Molecular recognition in thylakoid structure and function

John F. Allen and Jens Forsberg

In photosynthesis, light-harvesting chlorophyll molecules are shunted between photosystems by phosphorylation of the protein to which they are bound. An anchor for the phosphorylated chlorophyll–protein complex has now been identified in the reaction centre of chloroplast photosystem I. This finding supports the idea that molecular recognition, not membrane surface charge, governs the architecture of the chloroplast thylakoid membrane. We describe a model for the chloroplast thylakoid membrane that is consistent with recent structural data that specify the relative dimensions of intrinsic protein complexes and their dispositions within the membrane. Control of molecular recognition accommodates membrane stacking, lateral heterogeneity and regulation of light-harvesting function by means of protein phosphorylation during state transitions – adaptations that compensate for selective excitation of photosystem I or photosystem II. High-resolution structural description of membrane protein–protein interactions is now required to understand thylakoid structure and regulation of photosynthesis.

In oxygen-evolving photosynthesis, the conversion of light energy into electrochemical potential requires two photosystems working in close collaboration. In higher plants, these photosystems are integrated into the thylakoid membrane of the chloroplast, a cytoplasmic organelle (Fig. 1). The two photosystems – photosystem I and photosystem II – are wired electrically, in series, therefore the electrons flowing through one photosystem must travel at the same rate as those flowing through the other[1,2]. In all green plants and in most eukaryotic algae, photosystem I uses blue, red and far-red light, whereas photosystem II uses more blue than red light, and does not use far-light at all. In natural environments, both the intensity and the quality, or spectral composition, of natural, ambient light fluctuate with time. Such fluctuations occur for a variety of reasons, notably because of changes in shading and, for aquatic environments, in spectral filtering by water. If the energy available from a new, altered quality of light is not to be wasted when one photosystem is held back by the other, then there must be some way of redistributing light-harvesting antenna molecules to provide excitation energy where it is needed most.

Protein phosphorylation in light-state transitions

The broad outline of the mechanism that achieves this redistribution of absorbed excitation energy has been known for two decades[3–5] and is summarized in Fig. 2 (Box 1). When electrons pile up in plastoquinone (an electron carrier connecting photosystem I with photosystem II), a protein kinase acts to phosphorylate the apoproteins of the light-harvesting chlorophyll–protein complex, LHCII. Upon phosphorylation, LHCII leaves photosystem II, and acts, instead, as the light-harvesting antenna for photosystem I. Activation of an LHCII kinase therefore decreases absorption of light by photosystem II and increases absorption of light by photosystem I. Phosphorylation of LHCII is the basis of the transition to 'state 2', a state of adaptation to photosystem II-specific light. The transition to state 2 simultaneously clears the pile-up of electrons in plastoquinone in two ways: it decreases electron flow into plastoquinone from photosystem II, and increases electron flow out of plastoquinone to photosystem I. Conversely, a shortage of electrons in the plastoquinone pool between the photosystems is a signal that electrons are leaving faster than they are entering. The LHCII kinase is then switched off, LHCII becomes dephosphorylated by a phosphoprotein phosphatase, and the return of LHCII to photosystem II makes good the imbalance of energy distribution by driving a transition to 'state 1' (see, for example, Ref. 3).

Anchoring the mobile antenna

There are now new insights into the basis of the connection of LHCII with photosystem I and photosystem II in chloroplasts of eukaryotic plants and green algae. For example, Christina Lunde *et al.*[6] have shown that one protein subunit of photosystem I, called H, is required for the docking of phospho-LHCII when it acts as part of the light-harvesting antenna of photosystem I: H is part of the anchor that holds phospho-LHCII to photosystem I in light-state 2. Lunde *et al.*[6] used the model higher plant *Arabidopsis thaliana*, and inactivated the gene for the H subunit of photosystem I by antisense suppression. The consequences of this suppression point clearly to H as a component of the photosystem I anchor for phospho-LHCII. Without H, LHCII still became phosphorylated when plants were given photosystem II-specific blue light, but the light-harvesting ability of photosystem I was no longer enhanced. Thus the H-minus transformants were unable to perform the transition to state 2. Furthermore, because these transformants could not attain state 2, there was no longer a relaxation from state 2 to state 1. H-minus plants appear to be irreversibly locked in state 1, where phospho-LHCII never leaves photosystem II. Not only did the mutants exhibit phosphorylation of LHCII: the extent of phosphorylation appeared to be even greater than in the wild-type control[6]. Furthermore, light 1 became less effective in inducing dephosphorylation of

John F. Allen*
Jens Forsberg
Plant Biochemistry, Lund University, Box 117, SE-221 00 Lund, Sweden.
*e-mail: john.allen@plantbio.lu.se

http://plants.trends.com 1360-1385/01/$ – see front matter PII: S1360-1385(01)02010-6

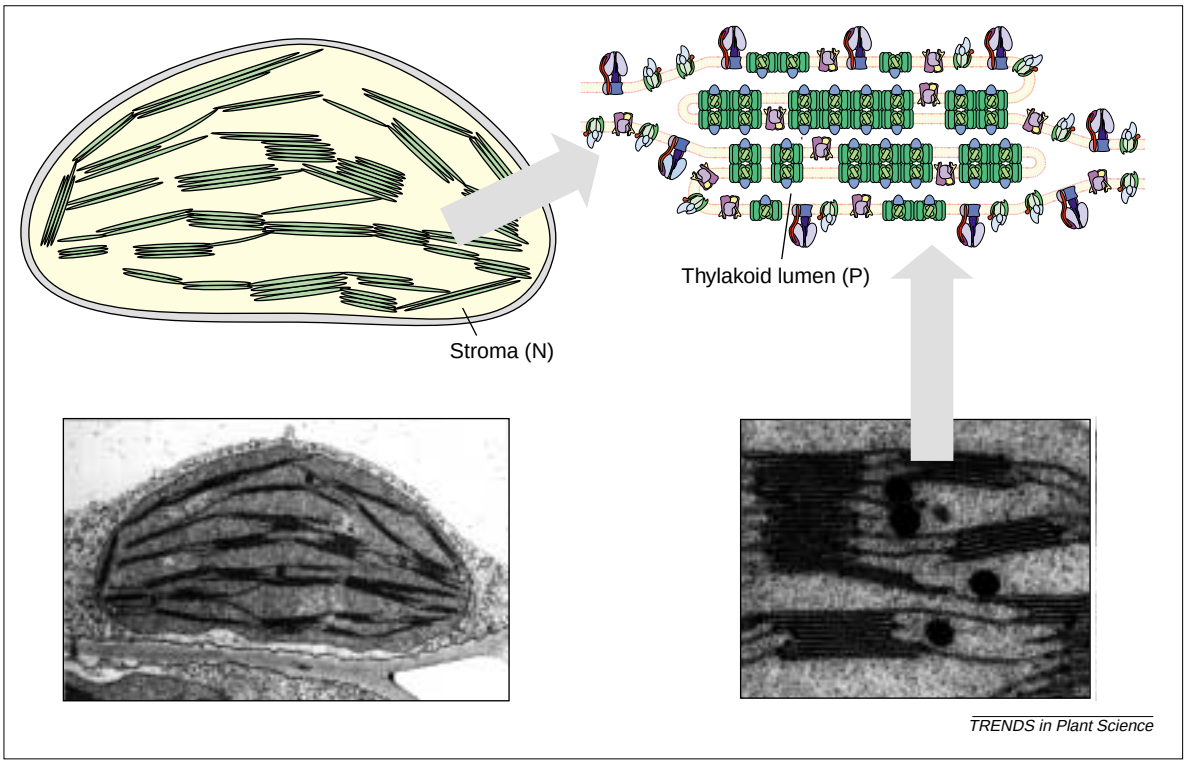

Fig. 1. Chloroplasts and thylakoids. The electron micrograph of a tobacco chloroplast (lower left) shows the familiar pattern of grana (membrane stacks) and stroma (aqueous phase surrounding the grana). Grana, long known as dark-green dots in light microscopy, contain the machinery of the light reactions of photosynthesis (and hence are coloured green in the cartoon at the upper left of the figure). Grana appear at higher magnification as stacks of membranous vesicles or 'thylakoids' (lower right). Thylakoids actually extend beyond the margins of the grana stacks as 'stromal thylakoids' or 'stroma lamellae'. The cartoon at the upper, right-hand side of the figure (adapted from an original diagram in Refs 21,69) indicates that a 'stack' of thylakoids might actually be composed of a single thylakoid vesicle folded in such a way as to give the impression of adjacent, discrete thylakoids when viewed in cross-section. The fold producing the effect (upper right) is schematic, and it is unclear whether precisely this fold is ever seen in electron micrographs. Nevertheless, the outline is a convenient representation of a topology for one small granum with a single internal, aqueous phase – the 'thylakoid lumen' or 'P (for 'positive')-phase'. The single, large thylakoid is folded back on itself because of adhesive contacts on its outer surface, facing the stroma, that is, the 'N (for 'negative')-phase'. Transmission electron micrographs of tobacco chloroplasts courtesy of Andrew Staehelin. Fig. 1 is modelled on a figure in Ref. 70.

phospho-LHCII (Ref. 6). Perhaps the phospho-LHCII phosphatase specifically recognizes its substrate when the substrate is bound to photosystem I, and dephosphorylation is thus inhibited in H-minus plants. In any case, the failure of H-minus plants to carry out the state 2 transition decreases their rate of plastoquinone re-oxidation by photosystem I, and this in itself tends to increase the phosphorylation of LHCII.

Molecular recognition or surface charge?

The results of Lunde *et al.*[6] identify the H subunit of photosystem I as the docking site for phospho-LHCII in state 2. This conclusion lends itself naturally to an interpretation based on molecular recognition: there is something about the phosphorylation of LHCII that increases its affinity for photosystem I at the expense of its interaction with photosystem II. It seems likely that this 'something' is a conformational change that alters the complementarity of a domain of LHCII so that it recognizes photosystem I when phosphorylated, but not when dephosphorylated. This 'molecular recognition' model was proposed in 1990 (Ref. 7) and developed to provide detailed predictions about the structural changes involved in state transitions[8,9]. By contrast, a different model states that the extra negative charge added to LHCII by the attached phosphate group repels fixed, negative charges inherent in photosystem II, causing a migration that is driven by electrostatic forces. This 'surface charge' model[10–12] was developed from the view that state transitions depend upon unstacking of thylakoids, and that unstacking results from electrostatic repulsion between negative charges of membrane surfaces[13–16]. Electrostatic repulsion would then predominate at low concentrations of screening cations[13,15]. It was originally thought that changes in free Mg^{2+} concentration, in themselves, produce state transitions by means of electrostatic effects on LHCII aggregation[10,16]. A more extreme view was that these organizing and regulatory effects of surface charge are a property of the membrane surfaces themselves, and that proteins, even LHCII, have no role in stacking or its control[17] (see also discussion in Ref. 10).

A 'surface charge' model – even one that admits a role for LHCII and its phosphorylation[11,12] – does not predict results such as those of Lunde *et al.*[6] One way to reconcile the surface charge model with the identification of the photosystem I 'anchor' might be to assume that the H subunit of photosystem I provides a centre of positive charge to which phospho-LHCII is held by electrostatic attraction. However, H

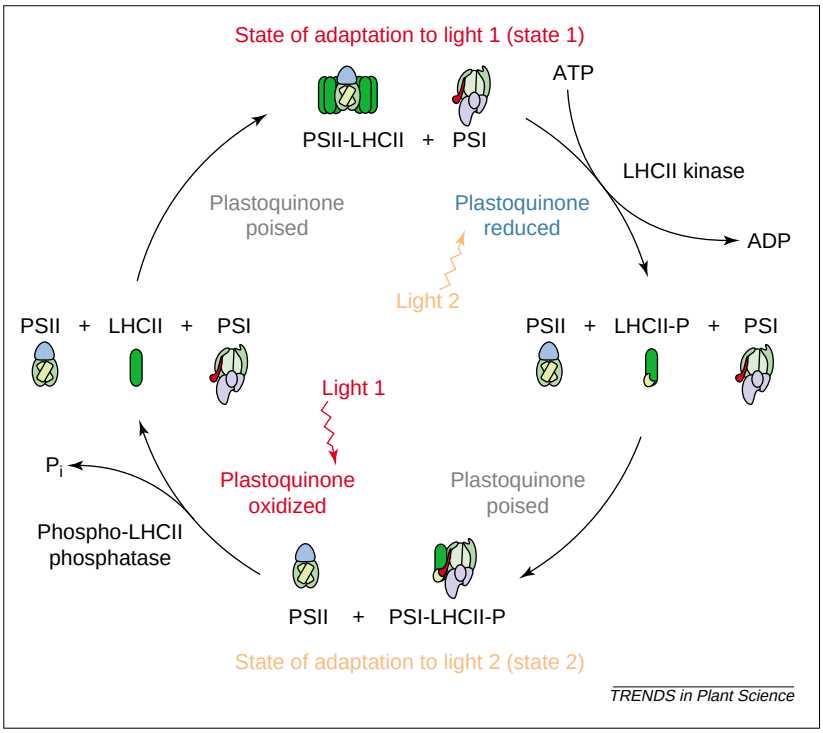

Fig. 2. An overview of redox-regulated phosphorylation of light-harvesting complex II (LHCII) as the basis of its functional re-association between photosystem I (PSI) and photosystem II (PSII) during transitions between light-states 1 and 2. Starting with poised plastoquinone in light-state 1 (12 o'clock), addition of light 2 will cause a reduction of plastoquinone. This activates the LHCII kinase, and phospho-LHCII then detaches from PSI (3 o'clock). Phospho-LHCII then acts, instead, as the antenna of PSI, resulting in light-state 2 (6 o'clock): redox poise of plastoquinone is thereby restored. Light 1 will then oxidize plastoquinone, thus inactivating the LHCII kinase. The action of the phospho-LHCII phosphatase then detaches LHCII from PSI (9 o'clock). This restores LHCII to PSI, resulting in a return to light-state 1 (12 o'clock).

is unlikely to be a basic protein: its pKa is predicted to be ~5. If electrical charge is the basis of the distribution of photosystems I and II and other proteins within the chloroplast thylakoid membrane[10,11], then a positively-charged H ought not to be a photosystem I subunit at all. If we accept the idea that the state 2 transition is driven by electrostatic repulsion between phospho-LHCII and photosystem II, then the phospho-LHCII of H-minus plants should be excluded from photosystem II just as much as in the wild type. But the results of Lunde *et al.*[6] suggest that the interaction of phospho-LHCII with photosystem II is maintained in H-minus plants.

According to the 'molecular recognition' viewpoint[7–9], these problems disappear because there is no need to seek a force that drives phospho-LHCII away from photosystem II and attracts it to photosystem I: the migration of LHCII is a simple consequence of its diffusion between two possible anchoring sites. The assumption of the molecular recognition model is that the photosystem I anchor is stronger for phospho-LHCII, whereas that of the photosystem II anchor is stronger for unphosphorylated or dephosphorylated LHCII. If LHCII merely changes its preferred binding site as a consequence of the structural change induced by phosphorylation, then state transitions are easily explained. The two photosystems compete for the attentions of LHCII and, for phospho-LHCII, photosystem I wins.

Before describing the structural re-alignment of LHCII between the photosystems as a result of guided molecular recognition, it is important to appreciate where photosystem I and photosystem II are located in thylakoid membranes, and why they are there. Our view is that lateral heterogeneity arises from protein–protein interactions, and regulation arises from their specific control. Thus thylakoid topology is dictated by the flexible, passive lipid bilayer going where its intrinsic proteins tell it.

Steric basis of stacking and lateral heterogeneity

Thylakoid membranes of higher plants (Fig. 1) are not structurally homogenous, but consist of two main domains: the grana, which are stacks of thylakoids; and the stroma lamellae, which are unstacked thylakoids[14,18,19]. These domains differ in protein composition and biochemical properties[10,20–23]. The grana stacks can be further divided into grana cores, grana margins and grana end membranes. Photosystem II is enriched in the grana whereas photosystem I is enriched in stroma lamellae, and in surface-exposed grana end membranes and margins[24,25].

We suggest that lateral heterogeneity in the distribution of photosystems I and II between different thylakoid domains arises from the tendency of LHCII to aggregate, and then from steric constraints on the occupancy of the appressed membranes that are thus produced. Figure 3 shows our current model for the topology of the chloroplast thylakoid membrane, and for the disposition within it of the major, intrinsic protein complexes. The idea of the topology deriving from protein–protein interactions, rather than lipid bilayer interactions, is now widely held[10,26]. The idealized membrane 'fork' of Fig. 3 is based on previously published models[10,21,26] that emphasize that folding of one long thylakoid vesicle with a single intra-thylakoid space (or 'lumen') can account for the appearance, in sections, of stacks of discs.

Appressed domains: photosystem II and LHCII

According to our model (Fig. 3), the close appression of grana membranes arises because the flat, N-phase-exposed surfaces of LHCII (Refs 27,28) form recognition and contact surfaces for each other, causing opposing surfaces of grana thylakoids to interact. There is no steric hindrance to this close opposition of stacked grana membranes, because photosystem II, like LHCII itself, presents a flat surface that protrudes no more than 10–20 Å beyond the membrane surface[29,30].

The functional significance of grana stacking is presumably to allow a large, connected, light-harvesting antenna to form both within and between membranes[10,19,21]. Within this antenna both lateral and transverse excitation energy transfer can take place, because excitons can pass between chlorophylls

Box 1. State transitions: discovery, mechanism, terminology

State transitions work by means of light- and redox-control of thylakoid protein phosphorylation. However, the existence of the two light states was recognized[a,b] eight years before the discovery of phosphoproteins in chloroplasts[c] and more than ten years before the explanation of plastoquinone redox-controlled light-harvesting complex II (LHCII) phosphorylation was described[d]. Direct and explicit descriptions of physiological redistribution of absorbed excitation energy between photosystem I and photosystem II were provided independently for the green alga *Chlorella pyrenoidosa*[a] and the red alga *Porphyridium cruentum*[b]. Subsequent research suggests that 'state 1–state 2 transitions' are a universal property of organisms that live by means of oxygen-evolving photosynthesis[e], from cyanobacteria to higher plants, even though neither cyanobacteria nor red algae such as *P. cruentum*[b] actually contain LHCII.

Lights and states

The terminology associated with the phenomenon of state transitions stands independently of their mechanism, and is as follows. Photosystem I is selected by a photosystem I-specific light, which can be termed 'light 1': photosystem II is correspondingly selected by 'light 2'. The state of adaptation to light 1 is called the 'light 1-state' or 'state 1'. The state of adaptation to light 2 is called the 'light 2-state' or 'state 2'. The transition from state 2 to state 1 is called the 'state 1 transition': by definition, it involves redirection of absorbed excitation energy to photosystem II at the expense of photosystem I. The transition from state 1 to state 2 is called the 'state 2 transition': it involves redirection of absorbed excitation energy to photosystem I at the expense of photosystem II. Misuse of this terminology has been a source of confusion, although these terms were clearly described and consistently applied[f], independently of the discovery that protein phosphorylation and redox control are involved.

Relative antenna size versus 'spillover'

One term that does imply a mechanism is 'spillover'. The term was introduced by Jack Myers[f], and strongly implies a mechanism that we now think is incorrect. The state 2 transition was originally supposed to result from a spillover, or overflow, of surplus excitation energy from photosystem II to photosystem I. 'Spillover' intuitively implies that the saturation of photosystem II leads to increased overflow of excitation energy to photosystem I, rather as a river, when dammed, might overflow its banks so that the water becomes diverted to a new destination. This metaphor is misleading because the consensus is now that state transitions involve complementary changes of the absorption cross-section of the two photosystems (reviewed in Ref. e). A better analogy is that a photosynthetic unit is a funnel that does not overflow because the diameters of the photosystem I and photosystem II funnels adjust themselves, both relative to each other and in absolute terms. *In vitro* treatments, such as cation depletion, can certainly induce a low-fluorescence state of high 'spillover' to photosystem I that superficially resembles state 2[g,h]. The term 'spillover' can legitimately be reserved for excitation energy transfer from photosystem II to photosystem I after non-physiological treatments that unstack thylakoids and thereby mix photosystem I and photosystem II. However, *in vivo*, the two photosystems are separated to some extent in space, by being enriched in adjacent but distinct thylakoid domains[i–m], and it is doubtful if spillover takes place even where these domains overlap at the grana margin. Treatments that induce spillover include cation-depletion and proteolysis of surface-exposed segments of LHCII that are required for membrane stacking[n]. Unstacking produces spillover in the form of a direct, one-way transfer of excitation energy after light absorption has taken place, but spillover is prohibited *in vivo* by spatial separation of the photosystems. Instead, state transitions involve complementary changes in the light-harvesting antenna sizes of photosystems I and II: in state transitions, the two photosystems alter their relative rates of light absorption. In state 2, photosystem II does not divert absorbed light energy, but simply receives less in the first place – the effect of the decreased antenna size is indistinguishable from a corresponding decrease in light intensity. As a physiological mechanism, state transitions have nothing to do with non-specific effects such as those induced by proteolysis or cation-depletion. In spite of its wide currency, even in teaching and text books[o–q], we recommend that use of the term 'spillover' is discontinued in this context.

References

a Bonaventura, C. and Myers, J. (1969) Fluorescence and oxygen evolution from *Chlorella pyrenoidosa*. *Biochim. Biophys. Acta* 189, 366–383

b Murata, N. (1969) Control of excitation transfer in photosynthesis. I. Light-induced change of chlorophyll *a* fluorescence in *Porphyridium cruentum*. *Biochim. Biophys. Acta* 172, 242–251

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

d Allen, J.F. *et al.* (1981) Chloroplast protein phosphorylation couples plastoquinone redox state to distribution of excitation energy between photosystems. *Nature* 291, 25–29

e Allen, J.F. (1992) Protein phosphorylation in regulation of photosynthesis. *Biochim. Biophys. Acta.* 1098, 275–335

f Myers, J. (1971) Enhancement studies in photosynthesis. *Annu. Rev. Plant Physiol.* 22, 289–312

g Arntzen, C.J. and Ditto, C.L. (1976) Effects of cations upon chloroplast membrane subunit interactions and excitation energy distribution. *Biochim. Biophys. Acta.* 449, 259–274

h 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

i Anderson, J.M. (1981) Consequences of spatial separation of photosystem 1 and 2 in thylakoid membranes of higher plant chloroplasts. *FEBS Lett.* 124, 1–10

j Anderson, J.M. (1982) Distribution of the cytochromes of spinach chloroplasts between the appressed membranes of grana stacks and stroma-exposed thylakoid regions. *FEBS Lett.* 138, 62–66

k Åkerlund, H-E. *et al.* (1976) Isolation of photosystem II enriched membrane vesicles from spinach chloroplasts by phase partition. *Biochim. Biophys Acta.* 449, 525–535

l Albertsson, P-Å. *et al.* (1990) The domain organization of the plant thylakoid membrane. *FEBS Lett.* 273, 36–40

m Armond, P.A. *et al.* (1977) Spatial relationship of photosystem I, photosystem II, and the light-harvesting complex in chloroplast membranes. *J. Cell Biol.* 73, 400–418

n Arntzen, C.J. (1978) Dynamic structural features of chloroplast lamellae. In *Current Topics in Bioenergetics* (Vol. 8) (Vernon, L. and Sanadi, R., eds), pp. 111–160, Academic Press

o Heldt, H-W. (1997) *Plant Biochemistry and Molecular Biology*, Oxford University Press

p Buchanan, B.B. *et al.* (2000) *Biochemistry and Molecular Biology of Plants*, American Society of Plant Physiologists

q Taiz, L. and Zeiger, E. (1998) *Plant Physiology*, W.H. Freeman, Sinauer Associates

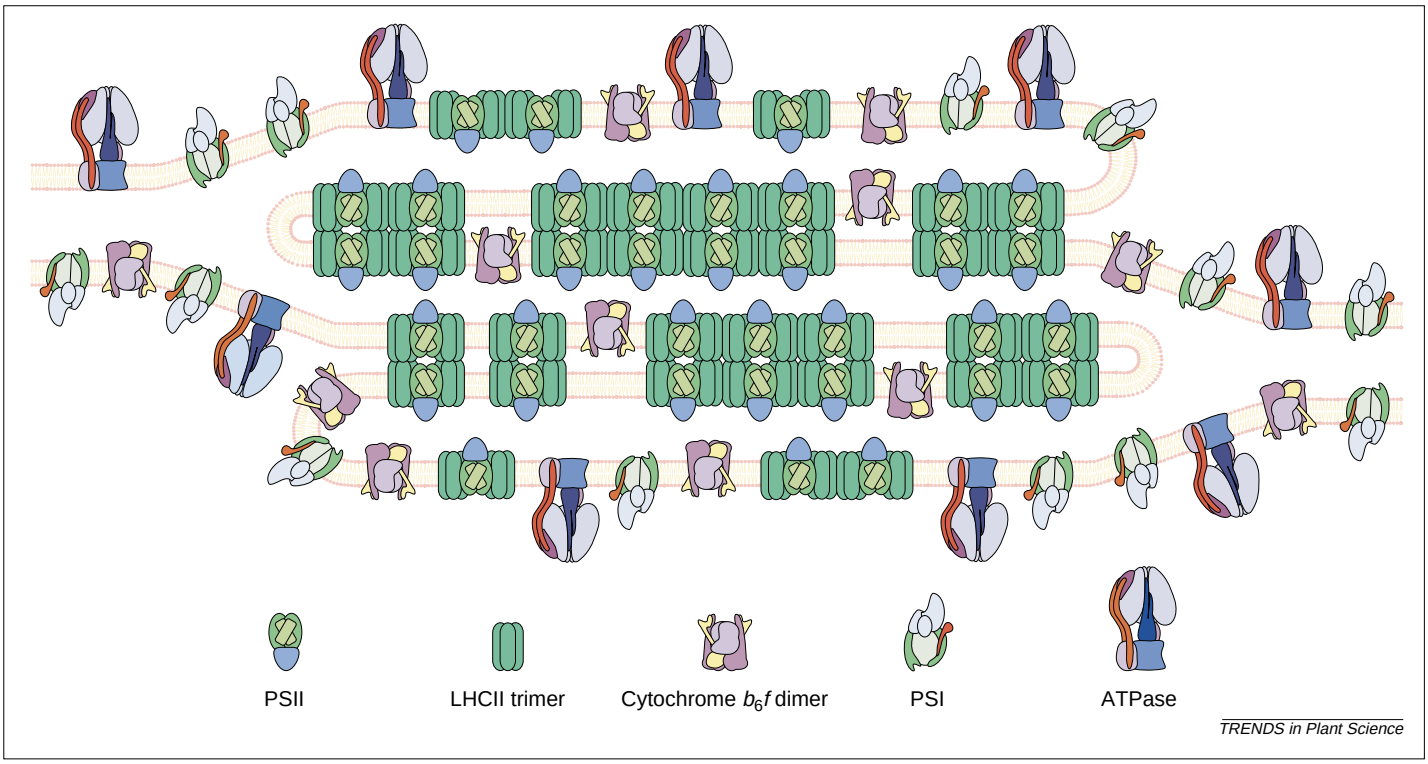

Fig. 3. Molecular recognition and steric hindrance determine both the topography of thylakoid stacks and lateral heterogeneity in the distribution of photosystems I and II. A high affinity of light-harvesting complex II (LHCII) for itself, both laterally and transversely, causes LHCII-rich regions of thylakoid to become tightly appressed, forming grana. Protein complexes that contain extended, stromal-phase projections into the aqueous phase are thereby excluded from the interior of grana stacks: these are photosystem I and the coupling ATPase. By contrast, photosystem II has a flat 'roof' that extends only 10–20 Å beyond the membrane surface on the outside (stromal, N-phase or cytoplasmic surface): photosystem II is easily accommodated within grana stacks. The lateral separation of photosystem I and photosystem II arises from the interaction of photosystem II with the membrane-'adhesive' antenna complex, LHCII. Photosystem I is excluded from the resulting, appressed, photosystem II-rich domains by means of steric hindrance. Membrane topology modified from Refs 21,71. The depictions of the major intrinsic complexes are schematic. CF1-CFo ATPase is adapted from Ref. 72 and based on the structure of bovine mitochondrial F1; cytochrome b_6f is depicted as a dimer and based on the structure of chicken mitochondrial cytochrome b-c_1 (Refs 73,74); photosystem I is adapted from a cartoon from Ref. 70, with dimensions for the surface-exposed domain consistent with results of Norbert Krauß *et al.*[35]; photosystem II and LHCII are schematic with the surface-exposed domains as specified by the cyanobacterial structure of Athina Zouni *et al.*[30] and, for pea LHCII, of Werner Kühlbrandt *et al.*[27]. Photosystem II is dimeric[29,30] but is here shown as a monomer along the x-axis for convenience – the second monomer could be envisaged as lying behind the first, along the z-axis. LHCII is a trimer in its dephosphorylated form[28]. As far as is known to date, each intrinsic complex is accurately represented for 'height', that is, for its extension on the y-axis, transverse to the membrane plane – this will be unaffected by oligomeric state. The cytochrome b_6f complex is depicted in all parts of the thylakoid membrane because there is no purely steric restriction to it being present in grana stacks. However, cytochrome b_6f, which transports electrons between photosystem II and I, might be localized predominantly in grana margins.

located in LHCII complexes that are adjacent to each other, both within a single membrane and between appressed membranes[28,31]. This arrangement also suggests a functional explanation for the observation made using electron microscopy that the extent of grana stacking is inversely correlated with growth light intensity[19,32,33]. This effect might occur because antenna size is at a premium at low light levels, where the larger the number of chlorophylls connected for energy transfer, the smaller the probability that an exciton will be lost as fluorescence before it can be trapped to perform useful photochemistry at a photosystem II reaction centre. Photosystem II centres would be expected to form a regular, geometrical pattern by virtue of being embedded within a matrix of LHCII complexes within the grana stacks. This has been suggested[10] as the origin of the 'quantasome' structure in freeze–fracture electron micrographs[18].

A further consequence of the oligomeric nature of LHCII arrays is that regulation of the LHCII structure might occur allosterically, as suggested both for phosphorylation[7] and for regulation of energy dissipation by decreased lumenal pH and the xanthophyll cycle[34]. The 'state 1' condition (Fig. 4) is one in which many LHCII complexes are connected, the total antenna size of photosystem II is large, and individual photosystem II reaction centres are served by a 'lake' of antenna chlorophyll molecules. This is a 'low light', high-quantum-yield arrangement, where the option of a switch to effective and rapid energy dissipation might be especially important.

Unappressed domains: photosystem I and coupling ATPase

X-ray crystallographic studies of photosystem I (Refs 35,36) reveal an extended structure on what is topologically the same side of the chloroplast thylakoid as that of the contact surfaces between opposing LHCIIs within the grana stacks. This electron acceptor-side (N-phase-exposed or stroma-exposed) feature of photosystem I, which contains the iron–sulfur proteins that carry electrons to ferredoxin and ultimately on to $NADP^+$, protrudes ~50 Å beyond the membrane surface[36,37]. Thus even a single photosystem I could not possibly be accommodated within the grana stacks, where adjacent thylakoids are separated by no more than 40 Å. A steric restriction on the distribution of photosystem I has also been proposed on the basis of freeze–fracture electron

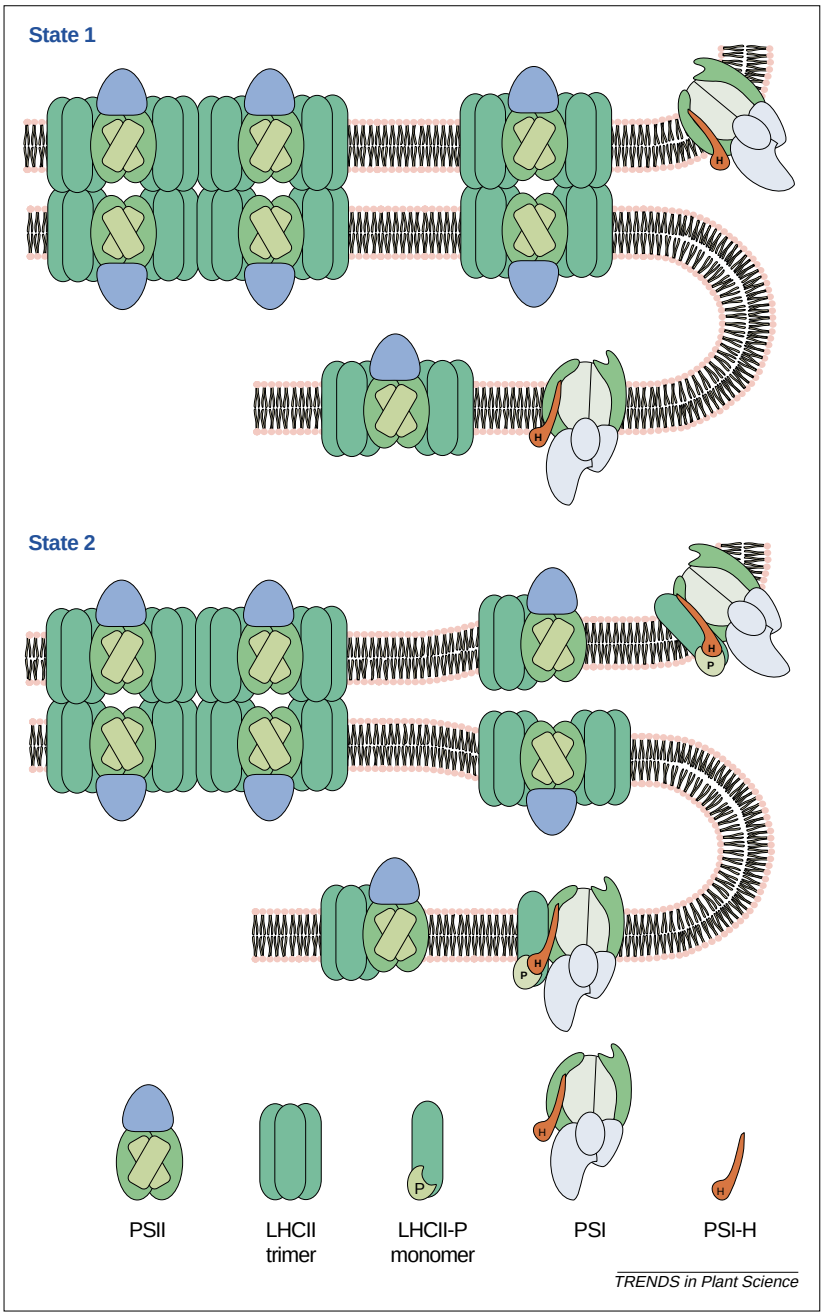

Fig. 4. Molecular recognition in thylakoid dynamics: a structural overview of states 1 and 2. In the transition from state 1 (upper) to state 2 (lower), phosphorylation-induced changes in the 3-D structure of light harvesting complex II (LHCII), disconnect it from photosystem II and reconnect it with photosystem I. This lateral migration, and the reverse migration in the transition from state 2 to state 1, are relatively short-range, and result from diffusion of LHCII between its two possible binding sites. The transition to state 2 gives a decreased absorption cross-section for photosystem II and an increased absorption cross-section for photosystem I. Modified after Ref. 8. Relative dimensions, shapes and oligomeric states of the complexes are depicted as described for Fig. 3. It should also be noted that there is evidence that the cytochrome b_6f complex (Fig. 3) might move laterally from photosystem II to photosystem I in *Chlamydomonas*[75], where state 2 is associated with an increase in cyclic photophosphorylation, driven by electron transport around photosystem I.

microscopy[38]. The enormous difference in the extent to which photosystem II and photosystem I project out of the cytoplasmic surface of the thylakoid membrane can be a simple requirement of their secondary electron acceptors – photosystem II supplies electrons to the membrane-intrinsic carrier plastoquinone, whereas photosystem 1 supplies electrons to the water-soluble carriers ferredoxin, ferredoxin-$NADP^+$-oxidoreductase and $NADP^+$. To transfer electrons out into the aqueous phase at optimal rates, photosystem I presents a relatively large surface area for diffusion, recognition and interaction of water-soluble acceptors both before and after electron transfer.

It is interesting to note that photosystem I and II also show differences in the extent to which their electron donor sides protrude from the thylakoid membrane. The differences in donor-side (P-phase-exposed or lumen-exposed) structures are the opposite of those on the acceptor-side (N-phase or stroma). Photosystem I has an donor side that projects ~20 Å into the thylakoid lumen, whereas photosystem II has a large structure that reaches 45 Å into the lumen from the inner surface of the thylakoid. As with the acceptor side, the donor side differences are consistent with the nature of the electron donors themselves. For photosystem I, the donor is the small, copper-protein, plastocyanin. Photosystem II takes electrons from its own water-oxidation complex, which is inherently bulky. Water oxidation might also require an extended diffusion path, for oxygen and for the protons released, perhaps delimited by the 33 kDa psbO beta cylinder seen in Athina Zouni and co-worker's[30] crystal structure.

Structural factors in migration of light harvesting complex II

The molecular recognition hypothesis for the mechanism of state transitions proposes that phosphorylation of LHCII decreases its affinity for photosystem II and increases its affinity for photosystem I (Refs 7–9), and that this change in binding specificity is the basis of lateral mobility of LHCII (Refs 39,40). Figure 4 presents the best model we can suggest in light of structural determinations of the photosystems[29,30,35,37], evidence for a structural change in LHCII (Ref. 41) and evidence of phospho-LHCII docking with photosystem I (Refs 6,42,43).

Light harvesting complex II changes three-dimensional structure upon phosphorylation

Following the demonstration of the role of plastoquinone redox-controlled phosphorylation of LHCII in state transitions, the idea that the phosphate group exerts its effect through electrostatic repulsion remained influential[3,4,12,44], and required no radical re-appraisal of the reigning paradigm of surface charge. By contrast, molecular recognition predicts that the charge on the phosphate group modifies electrostatic interactions only over atomic distances, typically <5 Å. These effects cause a 3-D structural change in LHCII upon phosphorylation. The interactions might take the form of electrostatic screening that offsets repulsion between fixed, basic side chains that flank the phosphorylation site, together with salt bridge contacts that stabilize a new structure for phospho-LHCII (Refs 7–9,41). The interactions are primarily intramolecular, although

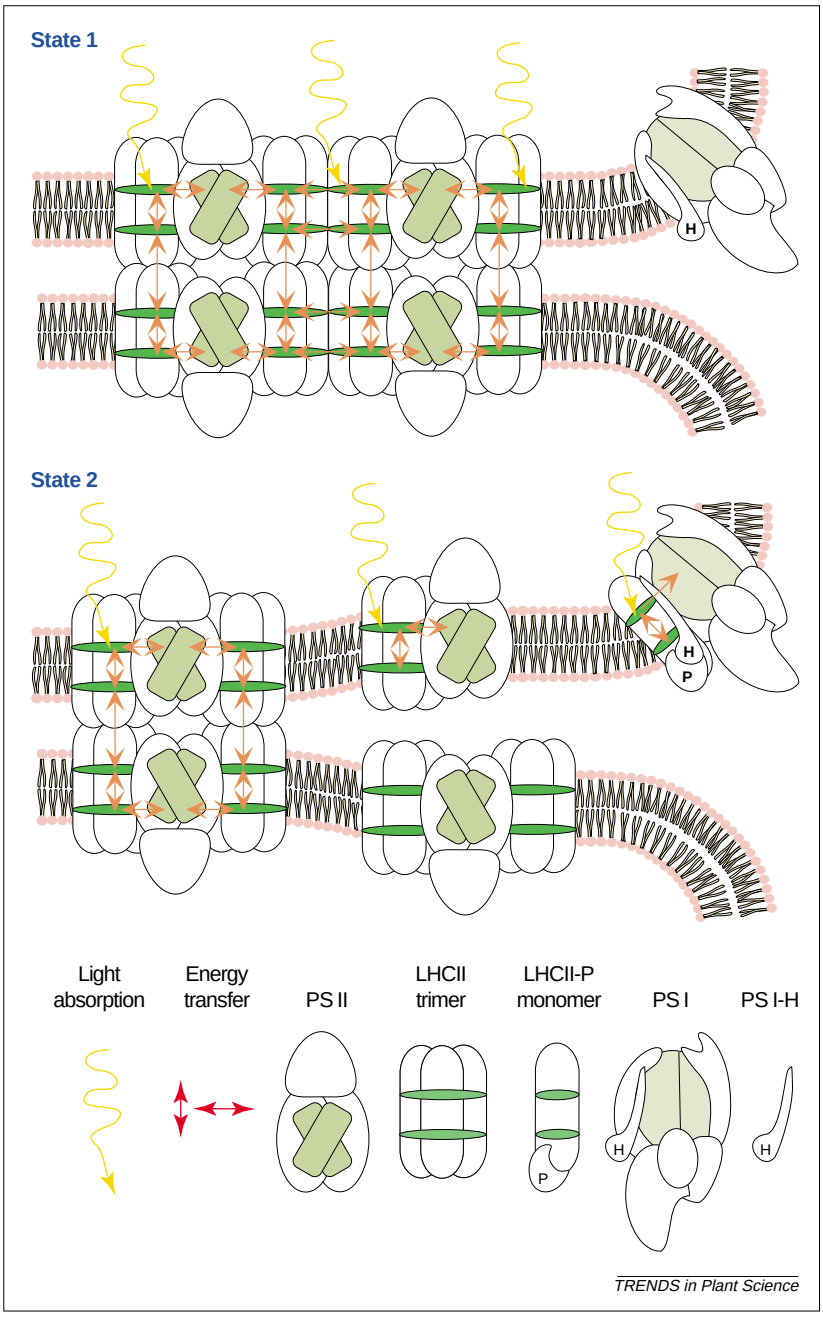

Fig. 5. Pathways of excitation energy transfer between light-harvesting complex II (LHCII), photosystem II and photosystem I. Light absorbed by a chlorophyll molecule in LHCII generates excitation energy that migrates between LHCII complexes and photosystem II. In state 2 (lower), some of this excitation energy migrates, instead, to photosystem I. In appressed membranes, LHCII forms oligomeric complexes that connect multiple photosystem II reaction centres for energy transfer, both laterally and transversely. During the transition from state 1 (upper) to state 2 (lower), phosphorylation-induced dissociation of LHCII units from each other causes partial disconnection of photosystem II reaction centres, transforming 'lakes' of photosystem II into more discrete 'puddles', where a smaller number of photosystem II units are connected for energy transfer. An extreme 'puddle' model is where each reaction centre carries it own, specific array or light-harvesting chlorophylls. Further phosphorylation of LHCII eventually results in separation of phospho-LHCII from photosystem II, giving transfer of absorbed excitation energy in state 2, from phospho-LHCII to photosystem I. Relative dimensions, shapes and oligomeric states of the complexes are depicted as described for Fig. 3. The green areas represent rings of chlorophyll molecules in LHCII. Excitation energy transfer between appressed membranes in state 1 might involve additional chlorophylls (not shown) buried within the membrane-extrinsic domains formed by aggregates of LHCII trimers.

salt bridges might also stabilize intermolecular contacts of phospho-LHCII, for example, with the psaH protein of photosystem I. The charge on the phosphate group can also be neutralized by protonation and should be expected to make a negligible contribution to the net charge of LHCII (Ref. 44). Even in closely appressed thylakoids at the centre of grana stacks, the two membrane surfaces are no more than 40 Å apart to allow for steric contact of LHCII surfaces[28]: isolated, fixed negative charges exert no repulsive force between membrane surfaces at this distance. Even the unstacking effect of Mg^{2+}-depletion probably arises primarily because of perturbation of salt bridges needed to stabilize the interactions between opposing LHCII complexes. LHCII is soluble in detergent solution, but only in the absence of Mg^{2+} ions. It aggregates when Mg^{2+} is present at millimolar concentrations[10].

Light harvesting complex II phosphorylation and cooperativity of photosystem II centres

In state 1, the extended excitation energy transfer pathway between photosystem II units is consistent with an observed sigmoidicity in fluorescence induction kinetics[45]. The transition to state 2 involves not only an increase in absorption cross-section of photosystem I and a decrease in that of photosystem II: it also produces a decrease in the cooperativity of photosystem II units. This decreased cooperativity causes a decrease in sigmoidicity by increasing the contribution of a first-order, single-exponential rise to the kinetics of fluorescence induction. This change in cooperativity of photosynthetic units takes place independently of complementary changes in absorption cross-section, and occurs in a related regulatory process in the single-photosystem purple bacteria[46].

It is important to know whether the decreased sigmoidicity of the fluorescence induction curve in state 2 is present or absent in the H-minus *Arabidopsis* mutants of Lunde *et al.*[6]. If it is absent, then decreased cooperativity of photosystem II units upon transition to state 2 remains even when there is no photosystem I anchor for phospho-LHCII. In this case, a feasible interpretation would be the creation, upon LHCII phosphorylation, of a steric obstacle to LHCII remaining in stacked membranes. Extension of the surface-exposed domain of LHCII so that it protrudes more than 20 Å beyond the thylakoid surface would exclude phospho-LHCII from appressed thylakoid domains. Such a phosphorylation-induced LHCII extension might decrease photosystem II cooperativity (Fig. 5), but would not necessarily induce a full transition to state 2 because there is no obstacle to phospho-LHCII taking a bound photosystem II with it into the grana margin. What is also required for a complete lateral re-association of phospho-LHCII is a decrease in its affinity for photosystem II relative to its affinity for photosystem I. The results of Lunde *et al.*[6] suggest that phosphorylated LHCII does not have to leave photosystem II centres. This suggestion was previously made on the basis of LHCII phosphorylation experiments carried out *in vitro* at

low temperatures, where decreased membrane fluidity limits lateral diffusion[47].

Decreased affinity of phospho-LHCII for itself and for appressed domains may be insufficient to cause phospho-LHCII to leave photosystem II. It is therefore possible that in the state 2 transition, phospho-LHCII detaches from photosystem II only when an intact photosystem I is available as a preferred binding site. Such direct exchange of LHCII is consistent with the domain organization envisaged by Per-Åke Albertsson and co-workers[25], and would eliminate the need for long-range diffusion of free LHCII monomers or trimers. Direct exchange is also possible without long-range migration between the photosystems in the thylakoid membrane because photosystems I and II are both present in the grana margin[20,24]. Steric considerations alone (Fig. 3) do not prohibit photosystem II, with or without a peripheral LHCII attached, from entering photosystem I-enriched, unappressed thylakoid domains[10,48]. The state 2 transition involves transfer of phospho-LHCII from photosystem II to photosystem I only as a transient process that occurs in grana margins. The return transfer of LHCII should also be expected to occur in grana margins. Once phospho-LHCII attached to photosystem I in unappressed membranes becomes dephosphorylated by a phosphoprotein phosphatase, the availability of a vacant and adjacent photosystem II will influence the rate of transition to state 1.

Novel phosphorylation sites

The original evidence for threonine phosphorylation at a site near the N-terminus of LHCII (Ref. 49) is supported by recent data from mass spectrometry[50,51]. Other phosphoproteins such as the core subunits of photosystem II and psbH can also now be precisely identified by the same technique[50,51]. The psbH protein, first discovered as a substrate for chloroplast protein kinase activity[52] can be phosphorylated at multiple sites[50,53]. The homology of psbH with part of LHCII (Refs 54,55) and its location in photosystem II, suggest that psbH might also play some part in the regulation of light-harvesting. LHCII can be phosphorylated at several sites[56] as well as on threonine. Tyrosine phosphorylation of LHCII occurs[57], and inhibitor and kinetic studies implicate tyrosine phosphorylation in the state 2 transition[58].

It is possible to imagine that the two types of LHCII phosphorylation (on threonine and tyrosine) have different roles in the state 2 transitions. One type of phosphorylation might serve to create a steric obstacle to LHCII remaining in tightly appressed membranes, whereas another might create the recognition motif required for detachment of LHCII from photosystem II and its reattachment with photosystem I. Once these phosphorylation sites are identified, site-directed mutagenesis should permit the resolution of the amino acids involved in these separate, functional effects.

Acknowledgements
We thank Per-Åke Albertsson and Hans-Erik Åkerlund for discussion and comments; and Andrew Staehelin for kindly providing the electron micrographs in Fig. 1. We also acknowledge the Swedish Natural Sciences Research Council's research grants to J.F.A. and studentship to J.F.

Reaction centre transcription and photosystem stoichiometry

All chloroplast thylakoid membranes are chimerical with respect to the location of the genes encoding their proteins[59]. Although state transitions (Fig. 2) serve to balance the two photosystems by post-translational modification of pre-existing proteins, it has been shown recently that the signal of redox imbalance that initiates state transitions – altered plastoquinone redox state – also governs transcription of reaction centre genes[60]. Transcriptional redox regulation of the initial steps in the biogenesis of photosystem I and II occurs rapidly, even before the redistribution of LHCII in state transitions is complete[61]. Rapid and direct redox control of reaction centre gene expression has been proposed as an over-riding functional requirement that accounts for the universal location of these genes in the chloroplast genome[62]. If correct, this hypothesis underlines the importance that regulation of photosystem stoichiometry[19] has had throughout evolution of oxygenic photosynthesis.

Conclusion and prospects

With the dramatically improving resolution of the three-dimensional structures of photosystem I (Refs 35–37) and photosystem II (Refs 29,30), it should soon be possible to see how each form of LHCII interacts with its preferred photosystem. For LHCII itself, a partial structure has been described for the unphosphorylated form[27,28]. Unfortunately, available structures for LHCII do not include information about the N-terminal threonine phosphorylation site itself, and little is known about the location of the phosphotyrosine. Nevertheless, there are several clear indications that threonine phosphorylation affects the 3-D structure of LHCII by means of a defined change in secondary structure – helix formation – in and around the phosphorylation site[41]. Clearly, we now need complete 3-D structures for both the phosphorylated and unphosphorylated forms of LHCII.

At atomic resolution, structural studies on the reaction centre[63] and the light-harvesting antenna complexes[64] of anoxygenic, purple photosynthetic bacteria[65] have blazed a trail along which oxygenic chloroplasts and cyanobacteria are beginning to follow[36]. Perhaps a topographical map of native thylakoid membranes at subnanometer resolution – and in states 1 and 2 – can come from atomic force microscopy[66], whereas high-resolution light and fluorescence microscopy[67] might also reveal subtle structural changes in thylakoids. In cyanobacteria, fluorescence recovery after photobleaching (FRAP) provides strong and direct support for the assumption that a peripheral light-harvesting complex truly migrates between photosystems I and II (Ref. 68). State transitions are fundamentally similar in chloroplasts and in cyanobacteria[8], in spite of their differences in membrane architecture, and there are

good reasons to think that molecular details of thylakoid structure, function and dynamics have been conserved throughout the evolution of photosynthesis.

A complete structural description of the intriguing ability of photosynthesis to balance the two photosystems is a long-term goal, but we can now be sure that it is, in principle, possible. State transitions do not result from action-at-a-distance, but from steric and allosteric control of creation and destruction of recognition motifs in proteins. Regulatory control requires specificity. Biological specificity resides in protein 3-D structure, and not in the indiscriminate physical properties of membrane surfaces. Guided molecular recognition controls light-harvesting function in photosynthesis by governing the structural interactions of the major protein complexes intrinsic to thylakoid membranes.

References

1 Hill, R. and Bendall, F. (1960) Function of the two cytochrome components in chloroplasts, a working hypothesis. *Nature* 186, 136–137

2 Duysens, L.N.M. and Amesz, J. (1962) Function and identification of two photochemical systems in photosynthesis. *Biochim. Biophys. Acta.* 64, 243–260

3 Allen, J.F. (1983) Protein phosphorylation – carburettor of photosynthesis? *Trends Biochem. Sci.* 8, 369–373

4 Bennett, J. (1983) Regulation of photosynthesis by reversible phosphorylation of the light-harvesting chlorophyll *a/b* protein. *Biochem. J.* 212, 1–13

5 Allen, J.F. *et al.* (1981) Chloroplast protein phosphorylation couples plastoquinone redox state to distribution of excitation energy between photosystems. *Nature* 291, 25–29

6 Lunde, C. *et al.* (2000) The PSI-H subunit of photosystem I is essential for state transitions in plant photosynthesis. *Nature* 408, 613–615

7 Allen, J.F. (1990) How does protein phosphorylation control protein–protein interactions in the photosynthetic membrane? In *Current Research in Photosynthesis* (Vol. 2) (Baltscheffsky, M., ed.), pp. 915–918, Kluwer Academic Publishers

8 Allen, J.F. (1992) Protein phosphorylation in regulation of photosynthesis. *Biochim. Biophys. Acta.* 1098, 275–335

9 Allen, J.F. (1992) How does protein phosphorylation regulate photosynthesis? *Trends Biochem. Sci.* 17, 12–17

10 Staehelin, L.A. and Arntzen, C.J. (1979) Effects of ions and gravity forces on the supramolecular organization and excitation energy distribution in chloroplast membranes. In *Chlorophyll Organization and Energy Transfer in Photosynthesis [Ciba Foundation Symposium 61 (new series)]*, pp. 147–175, Elsevier

11 Barber, J. (1982) Influence of surface charges on thylakoid structure and function. *Annu. Rev. Plant Physiol.* 33, 261–295

12 Barber, J. (1983) Membrane conformational changes due to phosphorylation and the control of energy transfer in photosynthesis. *Photobiochem. Photobiophys.* 5, 181–190

13 Arntzen, C.J. and Ditto, C.L. (1976) Effects of cations upon chloroplast membrane subunit interactions and excitation energy distribution. *Biochim. Biophys. Acta.* 449, 259–274

14 Arntzen, C.J. (1978) Dynamic structural features of chloroplast lamellae. In *Current Topics in Bioenergetics* (Vol. 8) (Vernon, L. and Sanadi, R., eds), pp. 111–160, Academic Press

15 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

16 Burke, J.J. *et al.* (1978) Involvement of the light-harvesting complex in cation regulation of excitation energy distribution in chloroplasts. *Arch. Biochem. Biophys.* 187, 252–263

17 Barber, J. (1979) Energy transfer and its dependence on membrane properties. In *Chlorophyll Organization and Energy Transfer in Photosynthesis [Ciba Foundation Symposium 61 (new series)]*, pp. 283–304, Elsevier

18 Park, R.B. and Sane, P.V. (1971) Distribution of function and structure in chloroplast lamellae. *Annu. Rev. Plant Physiol.* 22, 395–430

19 Anderson, J.M. (1986) Photoregulation of the composition, function and structure of thylakoid membranes. *Annu. Rev. Plant. Physiol.* 37, 93–136

20 Albertsson, P-Å. (1995) The structure and function of the chloroplast photosynthetic membrane – a model for the domain organization. *Photosynth. Res.* 46, 141–149

21 Anderson, J.M. (1981) Consequences of spatial separation of photosystem 1 and 2 in thylakoid membranes of higher plant chloroplasts. *FEBS Lett.* 124, 1–10

22 Anderson, J.M. (1982) Distribution of the cytochromes of spinach chloroplasts between the appressed membranes of grana stacks and stroma-exposed thylakoid regions. *FEBS Lett.* 138, 62–66

23 Åkerlund, H-E. *et al.* (1976) Isolation of photosystem II enriched membrane vesicles from spinach chloroplasts by phase partition. *Biochim. Biophys Acta.* 449, 525–535

24 Anderson, J. M. (1988) The grana margins of plant thylakoid membranes. *Physiol. Plant.* 76, 243–248

25 Albertsson, P-Å. *et al.* (1990) The domain organization of the plant thylakoid membrane. *FEBS Lett.* 273, 36–40

26 Arvidsson, P-O. and Sundby, C. (1999) A model for the topology of the chloroplast thylakoid membrane. *Aust. J. Plant Physiol.* 26, 687–694

27 Kühlbrandt, W. *et al.* (1994) Atomic model of plant light-harvesting complex by electron crystallography. *Nature* 367, 614–621

28 Kühlbrandt, W. (1994) Structure and function of the plant light-harvesting complex, LHC-II. *Curr. Opin. Struct. Biol.* 4, 519–528

29 Barber, J. and Kühlbrandt, W. (1999) Photosystem II. *Curr. Opin. Struct. Biol.* 9, 469–475

30 Zouni, A. *et al.* (2001) Crystal structure of photosystem II from *Synechococcus elongatus* at 3.8 Å resolution. *Nature* 409, 739–743

31 Rogl, H. and Kühlbrandt, W. (1999) Mutant trimers of light-harvesting complex II exhibit altered pigment content and spectroscopic features. *Biochemistry* 38, 16214–16222

32 Anderson, J.M. *et al.* (1973) Composition of the photosystems and chloroplast structure in extreme shade plants. *Biochim. Biophys. Acta* 325, 573–585

33 Melis, A. and Harvey, G.W. (1980) Regulation of photosystem stoichiometry, chlorophyll *a* and chlorophyll *b* content and relation to chloroplast ultrastructure. *Biochim. Biophys. Acta* 637, 138–145

34 Horton, P. *et al.* (2000) Allosteric regulation of the light-harvesting system of photosystem II. *Philos. Trans. R. Soc. London Ser. B* 355, 1361–1370

35 Krauß, N. *et al.* (1996) Photosystem I at 4 Å resolution represents the first structural model of a joint photosynthetic reaction centre and core antenna system. *Nat. Struct. Biol.* 3, 965–973

36 Jordan, P. *et al.* Three-dimensional structure of cyanobacterial photosystem I at 2.5 Å resolution. *Nature* (in press)

37 Schubert, W.D. *et al.* (1997) Photosystem I of *Synechococcus elongatus* at 4 Å resolution: comprehensive structure analysis. *J. Mol. Biol.* 272, 741–769

38 Hinshaw, J.E. and Miller, K.E. (1989) A novel method for the visualization of outer surfaces from stacked regions of thylakoid membranes. In *Techniques and New Developments in Photosynthesis Research* (Barber, J. and Malkin, R., eds), pp. 111–114, Plenum Press

39 Kyle, D.J. *et al.* (1983) Lateral mobility of the light-harvesting complex in chloroplast membranes controls excitation energy distribution in higher plants. *Arch. Biochem. Biophys.* 222, 527–541

40 Andersson, B. *et al.* (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

41 Nilsson, A. *et al.* (1997) Phosphorylation controls the three-dimensional structure of plant light harvesting complex II. *J. Biol. Chem.* 272, 18350–18357

42 Williams, R.S. *et al.* (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

43 Harrison, M.A. and Allen, J.F. (1992) Protein phosphorylation and Mg^{2+} influence light harvesting and electron transport in chloroplast thylakoid membrane material containing only the chlorophyll-*a*/*b*-binding light-harvesting complex of photosystem II and photosystem I. *Eur. J. Biochem.* 204, 1107–1114

44 Staehelin, L.A. and Arntzen, C.J. (1983) Regulation of chloroplast membrane function: protein phosphorylation changes the spatial organization of membrane components. *J. Cell Biol.* 97, 1327–1337

45 Kyle, D.J. *et al.* (1982) Thylakoid membrane protein phosphorylation leads to a decrease in connectivity between photosystem II reaction centers. *Biochim. Biophys. Acta* 680, 336–342

46 Holmes, N.G. and Allen, J.F. (1988) Protein phosphorylation in chromatophores from

Rhodospirillum rubrum. *Biochim. Biophys. Acta* 935, 72–78
47 Bingsmark, S. *et al.* (1990) Phosphorylation of LHC II at low temperatures. In *Current Research in Photosynthesis* (Vol. 2) (Baltscheffsky, M., ed.), pp. 799–802, Kluwer Academic Publishers
48 Mamedov, F. *et al.* (2000) Photosystem II in different parts of the thylakoid membrane: a functional comparison between different domains. *Biochemistry* 39, 10478–10486
49 Michel, H. *et al.* (1988) Tandem mass spectrometry reveals that three photosystem II proteins of spinach chloroplasts contain *N*-acetyl-*O*-phosphothreonine at their NH_2 termini. *J. Biol. Chem.* 263, 1123–1130
50 Vener, A.V. *et al.* (2001) Mass spectrometric resolution of reversible protein phosphorylation in photosynthetic membranes of *Arabidopsis thaliana*. *J. Biol. Chem.* 276, 6959–6966
51 Whitelegge, J.P. *et al.* (1998) Imaging the native state of thylakoid proteins by electrospray-ionization mass spectrometry. In *Photosynthesis: Mechanisms and Effects* (Vol. 5) (Garab, G., ed.), pp. 4381–4384, Kluwer Academic Publishers
52 Bennett, J. (1977) Phosphorylation of chloroplast membrane polypeptides. *Nature* 269, 344–346
53 Gómez, S.M. *et al.* (1998) Isolation and characterization of a novel xanthophyll-rich pigment-protein complex from spinach. In *Photosynthesis: Mechanisms and Effects* (Vol. 1) (Garab, G., ed.), pp. 353–356, Kluwer Academic Publishers
54 Hird, S.M. *et al.* (1986) The gene for the 10 kDa phosphoprotein of photosystem II is located in chloroplast DNA. *FEBS Lett.* 209, 181–186
55 Allen, J.F. and Findlay, J.B. (1986) Amino acid composition of the 9 kDa phosphoprotein of pea thylakoids. *Biochem. Biophys. Res. Commun.* 138, 146–152
56 Dilly-Hartwig, H. *et al.* (1998) Truncated recombinant light harvesting complex II proteins are substrates for a protein kinase associated with photosystem II core complexes. *FEBS Lett.* 435, 101–104
57 Tullberg, A. *et al.* (1998) A protein tyrosine kinase of chloroplast thylakoid membranes phosphorylates light harvesting complex II proteins. *Biochem. Biophys. Res. Commun.* 250, 617–622
58 Forsberg, J. and Allen, J.F. Protein tyrosine phosphorylation in the transition to light state 2 of chloroplast thylakoids. *Photosynth. Res.* (in press)
59 Race, H.L. *et al.* (1999) Why have organelles retained genomes? *Trends Genet.* 15, 364–370
60 Pfannschmidt, T. *et al.* (1999) Photosynthetic control of chloroplast gene expression. *Nature* 397, 625–628
61 Allen, J.F. and Pfannschmidt, T. (2000) Balancing the two photosystems: photosynthetic electron transfer governs transcription of reaction centre genes in chloroplasts. *Philos. Trans. R. Soc. London Ser. B* 355, 1351–1359
62 Allen, J.F. (1993) Control of gene expression by redox potential and the requirement for chloroplast and mitochondrial genomes. *J. Theor. Biol.* 165, 609–631
63 Deisenhofer, J. and Michel, H. (1989) Nobel lecture. The photosynthetic reaction centre from the purple bacterium *Rhodopseudomonas viridis*. *EMBO J.* 8, 2149–2170
64 Cogdell, R.J. *et al.* (1997) The structure and function of the LH2 (B800–850) complex from the purple photosynthetic bacterium *Rhodopseudomonas acidophila* strain 10050. *Prog. Biophys. Mol. Biol.* 68, 1–27
65 Papiz, M.Z. *et al.* (1996) A model for the photosynthetic apparatus of purple bacteria. *Trends Plant Sci.* 1, 198–206
66 Muller, D.J. *et al.* (2000) Atomic force microscopy of native purple membrane. *Biochim. Biophys. Acta* 1460, 27–38
67 Mehta, M. *et al.* (1999) Thylakoid membrane architecture. *Aust. J. Plant Physiol.* 26, 695–708
68 Mullineaux, C.W. *et al.* (1997) Mobility of photosynthetic complexes in thylakoid membranes. *Nature* 390, 421–424
69 Anderson, J. and Andersson, B. (1982) The architecture of thylakoid membranes: lateral and transverse organization. *Trends Biochem. Sci.* 7, 288–292
70 Buchanan, B.B. *et al.* (2000) *Biochemistry and Molecular Biology of Plants*, American Society of Plant Physiologists
71 Andersson, B. and Anderson, J.M. (1980) Lateral heterogeneity in the distribution of chlorophyll–protein complexes of the thylakoid membranes of spinach chloroplasts. *Biochim. Biophys. Acta* 593, 427–440
72 Junge, W. (1999) ATP synthase and other motor proteins. *Proc. Natl. Acad. Sci. U. S. A.* 96, 4735–4737
73 Zhang, Z. *et al.* (1998) Electron transfer by domain movement in cytochrome bc1. *Nature* 392, 677–684
74 Xia, D. *et al.* (1997) Crystal structure of the cytochrome bc1 complex from bovine heart mitochondria. *Science* 277, 60–66
75 Finazzi, G. *et al.* (1999) State transitions, cyclic and linear electron transport and photophosphorylation in *Chlamydomonas reinhardtii*. *Biochim. Biophys. Acta* 1413, 117–129

Plasmodesmata and plant cytoskeleton

Rachid Aaziz, Sylvie Dinant and Bernard L. Epel

Plant cell-to-cell communication is achieved by membranous conduits called plasmodesmata, which bridge the cytoplasm of neighboring cells. A growing body of immunolocalization data shows an association of the cytoskeleton machinery with plasmodesmata. The role of the cytoskeleton in the plasmodesmata-mediated transport has been well documented for virus movement. Because viruses are known to exploit existing host pathways and because the cytoskeleton is involved in intracellular trafficking, the cytoskeleton is thought to drive and target macromolecules to plasmodesmata. It is this link between plasmodesmata and the cytoskeleton that will be described here.

The plant cytoskeleton plays an important role in many biological processes, including cell division and expansion, organogenesis, tip growth and intracellular signaling[1,2]. The plant cytoskeleton is composed primarily of a network of microtubules and microfilaments (polymers of tubulin and actin, respectively) and diverse associated proteins. Whereas direct cell-to-cell communication is provided in animals and fungi by gap junctions and septal pores, respectively, within plants, cell-to-cell cytoplasmic trafficking takes place through plasmodesmata, wall spanning co-axial membranous organelles that bridge the cytoplasm of contiguous cells[3,4]. Plasmodesmata are considered to enable both physiological and developmental coordination of the plant[5]. Major insights in plasmodesmata functions have arisen from both viral movement studies and microinjection experiments[4], and by the use of transiently expressed green fluorescent protein (GFP) fusion proteins[6]. Functional studies have underlined that plasmodesmata are dynamic structures that

http://plants.trends.com 1360-1385/01/$ – see front matter PII: S1360-1385(01)01981-1