site opposite to that occupied by the four monomeric LHC I (lhca) components. This LHC II binding site is occupied by psaH, psaI and psaL, mutant plants of which are impaired in the ability to make the transition to light state 2 (Yadav et al. 2017). Cryo-EM also shows smaller electron densities attached to photosystem I in state 2 (Yadav et al. 2017), and these are suggested to correspond to CP29 or to monomeric LHC II. The presence of a chloroplast equivalent of respiratory complex I (NADH dehydrogenase) in *Arabidopsis* photosystem I particles (Yadav et al. 2017) is consistent with state 2 favouring an increase in quantum yield of cyclic photophosphorylation in photosystem I (Allen 1984, 2003a). There is agreement that LHC II from photosystem II binds to photosystem I in state 2 and contributes to its increased antenna size (Galka et al. 2012, Drop et al. 2014). From the viewpoint of the 'molecular recognition' model (Allen 1990, 1992b, 1992a, Allen and Nilsson 1997, Nilsson et al. 1997, Allen and Forsberg 2001, Ding et al. 2014) forces operating over atomic distances induce a local change of secondary structure in LHC II that entails tertiary structural changes and quaternary changes of LHC II oligomeric state and protein–protein interactions.

It should be noted that electrostatic forces over longer distances are envisaged in 'surface charge' models. Electrostatic forces acting perpendicular to the membrane plane contribute to thylakoid stacking (Barber 1982, Puthiyaveetil et al. 2017). Repulsion of phosphorylated LHC II from photosystem II and its attraction by photosystem I have been proposed to move LHC II laterally, within the thylakoid membrane (Barber 1982). This view tended to reconcile effects of protein phosphorylation with earlier ideas of regulation of excitation energy distribution by means of altered cation concentration on membrane surface electrical change and on the extent of thylakoid stacking (Arntzen and Ditto 1976, Burke et al. 1978a, 1978b, Barber 1980, 1982). The surface charge model for lateral movement of LHC II appears to lack testable predictions concerning effects of phosphorylation on protein 3-D structure.

Structures of cyanobacterial photosystem II reaction centres from femtosecond time-resolved free electron laser X-ray crystallography at room temperature focus on the electron donor side in order to resolve interatomic distance changes that occur within the catalytic mechanism of water oxidation (Kupitz et al. 2014). Two structures for the S1 and S3 state of the water oxidation cycle show little or no change at the acceptor side, although there are subtle differences from structures obtained at cryogenic temperatures (Young et al. 2016). These differences include rotation of transmembrane helices and amino acid side chains. After two flashes, plastoquinone is reduced by one electron at the Q_B site and structural changes accompany non-physiological reduction of the iron atom between Q_A and Q_B (Suga et al. 2017). It may be possible eventually to apply free-electron laser crystallography to the two whole photosystems in order to resolve changes in interatomic distances during the earliest events initiating state transitions. At lower resolutions, in situ cryo-electron tomography (Engel et al. 2015) in principle provides a means of studying the changes in photosynthetic membrane protein distribution and interactions.

High-resolution structures for complete photosystem II and photosystem I in both states 1 and 2 are required for further understanding of molecular recognition in state transitions. These structures could come from X-ray crystallography, single-particle cryo-EM or NMR spectroscopy. The structural changes occurring during the transitions themselves could then be inferred, addressing many of the questions surrounding the interactions of the peripheral antennae with reaction centre cores, as well as the plastoquinone redox signal that initiates these changes. State transitions take place at physiological temperatures. They therefore require protein conformational change, and structural flexibility; processes usually

considered to be absent at cryogenic temperatures and from crystals.

Taking all these factors into account, we need atomic-resolution structural information in order to increase understanding of the altered pathway of energy transfer that results from re-assignment of the light-harvesting function of LHC II between photosystem I and photosystem II. We may also find unexpected contexts by means of which to re-interpret the mechanism and role of regulation of light-harvesting function and membrane protein–protein interactions.

Acknowledgements – The author thanks Dalibor Stys for the atomic coordinate file Appendix S1 used in Fig. 5, Sujith Puthiyaveetil for communicating a paper prior to publication, Sujith Puthiyaveetil, Nobert Krauß and Jon Nield for comments on the manuscript, and The Leverhulme Trust for Emeritus Research Fellowship EM-2015-068.

References

Allen JF (1984) Protein-phosphorylation and optimal production of ATP in photosynthesis. Biochem Soc Trans 12: 774–775

Allen JF (1990) How does protein phosphorylation control protein-protein interactions in the photosynthetic membrane? In: Baltscheffsky M (ed) Current Research in Photosynthesis. Kluwer Academic Publishers, Dordrecht, pp 915–918

Allen JF (1992a) How does protein-phosphorylation regulate photosynthesis? Trends Biochem Sci 17: 12–17

Allen JF (1992b) Protein phosphorylation in regulation of photosynthesis. Biochim Biophys Acta 1098: 275–335

Allen JF (2003a) Cyclic, pseudocyclic and noncyclic photophosphorylation: new links in the chain. Trends Plant Sci 8: 15–19

Allen JF (2003b) State transitions – a question of balance. Science 299: 1530–1532

Allen JF, Findlay JBC (1986) Amino-acid-composition of the 9 KDa phosphoprotein of pea thylakoids. Biochem Biophys Res Commun 138: 146–152

Allen JF, Forsberg J (2001) Molecular recognition in thylakoid structure and function. Trends Plant Sci 6: 317–326

Allen JF, Horton P (1981) Chloroplast protein-phosphorylation and chlorophyll fluorescence quenching – activation by tetramethyl-para-hydroquinone, an electron-donor to plastoquinone. Biochim Biophys Acta 638: 290–295

Allen JF, Nilsson A (1997) Redox signalling and the structural basis of regulation of photosynthesis by protein phosphorylation. Physiol Plant 100: 863–868

Allen JF, Bennett J, Steinback KE, Arntzen CJ (1981) Chloroplast protein phosphorylation couples plastoquinone redox state to distribution of excitation energy between photosystems. Nature 291: 25–29

Allen JF, Sanders CE, Holmes NG (1985) Correlation of membrane-protein phosphorylation with excitation-energy distribution in the cyanobacterium *Synechococcus* 6301. FEBS Lett 193: 271–275

Amunts A, Toporik H, Borovikova A, Nelson N (2010) Structure determination and improved model of plant photosystem I. J Biol Chem 285: 3478–3486

Andersson B, Akerlund HE, Jergil B, Larsson C (1982) Differential phosphorylation of the light-harvesting chlorophyll protein complex in appressed and non-appressed regions of the thylakoid membrane. FEBS Lett 149: 181–185

Arntzen CJ, Ditto CL (1976) Effects of cations upon chloroplast membrane subunit interactions and excitation energy distribution. Biochim Biophys Acta 449: 259–274

Baena-González E, Barbato R, Aro E-M (1999) Role of phosphorylation in the repair cycle and oligomeric structure of photosystem II. Planta 208: 196–204

Baginsky S (2016) Protein phosphorylation in chloroplasts – a survey of phosphorylation targets. J Exp Bot 67: 3873–3882

Barber J (1980) 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 118: 1–10

Barber J (1982) Influence of surface charges on thylakoid structure and function. Annu Rev Plant Physiol Plant Mol Biol 33: 261–295

Barford D, Hu SH, Johnson LN (1991) Structural mechanism for glycogen phosphorylase control by phosphorylation and AMP. J Mol Biol 218: 233–260

Bassi R, Giacometti GM, Simpson DJ (1988) Changes in the organization of stroma membranes induced by in vivo state-1-state-2 transition. Biochim Biophys Acta 935: 152–165

Bellafiore S, Barneche F, Peltier G, Rochaix JD (2005) State transitions and light adaptation require chloroplast thylakoid protein kinase STN7. Nature 433: 892–895

Bennett J (1977) Phosphorylation of chloroplast membrane polypeptides. Nature 269: 344–346

Bennett J (1979a) Chloroplast phosphoproteins – phosphorylation of polypeptides of the light-harvesting chlorophyll protein complex. Eur J Biochem 99: 133–137

Bennett J (1979b) Chloroplast phosphoproteins – protein-kinase of thylakoid membranes is light-dependent. FEBS Lett 103: 342–344

Bennett J (1980) Chloroplast phosphoproteins – evidence for a thylakoid-bound phosphoprotein phosphatase. Eur J Biochem 104: 85–89

Bennett J, Allen JF, Steinback KE, Arntzen CJ (1980a) Phosphorylation of polypeptides of the light-harvesting Chl a/b complex. In: Akoyunoglou G (ed) Fifth International Congress on Photosynthesis. Nuclear Research Center "Demokritos", Halkidiki, Greece, pp 54
Bennett J, Steinback KE, Arntzen CJ (1980b) Chloroplast phosphoproteins – regulation of excitation-energy transfer by phosphorylation of thylakoid membrane polypeptides. Proc Natl Acad Sci USA 77: 5253–5257
Bennett J, Shaw EK, Michel H (1988) Cytochrome-b6f complex is required for phosphorylation of light-harvesting chlorophyll a/b complex II in chloroplast thylakoid membranes. Eur J Biochem 171: 95–100
Ben-Shem A, Frolow F, Nelson N (2003a) Crystal structure of plant photosystem I. Nature 426: 630–635
Ben-Shem A, Nelson N, Frolow F (2003b) Crystallization and initial X-ray diffraction studies of higher plant photosystem I. Acta Crystallogr D Biol Crystallogr 59: 1824–1827
Benson SL, Maheswaran P, Ware MA, Hunter CN, Horton P, Jansson S, Ruban AV, Johnson MP (2015) An intact light harvesting complex I antenna system is required for complete state transitions in *Arabidopsis*. Nature Plant 1: 15176
Biggins J, Campbell CL, Bruce D (1984) Mechanism of the light state transition in photosynthesis 2. Analysis of phosphorylated polypeptides in the red alga, *Porphyridium cruentum*. Biochim Biophys Acta 767: 138–144
Bonardi V, Pesaresi P, Becker T, Schleiff E, Wagner R, Pfannschmidt T, Jahns P, Leister D (2005) Photosystem II core phosphorylation and photosynthetic acclimation require two different protein kinases. Nature 437: 1179–1182
Bonaventura C, Myers J (1969) Fluorescence and oxygen evolution from *Chlorella pyrenoidosa*. Biochim Biophys Acta 189: 366
Bressan M, Dall'Osto L, Bargigia I, Alcocer MJP, Viola D, Cerullo G, D'Andrea C, Bassi R, Ballottari M (2016) LHCII can substitute for LHCI as an antenna for photosystem I but with reduced light-harvesting capacity. Nature Plant 2: 16131
Brinkert K, De Causmaecker S, Krieger-Liszkay A, Fantuzzi A, Rutherford AW (2016) Bicarbonate-induced redox tuning in photosystem II for regulation and protection. Proc Natl Acad Sci USA 113: 12144–12149
Burke JJ, Ditto CL, Arntzen CJ (1978a) Involvement of light-harvesting complex in cation regulation of excitation-energy distribution in chloroplasts. Arch Biochem Biophys 187: 252–263
Burke JJ, Steinback KE, Arntzen CJ (1978b) Cation regulation of grana stacking and excitation-energy distribution in a chlorophyll b-less barley mutant. Plant Physiol 61: 102
Cogdell RJ, Gardiner AT (2015) Activated OCP unlocks nonphotochemical quenching in cyanobacteria. Proc Natl Acad Sci USA 112: 12547–12548
Consoli E, Croce R, Dunlap DD, Finzi L (2005) Diffusion of light-harvesting complex II in the thylakoid membranes. EMBO Rep 6: 782–786
Crepin A, Caffarri S (2015) 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 1847: 1539–1548
Dekker JP, Boekema EJ (2005) Supramolecular organization of thylakoid membrane proteins in green plants. Biochim Biophys Acta 1706: 12–39
Depege N, Bellafiore S, Rochaix JD (2003) Role of chloroplast protein kinase Stt7 in LHCII phosphorylation and state transition in *Chlamydomonas*. Science 299: 1572–1575
Dietzel L, Braautigam K, Steiner S, Schuffler K, Lepetit B, Grimm B, Schottler MA, Pfannschmidt T (2011) Photosystem II supercomplex remodeling serves as an entry mechanism for state transitions in *Arabidopsis*. Plant Cell 23: 2964–2977
Ding JH, Li N, Wang ML, Zhang Y, Lu SQ, Long M (2014) The impact of N-terminal phosphorylation on LHCII conformation in state transition. Acta Mech Sin 30: 447–456
Dockter C, Muller AH, Dietz C, Volkov A, Polyhach Y, Jeschke G, Paulsen H (2012) Rigid core and flexible terminus. structure of solubilized light-harvesting chlorophyll a/b complex (LHCII) measured by EPR. J Biol Chem 287: 2915–2925
Drop B, Yadav KNS, Boekema EJ, Croce R (2014) Consequences of state transitions on the structural and functional organization of photosystem I in the green alga *Chlamydomonas reinhardtii*. Plant J 78: 181–191
Engel BD, Schaffer M, Cuellar LK, Villa E, Plitzko JM, Baumeister W (2015) Native architecture of the *Chlamydomonas* chloroplast revealed by in situ cryo-electron tomography. eLife 4: e04889
Farchaus J, Dilley RA (1986) Purification and partial sequence of the Mr 10,000 phosphoprotein from spinach thylakoids. Arch Biochem Biophys 244: 94–101
Fristedt R, Vener AV (2011) High light induced disassembly of photosystem II supercomplexes in *Arabidopsis* requires STN7-dependent phosphorylation of CP29. PLoS One 6(9): e24565
Galka P, Santabarbara S, Thi THK, Degand H, Morsomme P, Jennings RC, Boekema EJ, Caffarri S (2012) 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 24: 2963–2978

Goldschmidt-Clermont M, Bassi R (2015) Sharing light between two photosystems: mechanism of state transitions. Curr Opin Plant Biol 25: 71–78
Haldrup A, Jensen PE, Lunde C, Scheller HV (2001) Balance of power: a view of the mechanism of photosynthetic state transitions. Trends Plant Sci 6: 301–305
Harris D, Tal O, Jallet D, Wilson A, Kirilovsky D, Adir N (2016) Orange carotenoid protein burrows into the phycobilisome to provide photoprotection. Proc Natl Acad Sci USA 113: E1655–E1662
Harrison MA, Allen JF (1991) Light-dependent phosphorylation of photosystem-II polypeptides maintains electron-transport at high light-intensity – separation from effects of phosphorylation of LHC-II. Biochim Biophys Acta 1058: 289–296
Hellmich J, Bommer M, Burkhardt A, Ibrahim M, Kern J, Meents A, Muh F, Dobbek H, Zouni A (2014) Native-like photosystem II superstructure at 2.44 angstrom resolution through detergent extraction from the protein crystal. Structure 22: 1607–1615
Horton P (1983) Control of chloroplast electron-transport by phosphorylation of thylakoid proteins. FEBS Lett 152: 47–52
Horton P, Black MT (1980) Activation of adenosine 5′-triphosphate-induced quenching of chlorophyll fluorescence by reduced plastoquinone – the basis of state-I-state-II transitions in chloroplasts. FEBS Lett 119: 141–144
Horton P, Allen JF, Black MT, Bennett J (1981) Regulation of phosphorylation of chloroplast membrane polypeptides by the redox state of plastoquinone. FEBS Lett 125: 193–196
Huang CC, Couch GS, Pettersen EF, Ferrin TE (1996) Chimera: an extensible molecular modeling application constructed using standard components. Pac Symp Biocomput 1: 724
Hurley JH, Dean AM, Sohl JL, Koshland DE, Stroud RM (1990) Regulation of an enzyme by phosphorylation at the active-site. Science 249: 1012–1016
Jeschke G, Bender A, Schweikardt T, Panek G, Decker H, Paulsen H (2005) Localization of the N-terminal domain in light-harvesting chlorophyll a/b protein by EPR measurements. J Biol Chem 280: 18623–18630
Johnson LN, Barford D (1993) The effects of phosphorylation on the structure and function of proteins. Annu Rev Biophys Biomol Struct 22: 199–232
Jordan P, Fromme P, Witt HT, Klukas O, Saenger W, Krauss N (2001) Three-dimensional structure of cyanobacterial photosystem I at 2.5 angstrom resolution. Nature 411: 909–917
Kargul J, Barber J (2008) Photosynthetic acclimation: structural reorganisation of light harvesting antenna – role of redox-dependent phosphorylation of major and minor chlorophyll a/b binding proteins. FEBS J 275: 1056–1068
Kargul J, Turkina MV, Nield J, Benson S, Vener AV, Barber J (2005) Light-harvesting complex II protein CP29 binds to photosystem I of *Chlamydomonas reinhardtii* under state 2 conditions. FEBS J 272: 4797–4806
Kirilovsky D, Kerfeld CA (2016) Cyanobacterial photoprotection by the orange carotenoid protein. Nature Plant 2: 16180
Kouril R, Zygadlo A, Arteni AA, de Wit CD, Dekker JP, Jensen PE, Scheller HV, Boekema EJ (2005) Structural characterization of a complex of photosystem I and light-harvesting complex II of *Arabidopsis thaliana*. Biochemistry 44: 10935–10940
Kühlbrandt W (1994) Structure and function of the plant light-harvesting complex, LHC-II. Curr Opin Struct Biol 4: 519–528
Kupitz C, Basu S, Grotjohann I, Fromme R, Zatsepin NA, Rendek KN, Hunter MS, Shoeman RL, White TA, Wang DJ, James D, Yang JH, Cobb DE, Reeder B, Sierra RG, Liu HG, Barty A, Aquila AL, Deponte D, Kirian RA, Bari S, Bergkamp JJ, Beyerlein KR, Bogan MJ, Caleman C, Chao TC, Conrad CE, Davis KM, Fleckenstein H, Galli L, Hau-Riege SP, Kassemeyer S, Laksmono H, Liang MN, Lomb L, Marchesini S, Martin AV, Messerschmidt M, Milathianaki D, Nass K, Ros A, Roy-Chowdhury S, Schmidt K, Seibert M, Steinbrener J, Stellato F, Yan LF, Yoon C, Moore TA, Moore AL, Pushkar Y, Williams GJ, Boutet S, Doak RB, Weierstall U, Frank M, Chapman HN, Spence JCH, Fromme P (2014) Serial time-resolved crystallography of photosystem II using a femtosecond X-ray laser. Nature 513: 261–265
Leoni C, Pietrzykowska M, Kiss AZ, Suorsa M, Ceci LR, Aro EM, Jansson S (2013) Very rapid phosphorylation kinetics suggest a unique role for Lhcb2 during state transitions in *Arabidopsis*. Plant J 76: 236–246
Liu Z, Yan H, Wang K, Kuang T, Zhang J, Gui L, An X, Chang W (2004) Crystal structure of spinach major light-harvesting complex at 2.72 A resolution. Nature 428: 287–292
Liu W, Tu W, Liu Y, Sun R, Liu C, Yang C (2016) The N-terminal domain of Lhcb proteins is critical for recognition of the LHCII kinase. Biochim Biophys Acta 1857: 79–88
Lunde C, Jensen PE, Haldrup A, Knoetzel J, Scheller HV (2000) The PSI-H subunit of photosystem I is essential for state transitions in plant photosynthesis. Nature 408: 613–615
Mazor Y, Borovikova A, Nelson N (2015) The structure of plant photosystem I super-complex at 2.8 Å resolution. eLife 4: e07433
Michel HP, Bennett J (1987) Identification of the phosphorylation site of an 8.3 KDa protein from photosystem-II of spinach. FEBS Lett 212: 103–108

Michel H, Bennett J (1989) Use of synthetic peptides to study the substrate-specificity of a thylakoid protein-kinase. FEBS Lett 254: 165–170
Michel H, Hunt DF, Shabanowitz J, Bennett J (1988) Tandem mass-spectrometry reveals that 3 photosystem-II proteins of spinach-chloroplasts contain N-acetyl-o-phosphothreonine at their Nh2 termini. J Biol Chem 263: 1123–1130
Minagawa J (2011) State transitions – the molecular remodeling of photosynthetic supercomplexes that controls energy flow in the chloroplast. Biochim Biophys Acta 1807: 897–905
Minagawa J (2013) Dynamic reorganization of photosynthetic supercomplexes during environmental acclimation of photosynthesis. Front Plant Sci 4: 513
Mullineaux CW (1992) Excitation-energy transfer from phycobilisomes to photosystem-I in a cyanobacterium. Biochim Biophys Acta 1100: 285–292
Mullineaux CW (2008) Phycobilisome-reaction centre interaction in cyanobacteria. Photosynth Res 95: 175–182
Mullineaux CW, Holzwarth AR (1991) Kinetics of excitation-energy transfer in the cyanobacterial phycobilisome-photosystem-II complex. Biochim Biophys Acta 1098: 68–78
Mullineaux CW, Boult M, Sanders CE, Allen JF (1986) Fluorescence induction transients indicate altered absorption cross-section during light-state transitions in the cyanobacterium *Synechococcus* 6301. Biochim Biophys Acta 851: 147–150
Murata N (1969) Control of excitation transfer in photosynthesis I. Light-induced change of chlorophyll a fluorescence in *Porphyridium cruentum*. Biochim Biophys Acta 172: 242
Myers J (1971) Enhancement studies in photosynthesis. Annu Rev Plant Physiol 22: 289–312
Nawrocki WJ, Santabarbara S, Mosebach L, Wollman FA, Rappaport F (2016) State transitions redistribute rather than dissipate energy between the two photosystems in *Chlamydomonas*. Nature Plant 2: 16031
Nilsson A, Stys D, Drakenberg T, Spangfort MD, Forsen S, Allen JF (1997) Phosphorylation controls the three-dimensional structure of plant light harvesting complex II. J Biol Chem 272: 18350–18357
Olive J, Mbina I, Vernotte C, Astier C, Wollman FA (1986) Randomization of the EF particles in thylakoid membranes of *Synechocystis*-6714 upon transition from state-I to state-II. FEBS Lett 208: 308–312
Pietrzykowska M, Suorsa M, Semchonok DA, Tikkanen M, Boekema EJ, Aro EM, Jansson S (2014) The light-harvesting chlorophyll a/b binding proteins Lhcb1 and Lhcb2 play complementary roles during state transitions in *Arabidopsis*. Plant Cell 26: 3646–3660
Plöchinger M, Torabi S, Rantala M, Tikkanen M, Suorsa M, Jensen P-E, Aro EM, Meurer J (2016) The low molecular weight protein PsaI stabilizes the light-harvesting complex II docking site of photosystem I. Plant Physiol 172: 450–463
Pribil M, Pesaresi P, Hertle A, Barbato R, Leister D (2010) Role of plastid protein phosphatase TAP38 in LHCII dephosphorylation and thylakoid electron flow. PLoS Biol 8(1): e1000288
Puthiyaveetil S (2011) A mechanism for regulation of chloroplast LHC II kinase by plastoquinol and thioredoxin. FEBS Lett 585: 1717–1721
Puthiyaveetil S, Kirchhoff H (2013) A phosphorylation map of the photosystem II supercomplex C2S2M2. Front Plant Sci 4: 459
Puthiyaveetil S, van Oort B, Kirchhoff H (2017) Surface charge dynamics in photosynthetic membranes and the structural consequences. Nature Plant 3: 17020
Qin XC, Suga M, Kuang TY, Shen JR (2015) Structural basis for energy transfer pathways in the plant PSI-LHCI supercomplex. Science 348: 989–995
Reiland S, Finazzi G, Endler A, Willig A, Baerenfaller K, Grossmann J, Gerrits B, Rutishauser D, Gruissem W, Rochaix JD, Baginsky S (2011) Comparative phosphoproteome profiling reveals a function of the STN8 kinase in fine-tuning of cyclic electron flow (CEF). Proc Natl Acad Sci USA 108: 12955–12960
Rintamaki E, Martinsuo P, Pursiheimo S, Aro EM (2000) Cooperative regulation of light-harvesting complex II phosphorylation via the plastoquinol and ferredoxin-thioredoxin system in chloroplasts. Proc Natl Acad Sci USA 97: 11644–11649
Ruban AV, Berera R, Ilioaia C, van Stokkum IHM, Kennis JTM, Pascal AA, van Amerongen H, Robert B, Horton P, van Grondelle R (2007) Identification of a mechanism of photoprotective energy dissipation in higher plants. Nature 450: 575–578
Saito K, Williams WP, Allen JF, Bennett J (1983) Comparison of Atp-induced and state-1 state 2-related changes in excitation-energy distribution in Chlorella-Vulgaris. Biochim Biophys Acta 724: 94–103
Shapiguzov A, Ingelsson B, Samol I, Andres C, Kessler F, Rochaix JD, Vener AV, Goldschmidt-Clermont M (2010) The PPH1 phosphatase is specifically involved in LHCII dephosphorylation and state transitions in *Arabidopsis*. Proc Natl Acad Sci USA 107: 4782–4787
Shapiguzov A, Chai X, Fucile G, Longoni P, Zhang L, Rochaix JD (2016) Activation of the Stt7/STN7 kinase through dynamic interactions with the cytochrome b(6)f complex (vol 171, pg 82, 2016). Plant Physiol 171: 1533
Silverstein T, Cheng L, Allen JF (1993a) Chloroplast thylakoid protein phosphatase reactions are redox-independent and kinetically heterogeneous. FEBS Lett 334: 101–105
Silverstein T, Cheng LL, Allen JF (1993b) Redox titration of multiple protein phosphorylations in pea chloroplast thylakoids. Biochim Biophys Acta 1183: 215–220

Simpson DJ (1983) Freeze-fracture studies on barley plastid membranes 7. Structural-changes associated with phosphorylation of the light-harvesting complex. Biochim Biophys Acta 725: 113–120

Standfuss J, Terwisscha van Scheltinga AC, Lamborghini M, Kuhlbrandt W (2005) Mechanisms of photoprotection and nonphotochemical quenching in pea light-harvesting complex at 2.5 A resolution. EMBO J 24: 919–928

Subramanyam R, Jolley C, Brune DC, Fromme P, Webber AN (2006) Characterization of a novel photosystem I-LHCI supercomplex isolated from *Chlamydomonas reinhardtii* under anaerobic (state II) conditions. FEBS Lett 580: 233–238

Suga M, Akita F, Sugahara M, Kubo M, Nakajima Y, Nakane T, Yamashita K, Umena Y, Nakabayashi M, Yamane T, Nakano T, Suzuki M, Masuda T, Inoue S, Kimura T, Nomura T, Yonekura S, Yu L-J, Sakamoto T, Motomura T, Chen J-H, Kato Y, Noguchi T, Tono K, Joti Y, Kameshima T, Hatsui T, Nango E, Tanaka R, Naitow H, Matsuura Y, Yamashita A, Yamamoto M, Nureki O, Yabashi M, Ishikawa T, Iwata S, Shen J-R (2017) Light-induced structural changes and the site of O=O bond formation in PSII caught by XFEL. Nature Adv 543: 131–135

Takahashi H, Iwai M, Takahashi Y, Minagawa J (2006) Identification of the mobile light-harvesting complex II polypeptides for state transitions in Chlamydomonas reinhardtii. Proc Natl Acad Sci USA 103: 477–482

Takahashi H, Okamuro A, Minagawa J, Takahashi Y (2014) Biochemical characterization of photosystem I-associated light-harvesting complexes I and II isolated from state 2 cells of *Chlamydomonas reinhardtii*. Plant Cell Physiol 55: 1437–1449

Telfer A, Allen JF, Barber J, Bennett J (1983) Thylakoid Protein-phosphorylation during state-1-state-2 transitions in osmotically shocked pea-chloroplasts. Biochim Biophys Acta 722: 176–181

Theis J, Schroda M (2016) Revisiting the photosystem II repair cycle. Plant Signal Behav 11(9): e1218587

Thornber JP (1975) Chlorophyll-proteins – light-harvesting and reaction center components of plants. Annu Rev Plant Physiol Plant Mol Biol 26: 127–158

Tikkanen M, Nurmi M, Kangasjarvi S, Aro EM (2008) Core protein phosphorylation facilitates the repair of photodamaged photosystem II at high light. Biochim Biophys Acta 1777: 1432–1437

Tsinoremas NF, Hubbard JAM, Evans MCW, Allen JF (1989) P-700 photooxidation in state-1 and state-2 in cyanobacteria upon flash illumination with phycobilin-absorbed and chlorophyll-absorbed light. FEBS Lett 256: 106–110

Umena Y, Kawakami K, Shen JR, Kamiya N (2011) Crystal structure of oxygen-evolving photosystem II at a resolution of 1.9 Ångström. Nature 473: 55–60

Vainonen JP, Hansson M, Vener AV (2005) STN8 protein kinase in *Arabidopsis thaliana* is specific in phosphorylation of photosystem II core proteins. J Biol Chem 280: 33679–33686

Vener AV (2007) Environmentally modulated phosphorylation and dynamics of proteins in photosynthetic membranes. Biochim Biophys Acta 1767: 449–457

Vener AV, VanKan PJM, Rich PR, Ohad I, Andersson B (1997) 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 USA 94: 1585–1590

Vernotte C, Astier C, Olive J (1990) State-1-state-2 adaptation in the cyanobacteria *Synechocystis*-PCC-6714 wild-type and *Synechocystis*-PCC-6803 wild-type and phycocyanin-less mutant. Photosynth Res 26: 203–212

Wang P, Grimm B (2016) Comparative analysis of light-harvesting antennae and state transition in chlorina and cpSRP mutants. Plant Physiol 172(3): 1519–1531

Wei X, Su X, Cao P, Liu X, Chang W, Li M, Zhang X, Liu Z (2016) Structure of spinach photosystem II–LHCII supercomplex at 3.2 Å resolution. Nature 534: 69–74

Wientjes E, Drop B, Kouril R, Boekema EJ, Croce R (2013a) During state 1 to state 2 transition in *Arabidopsis thaliana*, the photosystem II supercomplex gets phosphorylated but does not disassemble. J Biol Chem 288: 32821–32826

Wientjes E, van Amerongen H, Croce R (2013b) LHCII is an antenna of both photosystems after long-term acclimation. Biochim Biophys Acta 1827: 420–426

Williams WP, Allen JF (1987) State-1/state-2 changes in higher-plants and algae. Photosynth Res 13: 19–45

Williams RS, Allen JF, Brain APR, Ellis RJ (1987) Effect of Mg-2+ on excitation-energy transfer between LHC-II and LHC-I in a chlorophyll-protein complex. FEBS Lett 225: 59–66

Wollman FA (2001) State transitions reveal the dynamics and flexibility of the photosynthetic apparatus. EMBO J 20: 3623–3630

Yadav KNS, Semchonok DA, Nosek L, Kouřil R, Fucile G, Boekema EJ, Eichacker LA (2017) Supercomplexes of plant photosystem I with cytochrome b6f, light-harvesting complex II and NDH. Biochim Biophys Acta 1858: 12–20

Young ID, Ibrahim M, Chatterjee R, Gul S, Fuller FD, Koroidov S, Brewster AS, Tran R, Alonso-Mori R, Kroll T, Michels-Clark T, Laksmono H, Sierra RG, Stan CA, Hussein R, Zhang M, Douthit L, Kubin M, de Lichtenberg C, Pham LV, Nilsson H, Cheah MH, Shevela D, Saracini C, Bean MA, Seuffert I, Sokaras D, Weng TC, Pastor E, Weninger C, Fransson T, Lassalle L, Brauer P, Aller P, Docker PT, Andi B, Orville AM, Glownia JM,

Nelson S, Sikorski M, Zhu DL, Hunter MS, Lane TJ, Aquila A, Koglin JE, Robinson J, Liang MN, Boutet S, Lyubimov AY, Uervirojnangkoorn M, Moriarty NW, Liebschner D, Afonine PV, Waterman DG, Evans G, Wernet P, Dobbek H, Weis WI, Brunger AT, Zwart PH, Adams PD, Zouni A, Messinger J, Bergmann U, Sauter NK, Kern J, Yachandra VK, Yano J (2016) Structure of photosystem II and substrate binding at room temperature. Nature 540: 453–457

Zito F, Finazzi G, Delosme R, Nitschke W, Picot D, Wollman FA (1999) The Qo site of cytochrome b(6)f complexes controls the activation of the LHCII kinase. EMBO J 18: 2961–2969

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. File Accept_Lund 2.pdb of atomic coordinates from NMR spectroscopy of a synthetic phosphopeptide corresponding to the N-terminus of LHC II (Nilsson et al. 1997). See Fig 5.

Edited by P. Cardol