REVIEW

Regulation of Chloroplast Membrane Function: Protein Phosphorylation Changes the Spatial Organization of Membrane Components

L. ANDREW STAEHELIN and CHARLES J. ARNTZEN
Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, Colorado 80309; and Michigan State University/Department of Energy Plant Research Laboratory, Michigan State University, East Lansing, Michigan 48824

ABSTRACT A chlorophyll-protein complex of chloroplast membranes, which simultaneously serves as light-harvesting antenna and membrane adhesion factor, undergoes reversible, lateral diffusion between appressed and nonappressed membrane regions under the control of a protein kinase. The phosphorylation-dependent migration process regulates the amount of light energy that is delivered to the reaction centers of photosystems I and II (PS I and PS II), and thereby regulates their rate of turnover. This regulatory mechanism provides a rationale for the finding that the two photosystems are physically separated in chloroplast membranes (PS II in appressed, grana membranes, and PS I in nonappressed, stroma membranes). The feedback system involves the following steps: a membrane-bound kinase senses the rate of PS II vs. PS I turnover via the oxidation-reduction state of the plastoquinone pool, which shuttles electrons from PS II via cytochrome *f* to PS I. If activated, the kinase adds negative charge (phosphate) to a grana-localized pigment-protein complex. The change in its surface charge at a site critical for promoting membrane adhesion results in increased electrostatic repulsion between the membranes, unstacking, the lateral movement of the complex to adjacent stroma membranes, which differ in their functional composition. The general significance of this type of membrane regulatory mechanism is discussed.

In all photosynthetic organisms, chlorophyll is noncovalently bound to proteins (1-3). These pigment proteins are nearly always hydrophobic and exist as multiprotein structural complexes embedded in the lipid phase of the chromatophore membranes of photosynthetic bacteria or the internal (thylakoid) membranes of chloroplasts. Greater than 99% of the chlorophyll in the membrane serves simply as a light-harvesting antenna (Figs. 1 and 2); the absorption of a photon by one pigment is followed by energy transfer throughout this antenna by resonance energy coupling of the dipole oscillations of the chromophores (4). The binding of chlorphylls to protein ensures the efficiency of this energy transfer (*a*) by maintaining high local concentrations of pigment (regulating both the orientation and distance among pigments, which influence the probability of dipole coupling), and (*b*) by creating unique local chemical domains that determine the absorption properties of the pigment. These local domains increase the width of the absorption band relative to that of free pigments and create defined patterns of spectral species that ensure energy migration down a favorable energetic gradient to pigments having the longest wavelength absorption properties (5).

Less than 1% of the total chlorophyll of chloroplasts is bound in unique reaction-center proteins in close association with an electron acceptor. In chloroplasts there are two reaction centers that act in series as shown in Figs. 1 and 2 (6). Photosystem II (PS II)[1] has a reaction-center chlorophyll absorbing near 680 nm and a bound plastoquinone (PQ_A) as the primary stable electron acceptor (7). Photosystem I (PS I) has a reaction-center chlorophyll absorbing at 700 nm and an iron-sulfur center acting as primary stable electron acceptor (6). PS I and PS II can be isolated and purified; each contains

[1] *Abbreviations used in this paper*: chl *a/b* LHC, chlorophyll *a/b* light-harvesting complex; PQ, plastoquinone; PS I and II, photosystems I and II.

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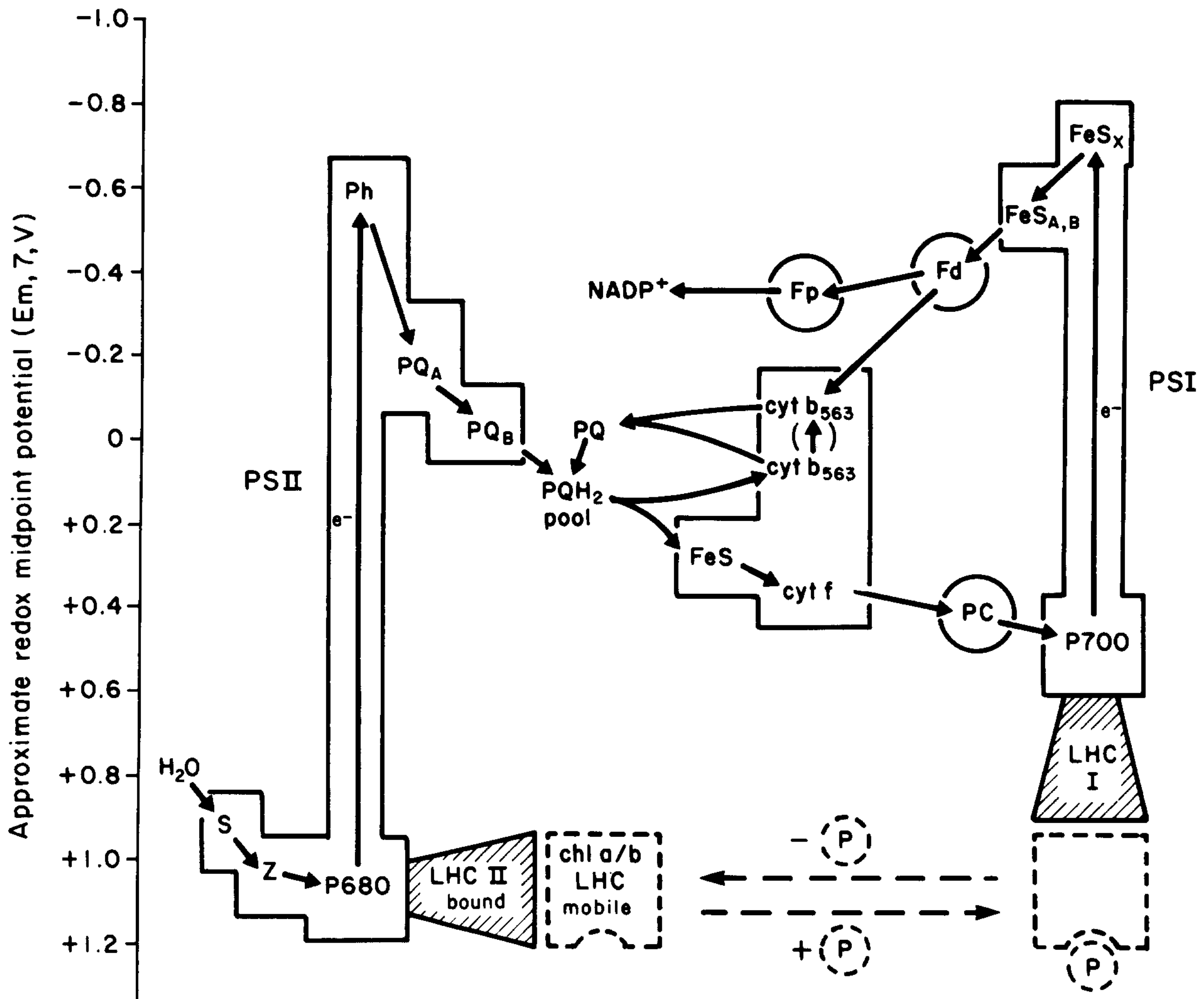


FIGURE 1 Pathway of electron transport in noncyclic and cyclic photosynthesis of green plants. The ordinate indicates the approximate midpoint potential of each redox carrier. (Note: dashed elements are NOT associated with this scale.) Boxes delineate molecules that are combined into integral membrane protein complexes; these relate directly to Fig. 2. Circles mark water soluble proteins. The Q-cycle, involving the oxidation of two molecules of plastohydroquinone (*PQH₂*) and subsequent reduction of one molecule of plastoquinone (*PQ*) by the components of the cytochrome *f/b*₆ complex, increases the ratio of protons moved across the membrane per pair of electrons transported through the chain. The phosphorylation-dependent, reversible migration of the chl *a/b* LHC (*LHC*) between PS II and PS I is shown schematically. Abbreviations, see legend to Fig. 2.

8–12 polypeptides plus the associated antenna chlorophylls and enzymatic co-factors (3). This will be discussed in more detail below. The following section will summarize the data showing that PS I and PS II are structurally segregated along chloroplast membranes. This requires that an electron "shuttle" serve to move electrons from PS II to PS I. To a large extent the shuttle function seems to be mediated by a lipid soluble "pool" of PQ molecules that are reduced by PS II and oxidized by the cytochromes of the electron transport chain, which transfer electrons to PS I (8, 9). In addition, the cytochrome-plastocyanin complex may also serve the lateral transfer of electrons to PS I within the chloroplast membrane (10; see Fig. 2).

Spatial Organization of Chloroplast Membranes

The membranes of chloroplasts, known as thylakoids, form an interconnecting network of flattened vesicles. Their main constituents are pigment proteins and complexes of polypeptides participating in the energy coupling reactions of photosynthesis. In higher plants and green algae the majority of thylakoid membranes are typically organized into closely appressed or stacked membrane regions, the grana thylakoids, which are interconnected with single, unstacked membranes, the stroma thylakoids (Fig. 3). The inner surface of these membranes encloses a space that is continuous between grana and stroma thylakoids.

Morphological and biochemical studies have demonstrated that stacked and unstacked membrane regions have a distinctly different structural organization and composition. Fig. 4 illustrates the nonuniform, lateral distribution of intramembrane protein complexes in pea thylakoid membranes as revealed by freeze-fracture electron microscopy. The origin of the four fracture faces, labeled EFs, EFu, PFs, and PFu (11), is illustrated in Fig. 5. Because of membrane splitting, stacked and unstacked membrane regions both give rise to P- and E-fracture faces, which exhibit complementary surface features (12). Thus, to obtain information on the total particle composition of a given membrane area, the particles on both faces have to be summed. Although the spatial differentiation of thylakoid membranes between stacked and unstacked regions is most evident on the E-fracture faces, quantitative measure-

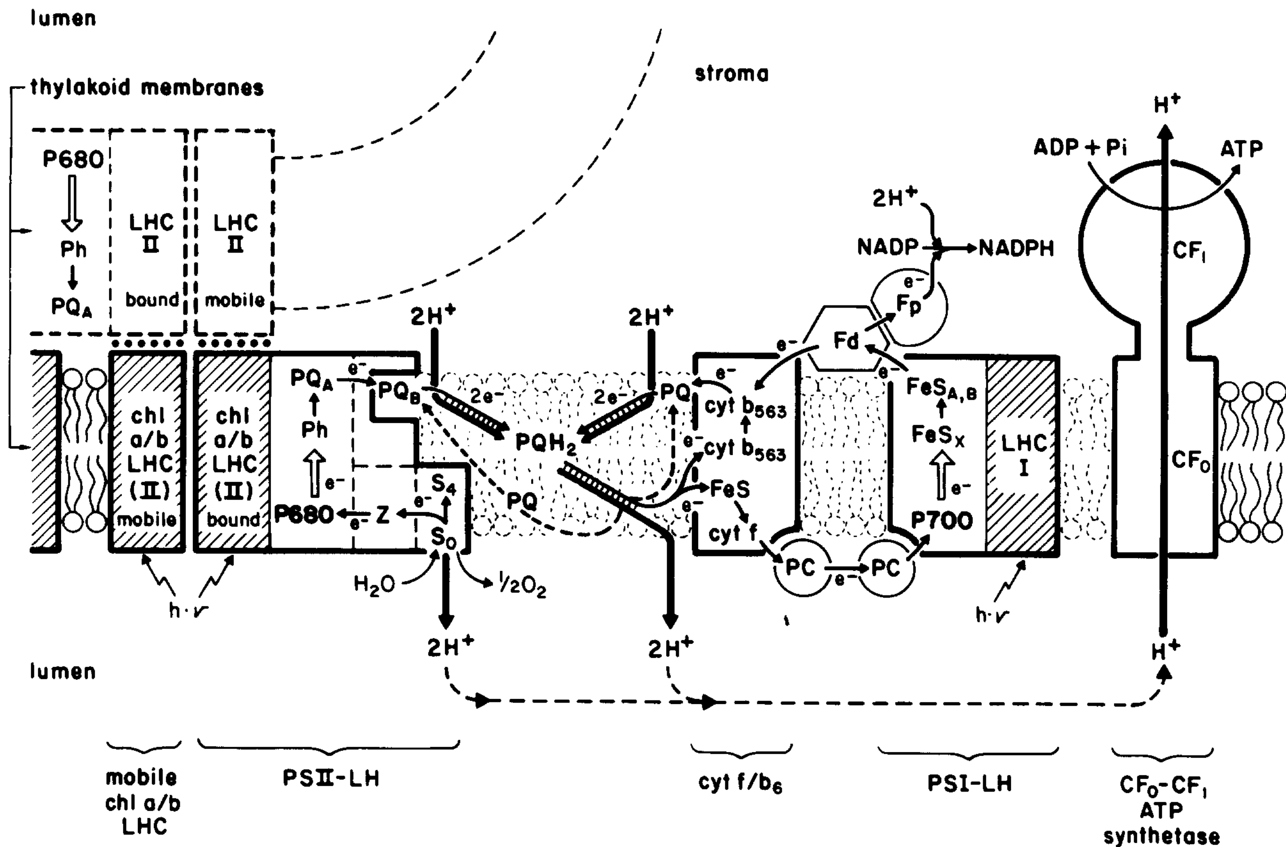
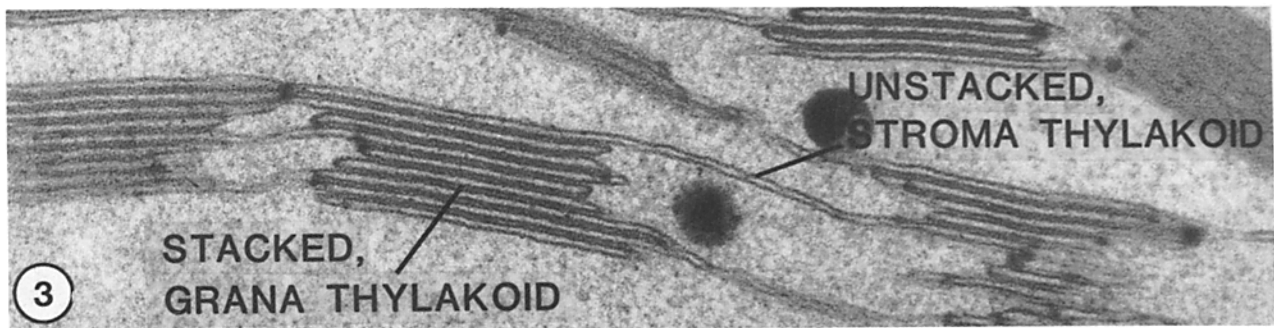
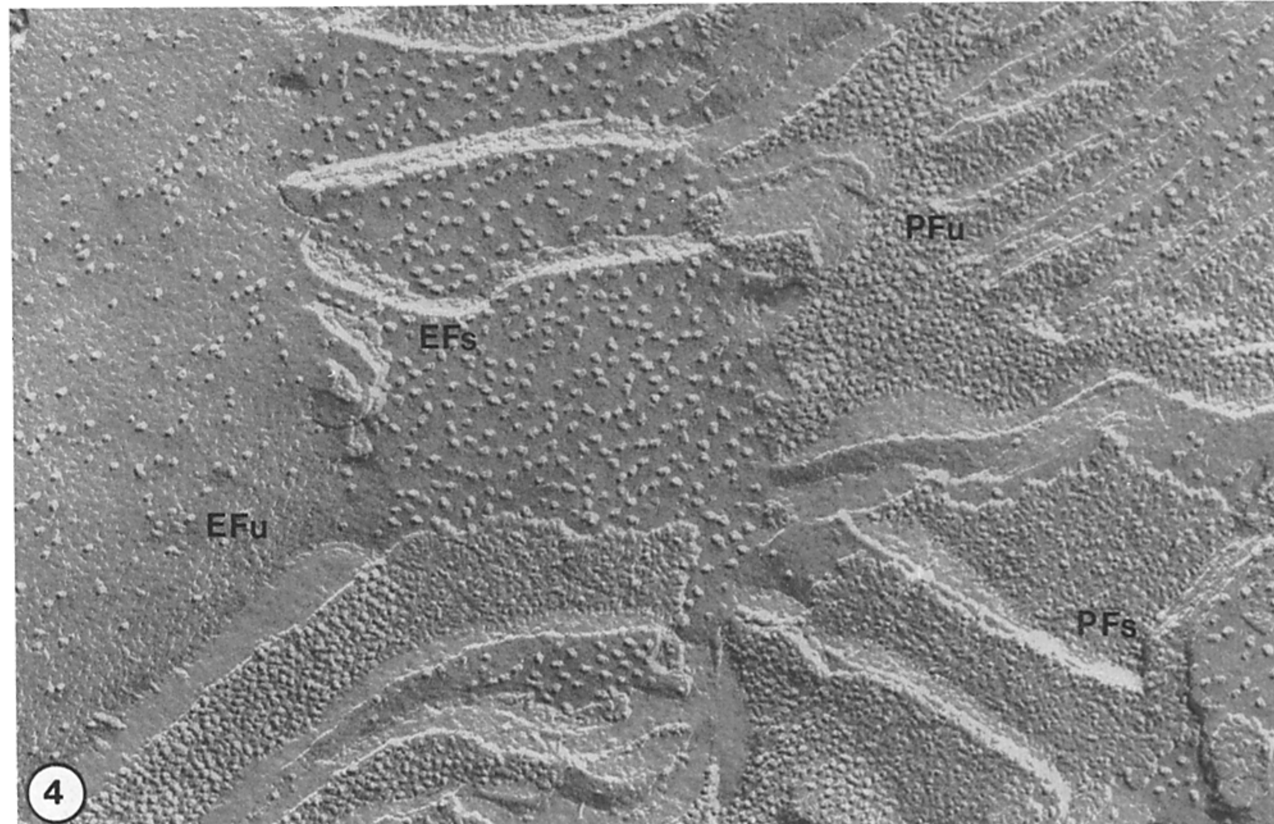


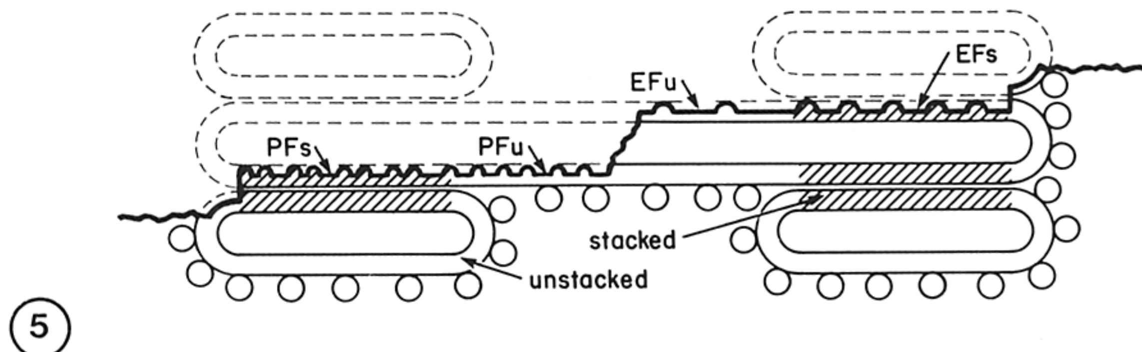
FIGURE 2 Organization of chloroplast membrane components participating in the electron coupling reactions of photosynthetic electron transport. Solid, thin arrows indicate electron transfer reactions; open arrows, chemical transitions; thick arrows, proton movements; dashed arrows, recycling routes for PQ. Three structurally distinct protein complexes participate in the linear electron transport pathway from water to NADP (6, 91): a PS II complex, which is linked by a lipid soluble pool of plastoquinone molecules to a cytochrome f/b_6 complex (7, 92), and a PS I complex, which receives electrons from the cytochrome complex via the water-soluble protein, plastocyanin (10). Also indicated is a quinone electron transport cycle, or Q cycle, which causes the translocation of one additional proton for every two electrons passing through the linear electron transport chain. Protons deposited within the thylakoid lumen exit through the proton-translocating CF_0-CF_1 ATP synthetase complex. Light-harvesting pigment-protein complexes (LHC) serve both photosystems; these preferentially associate with either PS I or PS II and are designated LHC I or LHC II, respectively. One population of the LHC II is mobile (30) and can serve either PS I or PS II by traveling laterally between the stroma lamellae (enriched in PS I centers) and grana stacks (enriched in PS II). Abbreviations: *chl a/b LHC*, chlorophyll *a/b* light-harvesting complex; *S* and *Z*, water-splitting and O_2-evolving enzymes that donate electrons to P680; *P680*, reaction center of PS II; *Ph*, bound pheophytin a (primary electron acceptor of PS II); PQ_A and PQ_B, special bound plastoquinone molecules (PQ_B is associated with the herbicide binding protein and can exchange with pool *PQ*); *PQ* and PQH_2, plastoquinone and reduced plastoquinone, FeS, Rieske iron sulfur protein; *cyt f* and *cyt* b_{563}, cytochromes *f* and b_{563} (b_6); *PC*, plastocyanin; *P700*, reaction center of photosystem I; FeS_x, special FeS center that serves as the primary acceptor of photosystem I; $FeS_{A,B}$, two special FeS centers; *Fd*, ferredoxin; *Fp*, flavoprotein (ferredoxin-NADP reductase); *NADP*, nicotinamide adenine dinucleotide phosphate; CF_0 and CF_1, basepiece and headpiece of ATP synthetase; *ADP* and *ATP*, adenosine di- and triphosphate; *h·ν*, light energy.

ments of particle sizes and densities reveal that each fracture face has a unique organization and composition. The EF particles have been shown to be structural equivalents of protein complexes composed of a PS II core surrounded by tightly bound light-harvesting complexes (bound chl *a/b* LHC; 13). (The PS II core complex is operationally defined as a protein complex that can be isolated from detergent-solubilized thylakoids and exhibits PS II reaction-center activity [3, 14]. The chl *a/b* LHC is a structural unit that can also be isolated and shown to consist of several polypeptides in the 24,000–29,000-mol wt size class and bound chlorophylls *a* + *b* in an *a/b* ratio of ~1.2 [15].) During greening of dark grown seedlings, the EFs particles increase in size in ~2.5-nm increments. This has led to the postulate that the different size classes of EFs particles (~10.5, ~13, and ~16 nm) arise from having an ~8-nm PS II core complex surrounded by various amounts of bound chl *a/b* LHC (13, 14). The analysis of PF particles is less advanced, but indirect evidence from studies of mutant thylakoid membranes and of purified membrane protein complexes suggest the following tentative relationship between functional membrane units and size classes of PF particles (reviewed in reference 16): PS I with bound light-harvesting antennae and 10–11-nm particles (17–19); cytochrome f/b_6 complexes and 8–9-nm particles (20); hydrophobic segment of the coupling factor and 9–10-nm particles (20, 21); mobile (i.e., not tightly bound to PS II centers) chl *a/b* LHC units and 8–9-nm particles (14, 22–24).

While we recognize that these structure/function assignments are still under experimental evaluation, we emphasize that they are fully consistent with the biochemical data concerning the distribution of functional membrane complexes between stacked grana and unstacked stroma membranes, as







FIGURES 3-5 Fig. 3: Thin section through a portion of a spinach chloroplast, showing interconnected stacked (grana) and unstacked (stroma) thylakoid membranes. × 100,000. Fig. 4: Freeze-fractured isolated pea thylakoids phosphorylated for 20 min in vitro before freezing. The membrane fracture faces EFs and PFs belong to stacked regions, the faces EFu and PFu to *unstacked* ones. Note the physical continuity of the stacked and unstacked membrane regions. × 90,000. Fig. 5: Diagram illustrating how the faces EFs, EFu, PFs, and PFu seen in Fig. 4 arise during the fracturing of thylakoid membranes.

shown in Table I. This table is based largely on membrane fractionation studies in which the grana and stroma regions of purified thylakoids were mechanically sheared apart in a pressure cell (Yeda Press) and then separated by means of polyethylene glycol-dextran phase partitioning methods (25). The details of this pattern of membrane subunit distribution

Table I
Spatial Distribution of Chloroplast Membrane Components

Component	Stacked (grana) membranes	Unstacked (stroma) membranes	Reference
	%	%	
PS II	85	15	8, 26
PS I	<15	>85	8
Cyt f/b_6	50 (?)	50 (?)	28, 29
Chl *a/b* LHC	More (70–90) (Phosphorylation dependent)	Less (10–30) (Phosphorylation dependent)	8, 30
ATP synthetase	0	100	27

have been recently reveiwed (9). Similar results have been obtained with detergent fractionation experiments that make use of the fact that stacked membrane regions are more resistant than unstacked regions to solubilization with detergents such as digitonin and Triton X-100 (2). An example of these observations is shown in Fig. 6; the SDS PAGE analysis of isolated grana and stroma lamellae fractions reveals a nonhomogeneous distribution of polypeptides. The assignment of function to individual polypeptides is now well advanced (reviewed in reference 3). Polypeptides of 65,000 mol wt, corresponding to the reaction center of PS I, and 56,000–58,000 mol wt, corresponding to the α and β subunits of the coupling factor, are strongly enriched in stroma lamellae. Two polypeptides of 43,000 and 48,000 mol wt, corresponding to the reaction-center components of PS II, and three in the 23,000–29,000-mol wt range, corresponding to the chl *a/b* LHC apoproteins, are largely localized in the grana (Fig. 6; compare lanes *1* and *4*).

Chloroplast membrane stacking can be experimentally manipulated by varying the concentration of cations in the medium used to suspend isolated thylakoids (11, 22, 31). Completely unstacked membranes are obtained by suspending the membranes in a low-cation solution such as 50 mM tricine-NaOH at neutral pH. This unstacking leads not only to the separation of the membranes, but also to intermixing of all thylakoid membrane components and thus to the loss of the lateral differentiation observed in control membranes. A return of the thylakoids to media containing >150 mM NaCl or >3 mM $MgCl_2$ produces both normal levels of stacked membranes and, given enough time (30 min), a complete resegregation of the different kinds of membrane particles into stacked and unstacked membrane regions as found in controls (11). Theoretical analyses (32–35) have shown that these and other salt effects on thylakoid membrane structure can best be modeled by assuming that grana thylakoid stacking is mediated by a combination of van der Waals attraction forces and electrostatic repulsive forces between the negatively charged membranes.

The biochemical basis for membrane stacking in green plant chloroplasts has been elucidated from reconstitution experiments using liposomes and purified chl *a/b* LHC material. When purified chl *a/b* LHC from Triton X-100-solubilized thylakoids (15) is reconstituted into artificial lipid bilayer membranes in a low-salt medium, ~8-nm particles appear in freeze-fracture images of such liposomes. The addition of >2 mM $MgCl_2$ or >60 mM NaCl to chl *a/b* LHC-containing liposomes leads to aggregation of the vesicles and to the clustering of the chl *a/b* LHC particles into the adhering membrane regions (24, 36, 37). Cyanobacteria and red algae do not contain chl *a/b* LHC and do not have stacked thylakoids; this is consistent with the role of the chl *a/b* LHC in mediating membrane appression.

Taken together, both microscopic and biochemical studies carried out during the last decade have provided convincing evidence that structural differentiation of thylakoid membranes into stacked and unstacked regions reflects an underlying functional differentiation of the membranes. The formation of stacked membrane regions, mediated largely by chl *a/b* LHC particles, seems to serve as the mechanism for spatially segregating PS II and PS I reaction-center units into separate membrane regions.

The "State" of the Chloroplast Pigment Bed

Because of their different chemical environments, the chlorophylls associated with the core complexes of PS I and PS II, although they are mostly chlorophyll *a*'s, have different absorption maxima. Thus, PS I is enriched in spectral forms absorbing at long wavelengths (680–710 nm; 17), whereas PS II possesses chlorophylls that absorb maximally at 670–680 nm (38, 39). Together, the chlorophylls associated with PS I and PS II comprise 40–50% of the total pigments of normal, fully developed chloroplasts; the remaining pigments are located in the chl *a/b* LHC. The red absorption maximum of chlorophyll *b* in this isolated complex is at 650 nm, whereas the chlorophyll *a* forms absorb primarily at wavelengths between 660 and 680 nm (36).

Action spectra have clearly established that the chlorophyll *b* of the chl *a/b* LHC primarily excites PS II (40). This finding can be explained by the fact that most PS II units and the bulk of the chl *a/b* LHC is localized in the grana thylakoids (Table I). On the basis of the pattern of pigment distribution

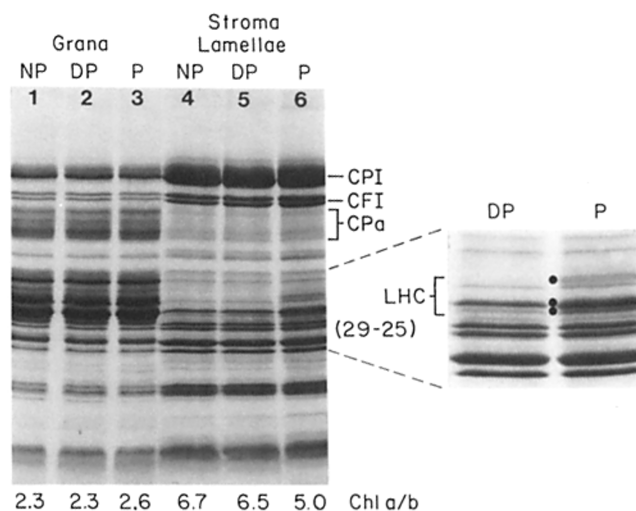


Figure 6 Migration of chl *a/b* LHC polypeptides from grana to stroma lamellae caused by phosphorylation of pea thylakoids as revealed by membrane fractionation and SDS PAGE techniques. Grana (lanes *1–3*) and stroma (lane *4–6*) lamellae fractions of control (nonphosphorylated, *NP*), 20 min light-phosphorylated in the presence of ATP (*P*), and 20-min dark-dephosphorylated (*DP*) thylakoid samples. The chlorophyll *a/b* ratios of the membrane samples before electrophoresis is shown at the bottom of each lane. The enlarged area shows the critical (20,000–30,000-mol-wt) regions of the gels of dephosphorylated and phosphorylated stroma lamellae samples. The dots mark the three LHC polypeptides, whose concentration increases in stroma membranes with phosphorylation of the chl *a/b* LHC. Molecular weight markers, × 10^3.

among PS I, PS II, and the chl *a*/*b* LHC, we can estimate that the combined pigment beds of chl *a*/*b* LHC and PS II in the grana will absorb ~70% of the initial quanta trapped by a given chloroplast.

In the context of noncyclic photosynthetic electron flow through PS II and PS I as envisaged by the well-known "two-light-reaction scheme" of electron transport (Fig. 1), the unequal trapping of light energy between PS II-rich grana and PS I-rich stroma membranes would appear to be wasteful. In particular, optimal efficiency of noncyclic electron flow demands that each reaction center undergo charge separations at the same rate to avoid over- or under-reduction of the connecting electron transport chain. This was first demonstrated by Emerson and Lewis (41) in their analysis of the quantum yield of photosynthesis. A severe "red drop" occurred at wavelengths >680 nm, where PS I preferentially absorbs. In the late 1960's it was recognized that plants possess a regulatory process that ensures equal turnover rates of the two reaction centers. In an elegant series of experiments, Bonaventura and Myers (42) and Murata (43) demonstrated that when algae were exposed sequentially to light of different spectral properties, their light-harvesting pigments underwent dynamic adaptations in response to the altered illumination, which in turn led to an improved balance in energy distribution between PS I and PS II. Similar changes have now been demonstrated in higher plants (44, 45). These are usually referred to as "state I–state II" transitions because they reflect an alteration in the state of the photosynthetic pigment bed in response to excess light trapping by PS I or PS II, respectively.

State I is defined as the condition that develops when a photosynthetic organism is exposed to light preferentially absorbed by PS I. Over a time scale of several minutes, an adaptation occurs that leads to more of the absorbed light energy being directed to PS II. This can be experimentally verified by measuring PS II activity at low light and observing increased quantum efficiency as well as an increased PS II fluorescence yield (34, 44–46). In contrast, state II results from a photosynthetic organism receiving excessive light preferentially absorbed by PS II; the adaptation response then involves distributing more light energy to PS I. It should be emphasized that these state changes are only important when light is limiting to the overall photosynthetic process. However, this is common under natural conditions, because plant growth patterns result in shading not only to surrounding species but also to lower leaves of individual plants. Supplemental illumination under field conditions has been shown to increase crop yields (47, 48), and seasonal variations in solar radiation have been found to correlate directly with crop productivity (49).

Two nonexclusive theoretical mechanisms have been proposed to explain light-harvesting state changes in algae. One suggests that the change occurs in the absorptive cross section of the photosystems (42); this is equivalent to an alteration in the numbers of antenna chlorophylls that are physically associated with the different reaction-center complexes. The other mechanism (43) favors a "spillover" of energy from the shorter-wavelength chlorophylls of PS II to the longer-wavelength-absorbing pigments of PS I; in essence, light initially absorbed in the PS II pigment bed would have a statistical probability of resonance energy transfer to the lower energetic sink in the pigment bed of PS I. This transfer could presumably be regulated by the distance between, and orientation among, the pigments of the two light-harvesting domains. Only recently has a biochemical mechanism been proposed that offers an explanation for how "state" changes may occur—these relate to covalent modification of the chl *a*/*b* LHC by protein phosphorylation.

Thylakoid Protein Phosphorylation

In a series of incisive experiments, Bennett (50–53) has demonstrated that a protein kinase and a protein phosphatase are bound to chloroplast membranes. These Mg-dependent enzymes control the phosphorylation/dephosphorylation of threonine residues exposed on the stromal surface of the chl *a*/*b* LHC. The kinase can be activated by light; an example of this effect is shown in Fig. 7. Chloroplast thylakoid polypeptides, when separated by SDS PAGE (stained for protein; lane *A*, Fig. 7) and analyzed by autoradiography (lanes *B* and *C*, Fig. 7), were found to incorporate ^{32}P into several membrane polypeptides. The preferred substrates for this ATP-dependent, uncoupler-insensitive reaction were the polypeptides of the chl *a*/*b* LHC (23,000–29,000 mol wt) (lane *B*, Fig. 7). If a sample was exposed to light, leading to protein phosphorylation, and then given a period of dark incubation, the chl *a*/*b* LHC was found to rapidly dephosphorylate (lane *C*, Fig. 7). It has subsequently been demonstrated (54, 55) that the key factor controlling the kinase activation is the oxidation/reduction state of the PQ, which acts as the intersystem electron carrier (Fig. 8). In the light, faster electron transfer by PS II than by PS I results in reduction of the PQ pool of electron carriers, which leads to kinase activation. The same activation can be induced in the dark by direct chemical reduction of the PQ pool (56). In vivo the degree of reduction of the PQ pool is not always controlled solely by the turnover rates of PS I and II. Some algae, such as *Chlamydomonas*, have respiratory enzymes associated with their chloroplasts (57), which can also control the redox state of the PQ pool.

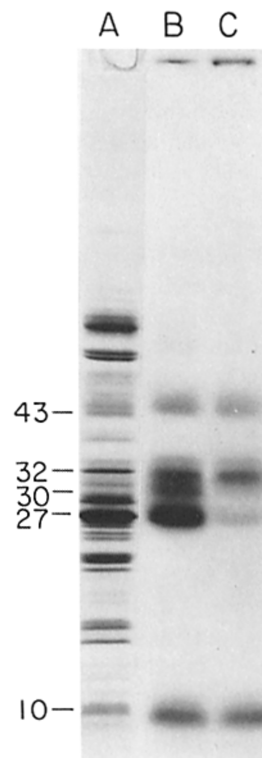


FIGURE 7 Phosphorylation of thylakoid polypeptides. Isolated washed pea thylakoids were illuminated in the presence of 200 μM [$\gamma^{32}P$]ATP. Thylakoid proteins were precipitated with acetone and fractionated by SDS PAGE. Lane *A*, Coomassie Blue-stained gel; lanes *B* and *C*, autoradiograms of light-phosphorylated (*B*) and dark-dephosphorylated (*C*) samples. The chl *a*/*b* LHC polypeptides (27,000–30,000 mol wt) show the heaviest labeling, which is also reversible (compare lanes *B* and *C*). The ^{32}P labeling of the other bands is both lighter and not as reversible under the conditions used for our experiments.

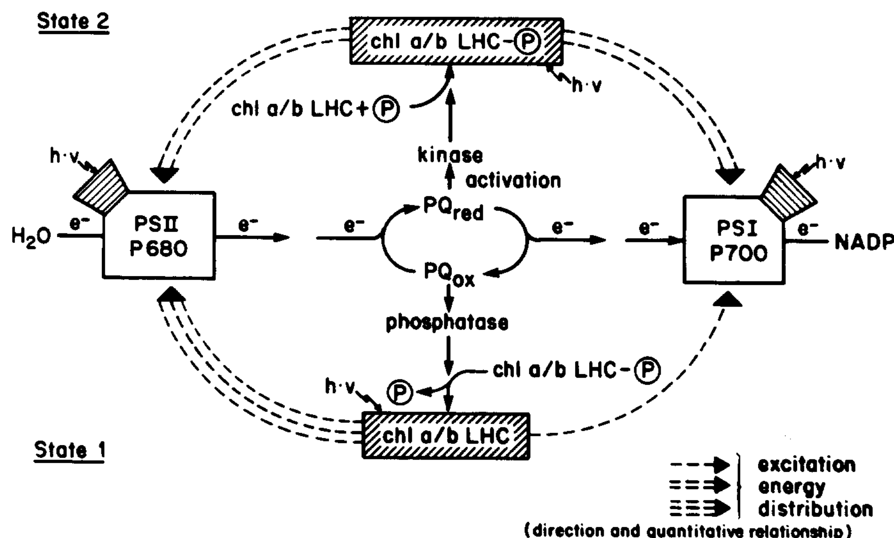


FIGURE 8 Schematic diagram of the feedback mechanism that controls the distribution of excitation energy between PS II and PS I, and thereby ensures maximal efficiency of photosynthesis. Striated boxes designate pigment-protein complexes involved in harvesting light energy (h · ν). Noncyclic electron transport from water to NADP is mediated by the reaction centers of PS II (P680) and PS I (P700). Reversible phosphorylation of the chlorophyll *a/b* light-harvesting complex (*chl a/b LHC*) is coupled to the redox state of plastoquinone (*PQ*), which is in a position to "sense" the turnover rates of the reaction centers P680 and P700. Reduction of plastoquinone by PS II leads to kinase activation and phosphorylation of the *chl a/b LHC*, and hence to increased excitation of *PS I*. Conversely, oxidation of platoquinone by *PS I* inactivates the kinase; the phosphatase then dephosphorylates the *chl a/b LHC* leading to more excitation of *PS II*. The structural basis for this regulation is diagrammed in Fig. 9.

The activity of this photosynthetic respiratory pathway explains a previous report (58) that light transitions do not strongly affect thylakoid protein phosphorylation in *Chlamydomonas* (F. Wollman, personal communication).

The fact that reversible thylakoid protein phosphorylation was largely directed at the chl *a/b* LHC suggested that this covalent modification could affect the light-harvesting properties of the membrane. Indeed, more of the absorbed quanta were found to be distributed to PS I in phosphorylated membranes (59). This work led to the hypothesis that protein phosphorylation of the chl *a/b* LHC was the underlying biochemical mechanism controlling state changes of the chloroplast pigment bed.

Several experimental systems have now been studied to test this hypothesis. First, mutant and developing chloroplasts were used to analyze protein phosphorylation and concomitant light-harvesting (state) changes. In chloroplasts deficient in the chl *a/b* LHC, there were no changes, which indicates that this complex is centrally involved (60). Further support has come from measurements of the quantum yield of the two reaction centers under state changes of the pigment bed that result in altered distribution of absorbed excitation energy to PS I and II (55, 61, 62). As predicted by the hypothesis, PS I quantum yields increased and PS II decreased in the phosphorylated membranes when measured at low light intensities.

Using the mathematical formulations for chlorophyll fluorescence inductions developed in the laboratory of Butler (63), it has been possible to analyze the physical mechanism by which protein phosphorylation leads to changes in the distribution of absorbed excitation energy in chloroplasts (64, 65). These studies demonstrated that both the absorptive cross section of PS I and the energy transfer from PS II to PS I increased in phosphorylated membranes. The significance of these changes with respect to alteration of structure of the chloroplast membranes is described below.

Membrane Structural Changes Associated with State 1–State 2 Transitions

The ability of chloroplast membranes to form stacked regions is governed largely by the counteractive nature of repulsive electrostatic forces and attractive van der Waals interactions (32, 33). Thus, incorporation of negatively charged phosphate groups into the membrane-bound chl *a/b* LHC particles by protein phosphorylation could be expected to influence the structural organization of such membranes. Alterations in the extent of membrane stacking that accompany state changes have been reported in the literature during the past decade (66–68), but none of these studies led to a clear understanding of the structural basis of the state 1–state 2 transition. With this in mind, we recently examined the effects of phosphorylation of the chl *a/b* LHC on the supramolecular organization and the extent of stacking of thylakoid membranes (30).

For these studies we examined three types of thylakoids: control, in vitro phosphorylated, and in vitro phosphorylated/dephosphorylated. Using freeze-fracture electron microscopy to probe thylakoid membrane structure, we found it nearly impossible to distinguish nonphosphorylated, phosphorylated, and dephosphorylated membranes based on the qualitative appearance of their four fracture faces. The phosphorylated thylakoids shown in Fig. 4 appear virtually identical to typical control (dark-treated, nonphosphorylated) chloroplast membranes. However, quantitative analysis of particle sizes, as well as direct measurements of appressed vs. nonappressed membranes in cross-fractured samples, revealed significant differences in their supramolecular organization. Thus, light-mediated phosphorylation of the chl *a/b* LHC led to a 23% decrease in the amount of stacked membranes in our preparations of pea thylakoids. Dark-induced dephosphorylation of the chl *a/b* LHC reversed the effect. Because this decrease in membrane stacking was accompanied by only minor changes in particle density, we concluded that the changes were brought about by a net transfer of intramembrane particles from stacked to unstacked membrane regions.

To determine which particles participated in the migration, we took advantage of the fact that each type of fracture face possesses a unique set of particles in terms of size distribution (11). Size measurements made on the different samples indicated that the lateral distribution of only one category of intramembrane particles (~8-nm PF particles) was affected by the reversible phosphorylation of the chl *a/b* LHC (30).

Phosphorylation led to the net transfer of ~8-nm PFs particles to PFu regions, whereas dephosphorylation produced a return of an equivalent number of ~8-nm PFu particles to PFs regions.

Biochemical Basis of Phosphorylation-induced Thylakoid Structural Changes

The biochemical identity of the mobile PF particles was established through the use of detergent fractionation of thylakoids into grana and stroma lamellae (30). Analysis by SDS PAGE (Fig. 6) showed that the stroma lamellae of phosphorylated membranes (lane *6*) contained increased levels of the 23,000–29,000-mol wt polypeptides of the chl *a/b* LHC relative to nonphosphorylated controls of dephosphorylated samples. These phosphorylation-induced changes are paralleled by a decrease in the chl *a/b* ratio of the stroma lamellae (from 6.7 to 5.0), and a concomitant increase in the chl *a/b* ratio in grana membranes (from 2.3 to 2.6; Fig. 6). A recent report (69), which showed that most of the phosphorylated chl *a/b* LHC is present in stroma thylakoids, is consistent with these findings. Taken together, these observations suggest that a subfraction of the chl *a/b* LHC moves from grana to stroma lamellae when the thylakoids become phosphorylated, but returns to the grana after protein dephosphorylation.

The combined structural and biochemical composition data are consistent with the hypothesis that the PF particles that move between grana and stroma lamellae in phosphorylated membranes are the morphological counterparts of a population of mobile chl *a/b* LHC; that is, protein-chromophore complexes that are not tightly bound to PS II reaction-center complexes. Therefore, we can estimate that 20–25% of the mobile chl *a/b* LHC units participate in the regulatory movements. These ideas are summarized in Fig. 9. This model also relates to two questions that can be developed from the conclusion that there is a mobile chl *a/b* LHC. First, why should the LHC move? Second, what will be the consequences of the lateral displacement?

Mechanistic and Functional Aspects of LHC Migration

The mechanism(s) regulating the LHC movement is probably related to surface charge density and surface configuration of the protein complexes involved. It is known that thylakoids have a net negative charge, and that cations decrease the surface potential, allowing two membranes to approach (32, 33). However, the membrane surface-exposed segment of the chl *a/b* LHC is a cationic peptide region rich in lysine/arginine residues (70). This segment is necessary to elicit normal grana stacking and may function via charge interactions with the adjacent membrane to stabilize regions of membrane contact (70–72). The surface-exposed chl *a/b* LHC polypeptide segment also contains two threonine residues. One or both are the site(s) of phosphate incorporation. Since each mobile chl *a/b* LHC particle contains probably six chl *a/b* LHC molecules (24), phosphorylation of the chl *a/b* LHC could add between 6 and 12 negatively charged phosphate groups to each particle. An increased negative charge has been detected in the vicinity of PS II after phosphorylation of chl *a/b* LHC (73). We propose therefore that it is the increase in negative surface charge and the resulting conformational changes of the mobile chl *a/b* LHC that cause destacking of the membranes and lateral migration of this complex from appressed to nonappressed regions (Fig. 9).

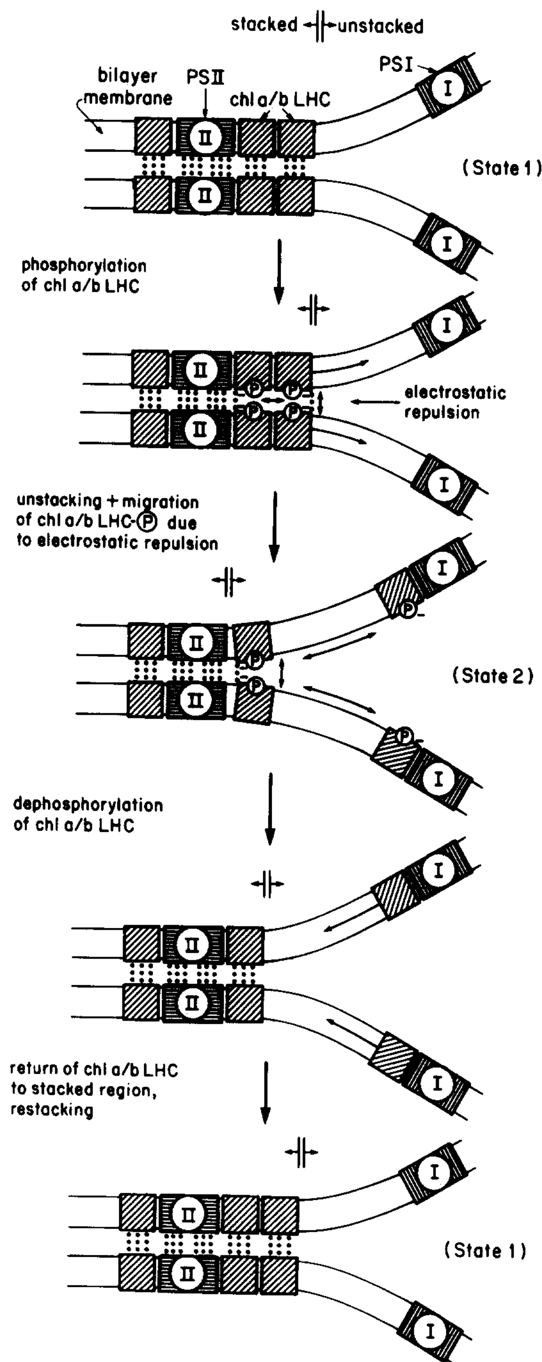


FIGURE 9 Schematic diagrams depicting how the reversible phosphorylation of the chl a/b LHC affects membrane stacking and the distribution of these particles between photosystem II (PS II)-rich stacked membranes and photosystem I (PS I)-rich unstacked membranes. The aggregation of the unphosphorylated chl a/b LHC particles in stacked regions is due to the fact that these particles serve not only as light harvesting antennae for photosystem II (PS II) but also as membrane adhesion factors. Upon phosphorylation, the added negative charge on the chl *a/b* LHC particles overbalances the attractive van der Waals interactions and thereby reduces the amount of membrane stacking. After release from the physical constraints of the stacked membrane regions, the phosphorylated chl *a/b* LHC particles can diffuse freely into the unstacked membrane regions where they harvest light energy for PS I units. The process is reversible.

The functional consequences of LHC migration to stroma lamellae can also be predicted. The transfer of light-harvesting chl proteins will result in the association of an increased

antennae pigment bed with PS I. A corresponding increase in α (the proportion of absorbed energy transferred to PS I; 63) has indeed been observed in phosphorylated membranes (64, 65). This observation also confirms the hypothesis of Bonaventura and Meyers (42) that state I–II changes alter the absorptive cross section of the two photosystems. As a second point, the removal of mobile LHC units from the grana, without a substantial increase in the density of the PS II centers remaining in the region of membrane appression, may explain the decreased connectivity between the PS II centers that has been measured in phosphorylated membranes (64, 65). The decrease in the probability of chl *a*/*b* LHC-mediated PS II to PS II energy transfer should lead to an increase in transfer from PS II to PS I, especially near the margin between appressed (PS II-enriched) and nonappressed (PS I-enriched) membranes. This explanation is consistent with the increased "spillover" of energy from PS II to I in phosphorylated membranes occurring during state changes that was hypothesized by Murata (43).

The concept that lateral migration of phosphorylated chl *a*/*b* LHC is essential for state changes of the pigment bed has recently been tested by changing the viscosity of thylakoid membranes (74). Incorporation of sufficient amounts of cholesterol hemisuccinate into thylakoids to drastically reduce lateral migration of membrane components did not affect the light activation of the protein kinase and phosphorylation of the chl *a*/*b* LHC, but it did prevent state I–state II transitions from occurring. These results, together with others discussed above, clearly demonstrate that although it is essential, protein phosphorylation alone is insufficient to bring about state changes. Lateral migration of the chl *a*/*b* LHC in the plane of the membrane is an equally important part of the mechanism responsible for regulating the amount of light energy that is delivered to PS I and PS II.

Why Do Chloroplasts Need Grana?

One of the persistently intriguing aspects of chloroplast membranes of higher plants and green algae has been their spatial differentiation into stacked (grana) and unstacked (stroma) regions. This feature clearly relates to the functional specialization of the membranes. Chloroplast membranes have one purpose: to trap radiant energy and produce stable high-energy chemical intermediates (ATP and $NADPH_2$). It is clear that grana are not required to carry out this energy conservation, since partially developed membranes having no chl *a*/*b* LHC or grana will catalyze photophosphorylation and electron transport (75). Analyses of membrane development and the appearance of grana lead to one conclusion: that the stacked membranes are correlated with increased efficiency of light trapping and utilization, particularly under limiting light conditions (75, 76). This idea is consistent with the observation that plants grown in shade or low light have more stacked membranes than those grown in high light (77). As pointed out above, the chl *a*/*b* LHC is not only the major light-harvesting system of higher plants and green algae, but is also the component that promotes adhesion between chloroplast membranes. Thus, when light conditions become the limiting factor in photosynthesis, the primary adaptation of plants is to increase proportionately the amount of chl *a*/*b* LHC in their thylakoids and concomitantly the proportion of stacked grana membranes (78).

Although these considerations can explain the relationship between light conditions and amounts of grana thylakoids, they do not answer the more basic question of why grana are needed in the first place. We propose the following explanation.

The chlorophylls associated with the chl *a*/*b* LHC absorb at shorter wavelengths than the reaction-center chlorophylls of PS II and PS I, thereby allowing the chl *a*/*b* LHC to transfer excitation energy very efficiently to both types of reaction centers by means of resonance energy transfer. However, because resonance energy transfer reactions are statistical events that depend on a number of parameters, such as absorption band overlap and distance between and orientation among the chlorophyll molecules, several other types of excitation energy transfers also occur in chloroplast membranes such as chl *a*/*b* LHC → chl *a*/*b* LHC and PS II → PS I transfers (40, 63). The question that therefore arises in this context is: what prevents all the energy trapped by the different antennae systems from ending up in PS I, since this reaction center has the most red-shifted chlorophyll absorbing species and is also more efficient in light trapping than PS II, as evidenced by the lower room temperature fluorescence (46)? Evolution appears to have provided a "simple" structural solution to this problem in green plants by segregating most of PS II and chl *a*/*b* LHC into grana and most of PS I into stroma membrane regions (Table I). The spatial segregation of the two types of reaction centers prevents excessive transfer of excitation energy from PS II to PS I. Nevertheless, this lateral segregation of reaction centers, each type competing for the available absorbed radiant energy, imposes certain limitations upon function. If the pigment bed was simply divided into grana vs. stroma lamellae, the light-absorption properties of the chloroplasts would be constant. Because environmental light conditions are not constant, and because cellular demands for PS I-mediated noncyclic and cyclic electron flow frequently change, such an invariant pigment bed would be a disadvantage. By developing a mobile antenna system that can be variably partitioned between PS II-rich grana and PS I-rich stroma membranes, the major problem of regulating energy distribution between the two photosystems has been overcome and the efficiency of photosynthesis in higher plants has been maximized.

Control of Excitation Energy Distribution in Thylakoids Lacking Stacked Regions

In light of the preceding discussion on the mechanism of regulation of excitation energy distribution between PS II and PS I, it seems somewhat ironic that the basic phenomenon of state I–state II transitions was first reported for the red alga *Porphyridium* (43), an alga that does not possess stacked thylakoids. Red algal thylakoids, like those of cyanobacteria, lack chl *b*-containing LHC; instead, they use light-harvesting antennae with different absorption characteristics known as phycobilisomes (79, 80). Phycobilisomes are large, water-soluble pigment-protein complexes that contain phycobilins as light-harvesting pigments. They are located on the stromal surface of thylakoids and appear to be both structurally and functionally linked to and positioned over the PS II complexes in the membrane (79–81). At present, little is known about the actual mechanism of state I–state II transitions in phycobilisome-containing thylakoids (82). However, in analogy to the green plant system, we propose that a similar, but smaller, charge-dependent lateral reorganization of membrane complexes could produce the same results. Since resonance energy

coupling decreases by the sixth power of the distance (83), an alteration in the spatial relationship between the central, allophycocyanin-containing subunits of phycobilisomes and the PS II and PS I complexes in the membrane by as little as 10 nm may be sufficient.

Phosphorylation-dependent Regulation of Other Membrane Activities

The studies described in this article have led to the discovery of a novel type of mechanism for regulating membrane activity in the fundamental process of photosynthesis, using a combination of chemical modification and structural reorganization of membrane components at the supramolecular level. Because phosphorylation of proteins is widely used by cells to regulate the activity of individual proteins and of metabolic pathways (reviewed in reference 84), it is probable that the type of regulatory mechanism outlined in this review will be found in other membrane systems as well. Similar mechanisms may operate in the control of cell-cell interactions in embryonic and mature tissues, in the regulation of cell surface receptors, and in the recycling of rhodopsin molecules. In the case of rhodopsin, it is well known that adsorption of light by a rhodopsin molecule leads to the isomerization of 11-*cis* retinal and eventually to the separation of the all-*trans* retinal from the opsin molecule (bleached rhodopsin). Less appreciated is the fact that the light-induced conformational changes in rhodopsin also make it available as a substrate for phosphorylation by a specific protein kinase and ATP. Each opsin molecule may carry as many as seven phosphate groups attached to serine and threonine residues (85). About 1 h is required for complete dephosphorylation both in vivo and in vitro (86). No specific function has yet been correlated with this phosphorylation of bleached rhodopsin. On the basis of the ideas developed in this review, it would seem possible that the phosphorylation of bleached rhodopsin may aid in the directed lateral migration of such molecules from the center to the periphery of rod outer segment disc membranes. This transfer to the disc periphery could aid in the regeneration of the opsin molecules, since "fresh" 11-*cis* retinal molecules are believed to be brought to the disc membranes through the cytoplasm (87–90). Thus, phosphorylation of the bleached rhodopsin molecules may speed up their recycling by electrophoresis of the highly negatively charged molecules to the disk periphery, and by keeping them there until they are refurbished with 11-*cis* retinal. In addition, this phosphorylation of opsin may prime the molecule for the refurbishing reactions.

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