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Molecular Recognition: How Photosynthesis Anchors the Mobile Antenna

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True to its name, light-harvesting complex II (LHC II) harvests light energy for photosystem II (PS II). However, LHC II can stray, harvesting light energy for photosystem I (PS I) instead. Cryo-electron microscopy (cryo-EM) now shows how this mobile antenna becomes so attached to its new partner.

Harnessing Sunlight

Light-harvesting complex II (LHC II) is a membrane protein that contains chlorophyll and carotenoid pigment molecules. The chlorophylls absorb light and transfer its energy to the photochemical reaction centre of chloroplast photosystem II (PS II). In the reaction centre itself, the absorbed excitation energy oxidises a specific chlorophyll molecule. This primary reaction initiates electron transport in photosynthesis. The oxidised chlorophyll is re-reduced with electrons supplied by the oxidation of water, yielding free molecular oxygen – upon which all aerobic life depends. The electrons from PS II arrive eventually at PS I, where the absorbed light energy is harnessed in a similar way, by chlorophyll oxidation, but in a separate and distinct reaction centre. PS II and PS I are therefore connected in series, which means that their electron currents are equal, as depicted in the Z-scheme of noncyclic photosynthetic electron transport. PS I can also pass electrons back to itself, in a cyclic pathway that is also coupled to ATP synthesis, but without the direct need for an initial electron donor or terminal electron acceptor. Working together in this way, PS I and PS II harness sunlight for carbon and nitrogen assimilation, providing all of our food and most of our fuel.

Some individual LHC II pigment–protein complexes are able to collect light energy either for PS I or for PS II. For optimal efficiency, the rates of absorbed excitation energy transfer to the two reactions centres must match their rates of electron transfer. Redirection and redistribution of LHC II between PS II and PS I are used to achieve this effect. Phosphorylation of a side chain of one of its polypeptides redirects LHC II to PS I; dephosphorylation returns it to PS II. This reversible, post-translational modification of LHC II produces balanced excitation energy distribution between the two photosystems of green plants and algae.

How does LHC II switch its antenna function between PS II and PS I? Protein phosphorylation in LHC II causes it to bind to PS I. A cryo-electron microscopy (cryo-EM) structure for the resulting supercomplex [1] now shows how the phosphoryl group stabilises a protein secondary structure that anchors PS I and LHC II together.

The Busy Life of LHC II

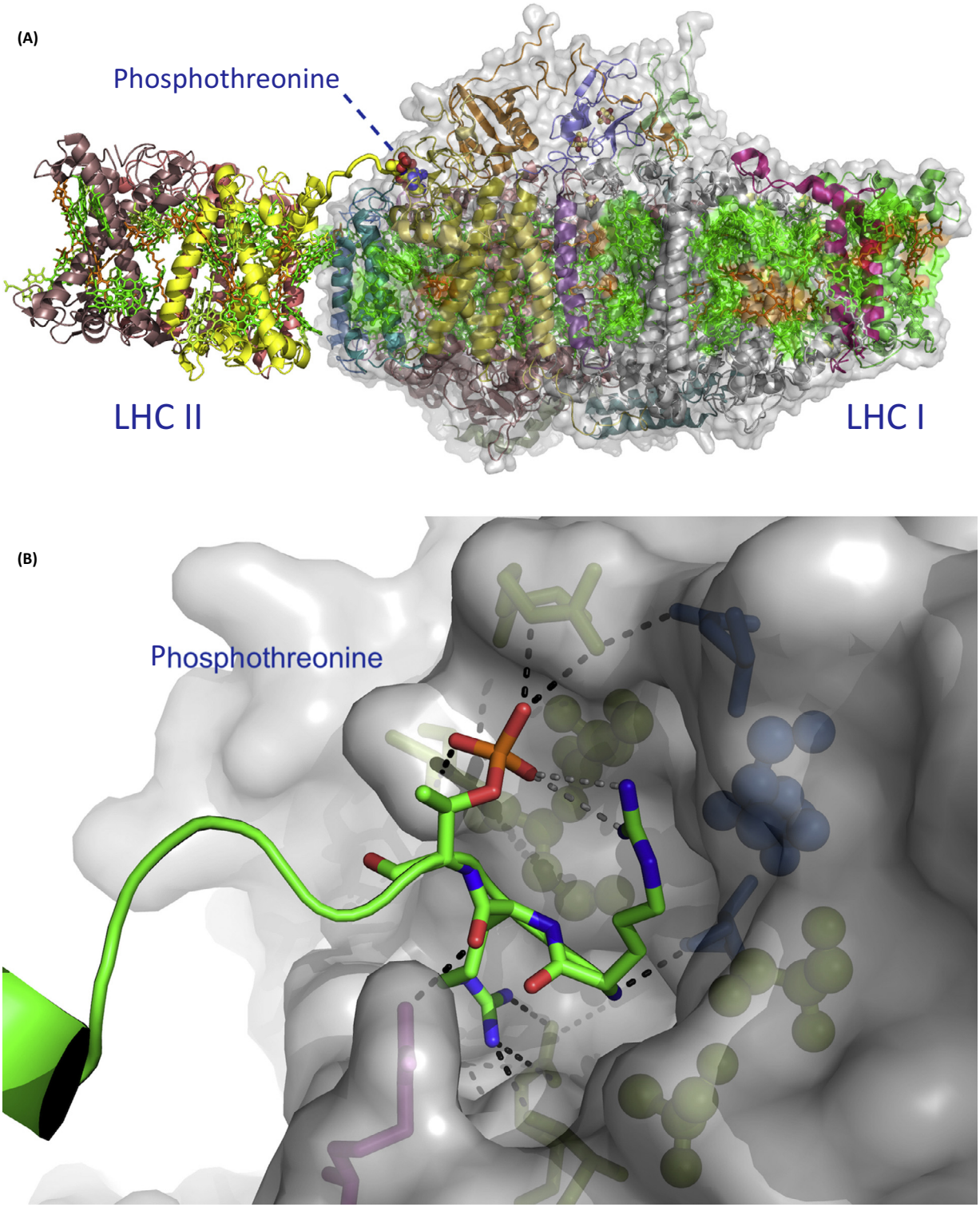
Pan *et al*. present a new model at 3.3 Å resolution of the maize (*Zea mays*) pLHC II–PS I supercomplex that is formed when leaves are exposed to orange light, or light 2, which initially favours PS II (Figure 1) [1]. This state of adaptation to light 2 is light-state 2 (or 'state 2') – in which a fraction of absorbed light energy has become redistributed to PS I at the expense of PS II [2]. LHC II is a trimer. Each component polypeptide (termed Lhcb1, Lhcb2, etc.) has three transmembrane helices and binds 14 chlorophyll molecules [3,4]. The new model shows one specific LHC II polypeptide, Lhcb2, in its phosphorylated form (pLhcb2), closely aligned with the PS I reaction centre complex (Figure 1A). pLhcb2 adopts a small, stable, compact structure at and around the phosphorylation site, which is a threonine residue close to its amino terminus. Two basic amino acid residues near the N-terminus of pLhcb2 point away from the threonine phosphorylation site (Figure 1B). Of these, Arg1 'folds back' (away from PS I) to stabilise the phosphate group by an ionic interaction. By forming a salt bridge (with Glu67) and a hydrogen bond with the Ser72 side chain, the Arg2 side chain in pLHC II-PS I plays a role in stabilising the interface of pLHC II with the PS I reaction centre polypeptide PsaL.

Pan *et al*. looked for the degree of conservation of the interface of the PsaL polypeptide of PS I with pLhcb2, and assumed Lhcb2 to be the phosphorylated subunit of LHC II that interacts with the PS I core, as indicated by biochemical studies with chloroplasts from *Arabidopsis thaliana* [5].

In the maize cryo-EM structure, the N-terminal portion of Lhcb2 resembles a rope with a grappling hook (containing the phosphate group) that has been thrown at the PS I core to become fixed into a binding pocket on the stromal side of PS I (Figure 1A). There seems to be no precedent for a structured, folded, but 'free-floating' polypeptide segment such as that suggested from residues 4–8 of the Lhcb2 N-terminus, which adopts a defined 3D structure but is not packed against another segment of polypeptide or any other molecule resolved in the structure.

Open Questions

A key point from these structural studies is that phosphorylation induces a change in structure that tips the affinity of LHC II in favour of PS I, at the expense of PS II. However, many questions remain. How large is the change in structure? What are the default contacts between LHC II and PS II, and how and why are these overridden? Is there an extensive structural change in LHC II upon phosphorylation, or does the N-terminus of pLhcb2 alone form something resembling a hook on the end of a piece of rope? A piece of rope is flexible, and this analogy is not obviously reconciled with precise orientation, as would be required for a docking event that might be necessary for efficient



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excitation energy transfer between chlorophylls of pLHC II and PS I. Can the 'rope' shorten or wind?

The structural model for pLHC II-PS I [1] has implications concerning chloroplast thylakoid membrane lateral heterogeneity with its separation of PS I and PS II between different membrane domains. The LHC II donor, PS II, and the LHC II acceptor, PS I, can be in close proximity, most likely in the grana margin or 'end membrane' [6]. This raises again questions concerning lateral migration of LHC II, and suggests that phospho-LHC II does not need to undertake any long, solitary journey from chloroplast thylakoid grana membranes (stacked) to stroma membranes (unstacked) during the transition state 2. A local, phosphorylation-induced anchor or grappling hook thrown out from LHC II could alter the interactions of closely adjacent proteins in such a way as to alter membrane topology and thylakoid membrane stacking. Thus a small, local, secondary structure could be viewed as a new recognition surface or as a 'plug-in' module for insertion and reattachment of phospho-LHC II to PS I. Initiation of a global conformational change might create a docking surface and a variety of contact points for functional interaction with polypeptides on PS I. PS I itself might undergo a conformational change to allow its own PsaL/H/O surface region to become more amenable to docking by pLHC II.

PS I from pea (*Pisum sativum*) chloroplasts is monomeric, unlike the oligomeric PS I of cyanobacteria [7]. Oligomer formation in plant PS I may be prohibited by the presence of a single-helix subunit not found in cyanobacteria – PsaH. Mutational analysis of PsaH suggests that it is required for the binding of PS I to phospho-LHC II in state 2. This binding requires additional small PS I subunits, including a 4 kDa protein termed Psal together with PsaL. The plant PS I complex contains four monomeric LHC I (Lhca) subunits in a 'crescent moon' configuration [8], each similar in overall structure to one Lhcb subunit of trimeric LHC II. The binding affinity of Psal/H/L for phospho-LHC II might also increase on the lumen-exposed side of the membrane [9], even though the phosphorylation site itself is on the stromal side.

Both the exact location of the N-terminal phosphorylation site and the polypeptide composition of LHC II oligomers differ between pea, spinach (*Spinacia oleracea*), arabidopsis (*Arabidopsis thaliana*), and chlamydomonas (*Chlamydomonas reinhardtii*). It seems likely that the composition of the phospho-LHC II–PS I supercomplex is species-specific. Across green plant species and phyla, how general is the anchoring mechanism revealed by Pan *et al*. for maize (*Zea mays*)?

Concluding Remarks and Future Directions

Structures of arabidopsis chloroplast PS I with LHC II in light-state 2 have been obtained by single-particle transmission EM [5,10], and show electron densities that correspond to those of the LHC II trimers resolved by X-ray crystallography. In agreement with the cryo-EM structure of Pan *et al*. [1], the phospho-LHC II trimer attaches to the periphery of PS I at a site opposite to that occupied by the four monomeric LHC I (Lhca) components. This LHC II binding site (Box 1 and Figure 1B) is occupied by PsaH, PsaO, and PsaL, and plants mutant for these proteins are impaired in the ability to make the transition to light state 2. Psal has an indirect effect by stabilising the interface. Single-particle EM also shows smaller electron densities attached to PS I in state 2 [11], and these are suggested to correspond to CP29 or to monomeric LHC II. The presence of a protonmotive photosynthetic complex I [12,13] in an arabidopsis PS I supercomplex [10,11] is consistent with light-state 2 supporting increased cyclic electron transport and photophosphorylation.

From the cryo-EM structural studies of Pan *et al*. [1], there can be little doubt that phosphorylated LHC II detaches from PS II and binds to PS I, thus accounting for the well-documented increase in PS I light-harvesting antenna size, and the decrease in PS II antenna size, in light state 2 [2]. The mechanism underlying this transition involves forces that operate over atomic distances – effects on membrane interactions, as well as on photosystem distribution between membrane domains, are consequences rather than causes.

Figure 1. Structural Model of Maize Chloroplast Supercomplex Containing Photosystem I (PS I) Bound to Phosphorylated Light Harvesting Complex II (LHC II). (A) The view is within and parallel to the horizontal thylakoid membrane plane, with the stromal surface to the top. The supercomplex is formed in light state 2. The path of electron transfer within the PS I reaction centre is vertically upward, from lumen to stroma. Light energy is absorbed and transferred by chlorophyll *a* (dark green), chlorophyll *b* (light green), and by carotenoids (orange), all rendered as sticks. Individual polypeptides are depicted as ribbons coloured arbitrarily to distinguish between them, although the single, phosphorylated LHC II polypeptide, pLhcb2, is shown in yellow. CPK-coloured spheres and sticks show residues 1–3 of LHC IIb and the Fe–S electron acceptors on the stromal side of the PS I reaction centre. PS I is shown in surface representation. (B) The PS I binding pocket for the LHC II polypeptide pLhcb2. Residues of the PS I core that form hydrogen bonds with pLhcb2 are shown as sticks, those which are only in van der Waals contact with pLhcb2 as sticks and spheres. For pLhcb2 only residues 1–3 are shown as sticks, the remaining portion as a cartoon. Residues of PsaH are shown in blue, those of PsaL in green, and Arg39 of PsaO in magenta. Black dashed lines indicate potential hydrogen bonds. The intrapolypeptide ionic interaction between Arg1 and pThr3 that stabilises the N-terminal structure of pLhcb2 is shown by grey dashed lines. Graphics prepared from 5zji.pdb [1] using the program PyMOL (The PyMOL Molecular Graphics System, Version 2.1 Schrödinger, LLC; https://pymol.org).

Phosphorylation induces a local change of secondary structure in LHC II that entails tertiary and perhaps quaternary structural changes with effects on the reaction centre cores of PS I and PS II.

Protein structural description of the mechanism of state 1–state 2 transitions has been a goal for decades [2]. The findings of Pan *et al*. [1] outline the molecular basis of these adaptations of the photosynthetic apparatus. Future structural investigation can now focus usefully on conformational changes involved in molecular recognition guided by reversible phosphorylation of both LHC II and PS II reaction centre proteins [14], as well as on other post-translational modifications that regulate photosynthesis [15].

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