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REVIEW ARTICLE

Regulation of photosynthesis by reversible phosphorylation of the light-harvesting chlorophyll *a/b* protein

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Photosynthetic CO_2 fixation in chloroplasts is driven by ATP and NADPH generated by electron and proton transport within the thylakoid membrane. The movement of an electron from water to $NADP^+$ along the electron transport chain requires two photons and involves two separate photosystems (PS I and PS II) operating in series. Each photosystem contains a reaction centre consisting of one or two chlorophyll *a* molecules capable of producing across the membrane a separation of reductant and oxidant that will initiate electron transport (Velthuys, 1980; Malkin, 1982). However, less than 1% of chlorophyll molecules possess reaction centre activity. The remainder have a less direct connection with electron transport. Their role is to absorb as much light as possible and channel the resulting excitation energy to the reaction centres. Without these light-harvesting chlorophyll molecules, the electron transport rate would be severely limited under any light intensity less than that of full sunlight.

Much of the current interest in the light-harvesting chlorophylls stems from the discovery that most if not all of the chlorophyll molecules of the membrane exist as pigment–protein complexes (Ogawa *et al.*, 1966; Thornber *et al.*, 1967, 1979; Thornber, 1975). The protein components are thought to give to the chlorophyll molecules the correct spectral qualities, orientation and spacing to enable them to perform their light-harvesting role quickly and efficiently. In green plants, the most abundant of the pigment–protein complexes is the light-harvesting chlorophyll *a/b* complex (LHC). It comprises about 50% of the total chlorophyll content of the photosynthetic membrane and about 30% of the total protein. The polypeptide component, the light-harvesting chlorophyll *a/b* protein (LHCP), is encoded in the nucleus, synthesized in the cytoplasm and transported into the chloroplast in precursor form. The accumulation of LHCP in the photosynthetic membrane is regulated by light at the transcriptional and post-translational levels and possibly also at the translational level, and light controls the phosphorylation of the protein. In this article, I review the structure, biosynthesis and function of LHC, giving special emphasis to the recent studies which show that reversible phosphorylation of LHC regulates the distribution of excitation energy from the complex to PS I and PS II in response to changes in the spectral quality of light.

Abbreviations used: LHC, light-harvesting chlorophyll *a/b* complex; LHCP, light-harvesting chlorophyll *a/b* protein; PQ, plastoquinone; P_r or P_{fr}, red- or far-red-absorbing forms of phytochrome; PS, photosystem; SDS, sodium dodecyl sulphate.

Structure of the LHC

Discovery

The LHC was discovered when the photosynthetic membranes (thylakoids) of green plants were subjected to SDS/polyacrylamide-gel electrophoresis without thermal denaturation (Ogawa *et al.*, 1966; Thornber *et al.*, 1967). The LHC was one of two chlorophyll–protein (CP) complexes visible in unstained gels. Initially known as CP II, LHC contained approximately equal amounts of chlorophyll *a* and chlorophyll *b* and had an apparent molecular weight of about 35000. The other complex (CP I) contained only chlorophyll *a* and had an apparent molecular weight of about 110000. Because PS I was known to be enriched in chlorophyll *a* and PS II in chlorophyll *b*, CP I and CP II were attributed to PS I and PS II, respectively. However, the discovery that the chlorina f2 mutant of barley contained neither chlorophyll *b* nor CP II and yet was photosynthetically competent (Thornber & Highkin, 1974) led to the idea that CP II functions in a purely light-harvesting role for PS II. Accordingly, Thornber (1975) proposed that the complex should be known as the light-harvesting chlorophyll *a/b* protein. This proposal has been almost universally accepted, although there is some confusion as to whether the name refers to the complex as a whole or just to the protein component. In this article I use the abbreviation LHC to denote the complex and LHCP to denote the protein component, whether bound to pigment or free of pigment.

Occurrence

The LHC is believed to occur in all eukaryotes containing chlorophyll *b*, that is, in all land plants and in the green algae and *Euglena*. In other algal groups, the function of the LHC as a light-harvesting complex for PS II is performed by other complexes such as the phycobiliproteins in red algae and cyanobacteria (Gantt, 1981) and the chlorophyll *a*/chlorophyll *c* complexes of brown algae (Anderson & Barrett, 1979). The LHC also occurs in the prokaryote *Prochloron*.

Purification of LHC

SDS/polyacrylamide-gel electrophoresis is rarely suited to the purification of the LHC. Yields are low and the preparations suffer from contamination by colourless proteins of the same electrophoretic mobility. In what is probably the best available method (Burke *et al.*, 1978), thylakoids are washed thoroughly in a medium of low ionic strength to convert the complex system of stacked and unstacked membranes into large, balloon-like vesicles which are then treated with the non-ionic detergent Triton X-100 to solubilize protein components with minimal disruption to pigment–protein interactions. The solubilized material is subjected to overnight sucrose density gradient centrifugation to separate the LHC from certain other thylakoid components (especially PS I). The addition of $MgCl_2$ to the most fluorescent gradient fractions induces specific precipitation of LHC by a mechanism that is not fully understood.

The fact that the LHC can only be defined operationally is unsatisfactory for several reasons. Firstly, there is no guarantee that different purification procedures will yield the same material in terms of polypeptide, pigment and lipid composition; this is especially worrying in the case of procedures which differ in the detergent employed. Secondly, there is no guarantee that different forms of the LHC will respond identically during the purification procedure. Thirdly, operational definitions are based on physical rather than functional criteria. An enzymologist, for example, would never think of defining an enzyme in terms of the physical properties that he exploits durings its purification [sedimentation coefficient, isoelectric point, solubility in $(NH_4)_2SO_4$, etc.] rather than in terms of its catalytic activity.

Composition

The LHC contains chlorophyll *a*, chlorophyll *b* and the two xanthophylls, neoxanthin and lutein (Thornber, 1975; Ryrie *et al.*, 1980; Lichtenthaler *et al.*, 1982). The molar proportions of these four components are, on average, approx. 7:6:1:2. Burke *et al.* (1978) estimate that each LHCP binds 13 chlorophylls, while Thornber *et al.* (1977) and Li & Hollingshead (1982) estimate that there are about 6 chlorophylls per LHCP.

The LHC is known to be heterogeneous with respect to polypeptide composition. Anderson & Levine (1974) were probably the first to provide evidence of multiple forms of LHCP and numerous groups have presented confirmation. The heterogeneity may be seen either at the level of chlorophyll–protein complexes (Delepelaire & Chua, 1979; Mullet *et al.*, 1981; Green & Camm, 1982) or at the level of fully denatured polypeptides (Apel *et al.*, 1975; Süss *et al.*, 1976; Machold *et al.*, 1977; Henriques & Park, 1977; Burke *et al.*, 1978; Machold & Meister, 1979; Delepelaire & Chua, 1979; Süss & Brecht, 1980; Ryrie *et al.*, 1980; Bellemare *et al.*, 1982; Andersson *et al.*, 1982*b*). A full assessment of the heterogeneity of the LHCP is beyond the scope of this article. However, it seems frequently to be the case that green plants contain two or three major species of LHCP that are structurally related as judged by amino acid analysis, partial proteolytic digestion, partial CNBr cleavage, or immunological cross-reactivity (Apel, 1977; Chua & Blomberg, 1979; Hoober *et al.*, 1980; Schmidt *et al.*, 1981; Bennett *et al.*, 1981). The LHCPs of most plant species have molecular weights in the range 23000–27000.

Other chlorophyll a/b complexes

Camm & Green (1980, 1981) and Green & Camm (1982) have shown that several higher plants and a green alga contain a chlorophyll *a*/chlorophyll *b* complex (CP29) that is distinct from the LHC. It is released from thylakoids by the detergent octylglucoside, it exhibits a higher chlorophyll *a*/chlorophyll *b* ratio than LHC and contains a single 29000 molecular weight polypeptide which gives a different proteolytic digestion pattern from those of the two major LHCPs. How closely related the LHC and CP29 may be is not yet clear.

Bellemare *et al.* (1982) have suggested that the peripheral light-harvesting complex of PS I may contain chlorophyll *b* in addition to the chlorophyll *a* reported by Mullet *et al.* (1980). In barley the three polypeptides of this complex have molecular weights of 20000, 21000 and 22000 compared with 24000, 25000 and 27000 for the three LHCPs.

LHC in the thylakoid membrane

Native LHC probably exists in the thylakoid membrane in the form of oligomers whose monomer units span the bilayer. The concept of LHC oligomers is derived from the sedimentation behaviour of LHC particles released from thylakoids by Triton X-100 (Burke *et al.*, 1978; Mullet & Arntzen, 1980) and from the electrophoretic behaviour of the LHC on recently introduced gel electrophoresis systems (Henriques & Park, 1978;

Anderson *et al.*, 1978; Markwell *et al.*, 1978; Machold *et al.*, 1979). Although the LHCP with its bound chlorophyll molecules is largely buried within the galactolipid bilayer, it protrudes sufficiently from the outer and inner surfaces of the thylakoid to be detectable immunologically in vesicles of normal orientation and in inverted vesicles (Andersson *et al.*, 1982*b*). LHCPs are not sensitive to tryptic digestion in inverted vesicles, but in normal thylakoids trypsin cleaves 10–20 amino acids from one end of the LHC polypeptides, reducing their apparent molecular weights by about 1000–2000. One of the released fragments has been sequenced: Ser-Ala-Thr-Thr-Lys-Lys (Mullet *et al.*, 1981). Almost all of the primary structure of the major LHCP of pea has been determined by sequencing a complementary DNA clone (Coruzzi *et al.*, 1983). It is clear from the primary structure that the above hexapeptide is located very close to the *N*-terminus of the protein. Thus, it is the *N*-terminus of the LHCP that is accessible to trypsin at the outer surface of the thylakoid.

Biosynthesis of the LHC

The biosynthesis of the LHC involves co-operation between nucleus, cytoplasm and chloroplast (Fig. 1). The protein components of the complex are encoded in nuclear DNA, synthesized on cytoplasmic ribosomes and taken up by chloroplasts in precursor form. In this they resemble the majority of chloroplast proteins (Ellis, 1981). The pigment components of the complex are synthesized entirely within the chloroplast. Many of the individual steps involved in the synthesis of the LHC have been reproduced *in vitro*.

LHCPs are encoded in the nucleus

The first evidence for nuclear encoding of these polypeptides was provided by Kung *et al.* (1972). They showed that structural differences in the major LHCP of different tobacco species are inherited in a Mendelian mode following reciprocal interspecific crosses. Another indication of nuclear encoding is the presence of LHCP mRNAs in the poly(A)-containing RNA fraction of barley, pea and *Lemna* leaves (Apel & Kloppstech, 1978; Schmidt *et al.*, 1981; Cuming & Bennett, 1981; Tobin, 1981). Unlike mRNA transcribed in the nucleus, chloroplast mRNA is not significantly polyadenylated (Wheeler & Hartley, 1975). LHCP mRNA sequences have been detected among the transcripts synthesized *in vitro* by isolated pea nuclei (Gallagher & Ellis, 1982).

LHCPs are synthesized as cytoplasmic precursors

The major LHCPs are labelled *in vivo* in plants supplied with radioactive amino acids or $^{35}SO_4^{2-}$. Labelling is blocked by inhibitors of cytoplasmic protein synthesis but not by inhibitors of chloroplast protein synthesis (Machold & Aurich, 1972; Ellis, 1975; Cashmore, 1976; Chua & Gillham, 1977;

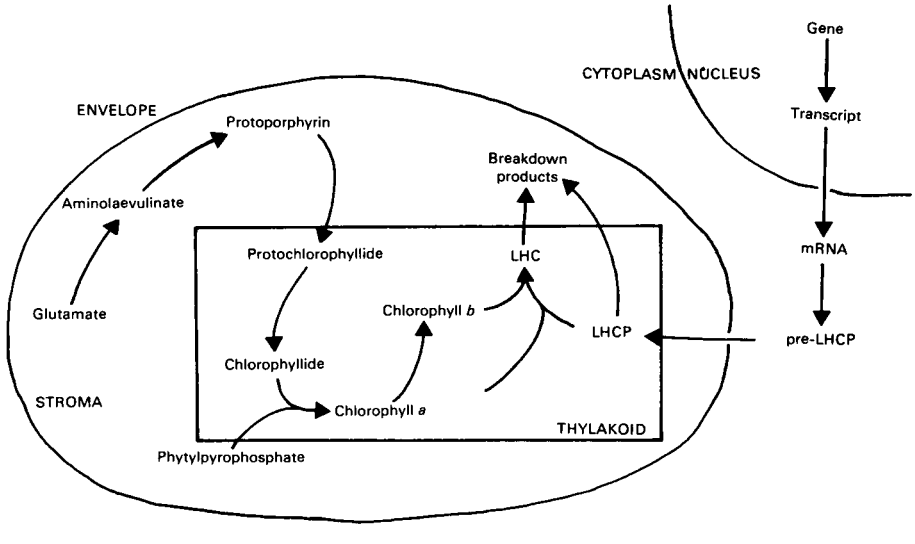

Fig. 1. *Biosynthesis of the light-harvesting chlorophyll a/b complex (LHC) involves nucleus, cytoplasm and chloroplast* The rectangular area is meant to represent the surface of the thylakoid where the final stages in the biosynthesis of the complex take place.

Schmidt *et al.*, 1981). However, even after the very shortest exposures to radioisotope *in vivo*, there is no hint that the LHCPs are synthesized in precursor form. To observe the pre-LHCPs, it is necessary to translate leaf mRNA *in vitro* and then to use antibodies raised against LHCPs to precipitate the precursors. These are generally 4000–6000 larger in molecular weight than the mature LHCPs as judged by SDS/polyacrylamide-gel electrophoresis (Apel & Kloppstech, 1978; Schmidt *et al.*, 1981; Cuming & Bennett, 1981; Tobin, 1981). Two pre-LHCPs have been detected in translation products of pea leaf mRNA (32000 and 30000 molecular weight). Broglie *et al.* (1981) have shown that the 32000 molecular weight pre-LHCP is taken up by isolated chloroplasts (see the next section) and cleaved to generate the 26000 molecular weight mature LHCP in pea. The additional 6000 molecular weight of sequence in the pre-LHCP is probably present entirely as an *N*-terminal extension of the mature protein.

Pre-LHCPs enter chloroplasts by a post-translational mechanism

Uptake, cleavage and membrane attachment of pre-LHCPs have also been achieved *in vitro*. When intact chloroplasts are added to a preparation of radioactive *in vitro* translation products specified by leaf poly(A)-containing mRNA, over 100 radioactive polypeptides appear within the organelles (Grossman *et al.*, 1982). Included among the transported products are the two major LHCPs (Schmidt *et al.*, 1981). The radioactive LHCPs have the same molecular weights as authentic mature LHCPs, they are found exclusively in the thylakoid membranes and they have the same sensitivity to proteinases *in situ*, the same solubility in chloroform/methanol and the same electrophoretic behaviour as authentic LHC. Thus, the pre-LHCPs are transported across the double envelope membrane, cleaved to their mature size, inserted into the photosynthetic membrane and ligated with chlorophylls. However, it is not clear whether cleavage occurs during transport across the envelope, in the stroma or in the thylakoids. That transport is post-translational rather than co-translational is indicated by the fact that the ribosomes of the cell-free translation system may be removed by centrifugation after translation but prior to addition of the chloroplasts without inhibiting transport. Transport is energy-dependent; energy may be supplied by light through photophosphorylation or by exogenous ATP (Grossman *et al.*, 1980).

Chlorophyll synthesis

Chlorophyll is an ester of a porphyrin (chlorophyllide) and a C_{20} terpene (phytol). The chlorophyllide biosynthetic pathway may be divided into two parts based on the location of the relevant enzymes: (i) the conversion of glutamate to protoporphyrin via δ-aminolaevulinic acid catalysed by soluble enzymes, and (ii) the conversion of protoporphyrin to chlorophyllide via protochlorophyllide catalysed by thylakoid-bound enzymes (Rebeiz & Castelfranco, 1973; Kannangara *et al.*, 1978; Lütz *et al.*, 1981). Phytol is synthesized from the C_5 precursor isopentenyl pyrophosphate via geranylgeranyl pyrophosphate, by enzymes that are either soluble or associated with the chloroplast envelope (Block *et al.*, 1980). It is not clear whether the hydrogenation of geranylgeranyl to phytyl takes place before or after esterification to chlorophyllide (Schoch *et al.*, 1977; Benz *et al.*, 1980; Rüdiger *et al.*, 1980; Soll & Schultz, 1981). Chlorophyll *b* is probably synthesized from a specific subfraction of the chlorophyll *a* pool (Shlyk, 1971; Oelze-Karow *et al.*, 1978; Melin *et al.*, 1981; Fradkin *et al.*, 1981; Tanaka & Tsuji, 1982). Until recently it had been assumed that only one chemical form of chlorophyll *a* and chlorophyll *b* existed. However, there is spectrofluorimetric evidence for up to four chemically distinct forms of both chlorophylls (Rebeiz *et al.*, 1981).

Chlorophyll synthesis is light-dependent in most angiosperms

In most angiosperms (flowering plants), but apparently not in all (Adamson & Hiller, 1981), chlorophyll synthesis is rendered light-dependent by the enzyme protochlorophyllide reductase. This NADPH-linked enzyme requires an excited protochlorophyllide substrate as part of its reaction mechanism (Griffiths, 1978). Thus, continuous illumination is required for continuous chlorophyll(ide) synthesis. However, protochlorophyllide fails to accumulate in darkness because of a negative feed-back control exerted on the δ-aminolaevulinate-synthesizing enzyme, very possibly by haem or Mg-protoporphyrin (Gough *et al.*, 1981). The synthesis of this enzyme may also be regulated by phytochrome (Masoner & Kasemir, 1975; Klein *et al.*, 1977; Kannangara & Gough, 1979).

Photoregulation of LHCP accumulation

The absence of chlorophyll *a* and chlorophyll *b* from most dark-grown angiosperms raises questions about the synthesis of LHCP and its photoregulation. Is LHCP synthesis co-ordinated with chlorophyll synthesis? If so, how is the co-ordination achieved, considering that the protein is synthesized outside the chloroplast, whereas chlorophylls are synthesized inside the organelle? If not, does LHCP accumulate in darkness? Although a full discussion of these questions is beyond the scope of this article, it is important to record that there are at least two

distinct photocontrols on the accumulation of LHCP. Firstly, the level of LHCP mRNA is to some degree regulated by phytochrome and, secondly, in the absence of certain stabilizing factors the LHCP is subject to breakdown.

Phytochrome controls the LHCP mRNA levels

Phytochrome is the photoreceptor for many light-dependent responses of plants, including the light-dependent formation of chloroplasts in angiosperms (Mohr, 1977). It is a photochromic protein, which exists in two interconvertible forms, P_r and P_{fr}, corresponding to forms that absorb red light and far-red light respectively. Since many phytochrome-mediated effects are elicted in response to a single flash of red light, which converts some P_r to P_{fr}, P_{fr} is conventionally referred to as the 'active form' of phytochrome. If P_{fr} is converted back to P_r by a pulse of far-red light before it has been able fully to initiate the response under study, then the response may be greatly reduced in magnitude. The reversibility by far-red light of the effects of red light is characteristic of many phytochrome-mediated responses.

The level of LHCP mRNA has been studied in several species of plant, including barley, *Lemna* and pea (Apel & Kloppstech, 1978; Apel, 1979; Tobin, 1981; Cuming & Bennett, 1981). In each case light was found to increase the level of LHCP mRNA, as measured by translation *in vitro* of poly(A)-containing mRNA followed by immunoprecipitation to detect LHCP precursors. That the increase was mediated by phytochrome was indicated by the fact that a pulse of red light led to an increase in the LHCP mRNA level, but not when followed immediately by a pulse of far-red light (Apel, 1979; Tobin, 1981). Dark-grown and light-grown pea seedlings have been compared with respect to their LHCP mRNA content (Cuming & Bennett, 1981) and the ability of their nuclei to transcribe the LHCP genes *in vitro* (Gallagher & Ellis, 1982). The level of polyadenylated LHCP mRNA increased about 12-fold on illumination, while the level of LHCP mRNA among *in vitro* transcripts increased about 10-fold. These results suggest that phytochrome increases the level of LHCP mRNA by stimulating events in the nucleus (such as transcription) rather than by inhibiting breakdown of the mRNA in the cytoplasm.

LHCP is subject to turnover in the absence of stabilizing factors

Under certain circumstances plant tissue may contain substantial levels of LHCP mRNA without accumulating more than a trace of LHCP. Examples include:

(i) dark-grown intermittently illuminated peas (Cuming & Bennett, 1981);

(ii) barley and *Lemna* exposed to a pulse of red light or intermittent illumination (Apel, 1979; Apel & Kloppstech, 1980; Tobin, 1981; Viro & Kloppstech, 1982);

(iii) pea seedlings transferred from light to darkness (Bennett, 1981);

(iv) a chlorophyll *b*-less mutant of barley (Apel & Kloppstech, 1978; Bellemare *et al.*, 1982).

Does the low level of LHCP in these tissues indicate that there is a second photocontrol on LHCP synthesis which permits translation of LHCP mRNA only when both chlorophyll *a* and chlorophyll *b* can be synthesized? Or is the LHCP synthesized only to be degraded in the absence of chlorophyll; that is, is LHCP subject to turnover?

Of these two possibilities, only LHCP turnover has direct experimental support. Bennett (1981) showed that turnover of LHCP prevents its accumulation when greening pea leaves are transferred from light to darkness. Slovin & Tobin (1982) exploited the aquatic mode of growth of *Lemna* to demonstrate LHCP turnover by means of pulse-chase labelling. However, they concluded that the turnover rate of LHCP was too slow to account for the very low level of labelling of LHCP seen in *Lemna* grown under intermittent red light. They suggested that there is a special control on the translation of LHCP mRNA that prevents labelling of the protein under intermittent illumination. Since, in intermittently illuminated leaves, LHCP mRNA is associated with polysomes and may be translated in run-off experiments *in vitro* (Cuming & Bennett, 1981; Slovin & Tobin, 1982; unpublished work cited by Viro & Kloppstech, 1982), any such translational control would presumably have to be exerted after recruitment of LHCP mRNA into polysomes. Turnover of LHCP has also been inferred by other workers (Apel & Kloppstech, 1980; Cuming & Bennett, 1981; Bellemare *et al.*, 1982; Viro & Kloppstech, 1982).

The presence of both chlorophyll *a* and chlorophyll *b* is certainly a necessary condition for the accumulation of LHCP, but is it sufficient? The fact that pre-existing LHC is degraded when immature greening pea leaves (Bennett, 1981) or immature radish cotyledons (Lichtenthaler *et al.*, 1981) are placed in darkness, whereas the LHC of mature chloroplasts is stable in darkness, suggests that the LHC needs to be further stabilized by some event associated with chloroplast maturation. It is possible that the lateral differentiation of thylakoids into appressed and non-appressed regions (see the next section) is an important aspect of the stabilization of the LHC. Until sufficient LHC units accumulate to enable the appressed regions rich in LHC to form, each LHC may be vulnerable to degradation in darkness.

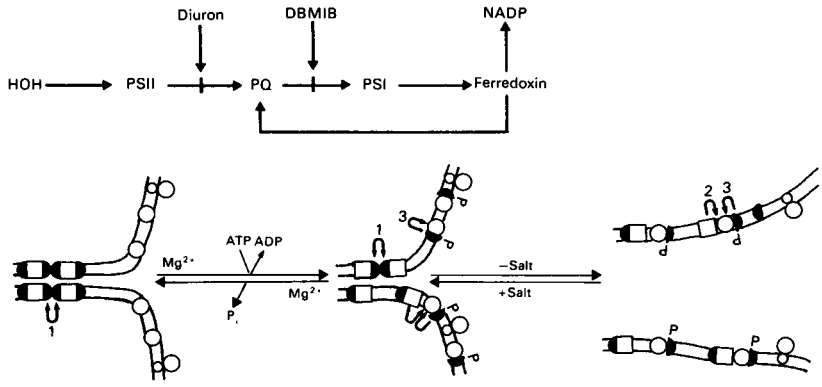

Fig. 2. *Phosphorylation of LHC reversibly alters the structure and function of the thylakoid membrane*
A simplified scheme of the cyclic and non-cyclic electron transport pathways is provided, with the site of inhibition by diuron and the primary (high-affinity) site of inhibition by 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone (DBMIB) indicated. Three types of excitation energy transfer are depicted: 1, transfer between PS II units, promoted by LHC units (Butler, 1980); 2, spillover from PS II to PS I; 3, α-transfer from LHC to PS I. Symbols: ⊂○, ATP synthase; ○, PS I; □, PS II; ◗, LHC.

Function of the LHC

State 1–State 2 transitions and adaptive changes in thylakoid structure

The LHC was originally believed to transfer excitation energy exclusively to PS II. However, it is becoming increasingly clear that the LHC is not a static structure but is able to interact with both photosystems to maximize photosynthetic efficiency. Since the two photosystems must operate in series in transporting electrons from water to $NADP^+$ (Fig. 2), light energy is used most efficiently when it is delivered in a balanced fashion to the photosystems. However, since PS I and PS II are composed of different pigment–protein complexes, with different absorption spectra, it is possible to select wavelengths that preferentially excite either PS I ('light 1') or PS II ('light 2'). Is the photosynthetic membrane capable of adjusting its structure to ensure that a balanced distribution of light 1 or light 2 is achieved?

As the early work pointing to the existence of two photosystems showed (Williams, 1977), an approximately balanced distribution of excitation energy can be achieved for light 2 but not for light 1. Since light 1 is usually 690–720 nm far-red light, chosen to excite the chlorophylls found in and around the reaction centre of PS I (P700), transfer of excitation energy to the reaction centre of PS II (P680) and associated pigments is energetically unfavourable. In contrast, 645 nm red light, often used as light 2 because of its preferential absorption by chlorophyll *b* molecules, is readily transferred to either P680 or P700, provided that the appropriate pigment organization is available (Bonaventura & Myers, 1969; Myers, 1971; Williams, 1977).

When algae are exposed to light 2 alone, PS II is initially overexcited relative to PS I. However, as Bonaventura & Myers (1969) showed, this situation of gross imbalance between the rates of excitation of the two photosystems does not persist. Over a period of about 5 min, the rate of electron transport (measured as O_2 evolution) gradually increases as a greater proportion of light 2 is delivered to PS I. The configuration of the membrane which enables light 2 to be distributed more equally between the two photosystems is known as State 2. When algae in State 2 are illuminated simultaneously with light 2 and excess light 1, PS I is initially overexcited relative to PS II since PS I receives not only light 1 but also a proportion of light 2. However, the membrane is gradually driven into another configuration (State 1) in which a greater proportion of light 2 is delivered to PS II at the expense of PS I. In this way the imbalance in the rates of excitation of PS I and PS II is minimized. These reversible responses to changes in the wavelength of light are known as State 1–State 2 transitions and are regarded as being important in ensuring the balanced delivery of excitation energy to the two photosystems under natural conditions (Barber *et al.*, 1981). They are observed both in algae and in higher plants (Chow *et al.*, 1981; Barber, 1982).

Cation-induced changes in the thylakoid structure and function

What is the molecular basis of State 1–State 2 transitions? For more than 10 years the most popular model of these transitions was based on the observed effects of cations on thylakoid structure

and function (Murata, 1969*b*; Williams, 1977; Barber, 1980, 1982). A characteristic feature of most green plants is that their photosynthetic membranes are differentiated into appressed and non-appressed regions (Fig. 2). The appressed regions are enriched for PS II and LHC while the non-appressed regions are enriched for PS I and ATP synthase (Anderson & Andersson, 1982). This lateral heterogeneity is preserved *in vitro* in the presence of a high concentration of monovalent cations (e.g., 100–150mM-NaCl) or a lower concentration of divalent cations (e.g., 2–10mM-$MgCl_2$). However, when thylakoids are resuspended in 1–5mM-NaCl, the appressed membranes separate and the lateral heterogeneity of PS I, PS II and LHC is lost as the particles become randomly distributed (Staehelin, 1976; Staehelin & Arntzen, 1979). Membrane appression and the lateral heterogeneity of particles are regained on the restoration of the original cation concentrations.

The removal and restoration of cations also results in reversible changes in the transfer of excitation energy between chlorophyll–protein complexes (Arntzen *et al.*, 1977; Butler, 1977, 1978). Chlorophyll fluorescence measurements indicate that in thylakoids displaying membrane appression and lateral heterogeneity, PS II and LHC interact strongly with one another but there is little excitation energy transfer from LHC to PS I ('α-transfer') or from PS II to PS I ('spillover'). However, in 1–5mM-NaCl, the randomization of particles is accompanied by an increase in both α-transfer and spillover.

Barber (1980, 1982) has interpreted these cation-induced changes in thylakoid structure and function in terms of surface charges. It is supposed that PS II and LHC have a natural tendency to aggregate as a result of hydrophobic interactions, but negative charges that they exhibit at the outer surface of the membrane provide repulsive forces that must be screened before the weaker hydrophobic interactions can come into play. In 1–5mM-NaCl, the fixed negative charges are not adequately screened, and so PS II and LHC particles cannot aggregate and neighbouring membrane surfaces cannot become appressed. However, in 2–10mM-$MgCl_2$ or 100–150mM-NaCl, the negative charges are effectively screened, and both particle aggregation and membrane appression can occur. According to this view, the lateral distribution of major thylakoid components is in principle variable and depends on the balance of ionic and hydrophobic forces at the membrane surface.

As mentioned above, trypsin cleaves 10–20 amino acids from the *N*-terminus of the LHCP. This comparatively minor alteration in LHC structure is thought to be sufficient to prevent thylakoid appression and particle aggregation even in the presence of cations (Steinback *et al.*, 1979). It also abolishes the ability of Mg^{2+} to promote the aggregation of liposomes containing purified LHC (Mullet & Arntzen, 1980; Ryrie *et al.*, 1980). These results indicate that the surface-exposed segment of LHC plays a major role in maintaining membrane appression. It is possible to rationalize this effect by noting that the tryptic hexapeptide is basic and its removal will increase the effective surface negative charge on the membrane and will therefore strengthen coulombic repulsion between particles and between membranes.

For several years, this electrostatic model of thylakoid structure has been used to explain State 1–State 2 transitions. Observed changes in the distribution of light 2 between PS I and PS II have been atrributed to light-induced changes in the divalent cation concentration surrounding the thylakoids. According to this model, in order to generate State 1, light 1 would have to increase the cation concentration in the stroma of the chloroplast, thereby promoting membrane appression and reducing α-transfer and spillover of energy to PS I. On the other hand, light 2, in order to generate State 2, would have to reverse these effects. However, it is very doubtful that light 1 and light 2 differ greatly in the degree to which they promote Mg^{2+} extrusion from thylakoids (in response to the pumping of H^+ into the thylakoids). It is also doubtful that the 'basal' level of Mg^{2+} surrounding thylakoids is low enough in light 2 to prevent membrane appression. Two recent estimates of the free Mg^{2+} concentration in chloroplasts in the dark are 1–3mM (Portis, 1981 and 1–4mM (Ben-Hayyim & Krause, 1980). Thus, the cation concentration of the stroma is likely to be too high at all times to explain the occurrence of State 2. Does this mean that the electrostatic model of State 1–State 2 transitions should be abandoned? I think that the answer to this question is that the electrostatic model should be retained but emphasis should be placed not on light-induced changes in stromal cation concentrations but not light-induced changes in the surface charges on specific thylakoid proteins. In the next section I summarize the evidence that State 1 and State 2 correspond respectively to the dephosphorylated and phosphorylated states of the LHC.

Phosphorylation of the LHC

Thylakoid protein phosphorylation

When isolated intact chloroplasts from higher plants are incubated in the light with [^{32}P]orthophosphate, several thylakoid proteins become phosphorylated (Bennett, 1977, 1979*a*). The two most conspicuous phosphoproteins are LHCPs (24000 and 26000 molecular weights). Four other phosphoproteins (9000, 33000, 35000 and 45000

molecular weights) are thought to belong to PS II (Steinback *et al.*, 1982; Owens & Ohad, 1982).

The protein kinase which phosphorylates LHCP and the other thylakoid proteins is itself attached to the membrane (Bennett, 1979*b*; Alfonzo *et al.*, 1980). When washed thylakoids are incubated in darkness with [γ-^{32}P]ATP and 5–10 mM-$MgCl_2$, no kinase activity is observed, but when the thylakoids are illuminated, all the thylakoid proteins that are labelled in intact chloroplasts become labelled. The kinase is not, however, strictly light-dependent because it can be activated in the dark with strong reducing agents such as reduced ferredoxin (Bennett, 1979*b*) and dithionite (Allen *et al.*, 1981).

Thylakoid protein kinase activity is sensitive to the redox state of the plastoquinone pool

The implication of the above results is that some component of the electron transport chain must be reduced before the protein kinase is activated. There are several lines of evidence that the crucial component is plastoquinone (PQ).

(i) Activation of the kinase in the light is abolished by diuron which inhibits the reduction of PQ by PS II, but it is not prevented by the low concentrations of 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone which inhibit PQH_2 oxidation (Bennett, 1979*b*; Allen *et al.*, 1981).

(ii) The kinase is activated in the dark by duroquinol, which donates electrons directly to PQ (Allen & Horton, 1981).

(iii) A sequence of single-turnover flashes that progressively reduces the PQ pool also progressively activates the kinase (Allen *et al.*, 1981).

(iv) Redox titrations in the dark show that protein kinase activation has the same redox midpoint potential as PQ reduction (about 0 mV at pH 7.8), and furthermore, the titration curve for kinase activation is that of a two-electron carrier, consistent with the reduction of PQ (Horton *et al.*, 1981).

Enzymology

The kinase has a K_m for ATP of about 90 μM (Bennett *et al.*, 1980) and its Mg^{2+} optimum is about 3–5 mM. The kinase is not washed off the membrane by extremes of ionic strength but it is reported to be solubilized by the non-ionic detergent β-octylglucoside (Alfonzo *et al.*, 1980). The enzyme is thought to contain a thiol group at the active site (Millner *et al.*, 1982). Thylakoids also carry phosphatase activity (Bennett, 1980; Owens & Ohad, 1982) which is inhibited *in vitro* by fluoride and molybdate and acts preferentially on LHCP, both *in vivo* and in isolated chloroplasts and thylakoids.

Light 1 and light 2 regulate kinase activity

Since the kinase is responsive to the redox state of the PQ pool and since the latter is usually sensitive to the relative rates of excitation of PS I and PS II, light 2 should activate the kinase by reducing the pool and light 1 should inhibit the enzyme by oxidizing the pool. This is illustrated in Fig. 3 for barley leaves supplied with [^{32}P]orthophosphate. Light 1

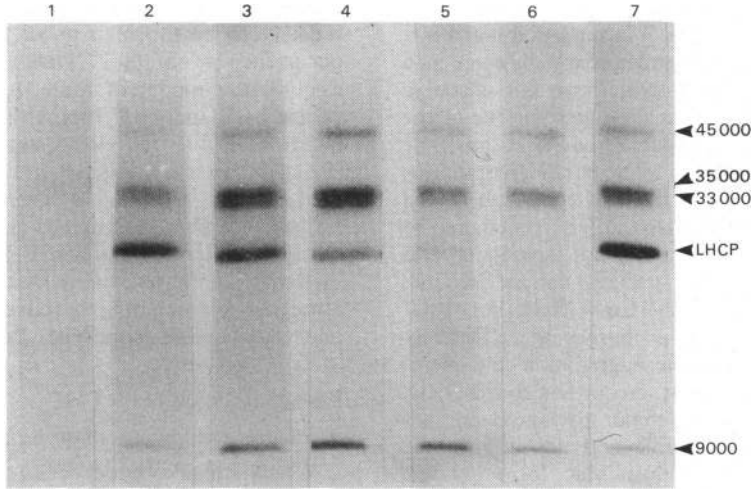

Fig. 3. *Light 1 and light 2 regulation of in vivo thylakoid protein phosphorylation*
Barley leaves were detached and allowed to take up [^{32}P]orthophosphate under either light 1 (714 nm) or light 2 (645 nm) prior to analysis for thylakoid phosphoproteins. The autoradiogram shows the degree of labelling of the LHCPs (24000 and 26000 molecular weight) and four PS II polypeptides (9000–45000 molecular weight). 1, 4 h under light 1; 2, 4 h under light 1, 10 min under light 2; 3, 4 h under light 2; 4–6, 4 h under light 2, then 8, 16 and 32 min under light 1; 7, 4 h under light 2, 32 min under light 1, then 10 min under light 2.

fails to activate the enzyme over a 4h period but light 2 activates and leads to extensive phosphorylation of the LHCP and PS II phosphoproteins within 10min. Transfer from light 2 to light 1 inhibits kinase activity and leads to dephosphorylation of LHCP with a half-life of about 8min. The PS II phosphoproteins are dephosphorylated much more slowly than is LHCP. This is an important point because it suggests that any rapid changes in structure or function that accompany dephosphorylation are most likely due to the dephosphorylation of the LHC. Steinback *et al.* (1982) have made a similar point, based on studies *in vitro*.

LHCP phosphorylation regulates excitation energy distribution between PS I and PS II

State-1–State 2 transitions reveal the ability of the photosynthetic membrane to detect and then at least partially correct any imbalance in the rate of excitation of PS I and PS II. It is clear that the detection of an imbalance in excitation is achieved through the sensitivity of the kinase to the redox state of the PQ pool, but how does protein phosphorylation or dephosphorylation correct the imbalance?

Several lines of evidence lead to the conclusion that LHC phosphorylation increases (and dephosphorylation decreases) the transfer of excitation energy to PS I at the expense of PS II.

1. ATP induces a slow decrease of chlorophyll fluorescence in isolated thylakoids at 20°C (Bennett *et al.*, 1980; Horton & Black, 1980, 1981, 1982; Horton *et al.*, 1981; Allen & Horton, 1981; Chow *et al.*, 1981; Telfer & Barber, 1981). Since at this temperature PS II and LHC fluoresce far more than PS I, such a decline is consistent with the notion that in the phosphorylated state of the thylakoid an increased proportion of excitation energy is delivered to PS I. The decline in fluorescence occurs only under conditions that are conducive to phosphorylation. It is promoted by light 2 but not by light 1 (Horton & Black, 1980; Chow *et al.*, 1981; Telfer & Barber, 1981). There is a linear relationship between the extent of LHC phosphorylation and the extent of the slow ATP-induced decrease in fluorescence (Horton *et al.*, 1981). The decline is reversed under conditions where the kinase is inhibited but the reversibility is blocked by NaF.

2. ATP induces a slow change in the chlorophyll fluorescence emission spectrum recorded at 77K (Bennett *et al.*, 1980; Horton & Black, 1981; Allen *et al.*, 1981; Chow *et al.*, 1981; Steinback *et al.*, 1982). At 77K, PS I, PS II and LHC fluoresce strongly with characteristic emission maxima. It is therefore possible to obtain direct evidence for the redistribution of excitation energy between the two photosystems. All authors report an ATP-dependent increase in the proportion of fluorescence emitted from PS I. When the kinase is inhibited, the fluorescence from PS I declines but the decline is blocked in the presence of NaF. The decline in the fluorescence from PS I is too rapid to be explained in terms of the dephosphorylation of any thylakoid protein other than LHC (Steinback *et al.*, 1982).

3. ATP causes a decrease of approx. 15% in the rate of electron transport through PS II (Steinback *et al.*, 1982; Farchaus *et al.*, 1982) and a corresponding increase in the rate of electron transport through PS I (Horton & Black, 1982; Farchaus *et al.*, 1982). These important results, which were obtained at limiting light intensities, show that phosphorylation of LHC can produce the changes in photosynthetic electron transport required to explain the optimization of photosynthetic quantum yield observed during State 1–State 2 transitions.

Molecular mechanisms

Increased excitation energy distribution to PS I at the expense of PS II may thus be achieved by protein phosphorylation, by cation depletion and by trypsin treatment. However, the response to phosphorylation is mechanistically different from the responses to the other two factors. Cation depletion and trypsin treatment result in the complete loss of grana and membrane appression and in the randomization of LHC, PS I and PS II in the plane of the membrane, as evidenced by increased transfer of energy to PS I from both LHC (α-transfer) and particularly from PS II (spillover). (Butler, 1978; Steinback *et al.*, 1979). Phosphorylation, on the other hand, results in only small reductions (10–15%) in the extent of membrane stacking (Staehelin *et al.*, 1982; Biggins, 1982), and α-transfer tends to be more marked than spillover (Horton & Black, 1981, 1982; Haworth *et al.*, 1982; Kyle *et al.*, 1982). From chlorophyll fluorescence induction transitions, recorded at 20°C, Horton & Black (1981, 1982) calculate that at 5–10mM-Mg^{2+} the ATP-induced decrease in excitation of PS II is due to the detachment of (phosphorylated) LHC units from PS II units, without a marked change in the average interaction among PS II units. This suggests that the ATP-induced increase in excitation of PS I is due exclusively to α-transfer. The data of Horton & Black (1982) suggest further that only at lower $MgCl_2$ concentrations (about 1mM) does spillover make an important contribution to the excitation of PS I. Kyle *et al.* (1982) and Haworth *et al.* (1982), using somewhat similar approaches, disagree with the results of Horton & Black (1981, 1982) and find that at 5mM-$MgCl_2$ spillover does make some contribution to the excitation of PS I. Furthermore they find that ATP tends to reduce the degree of interaction ('connectivity') of PS II units.

The simplest molecular explanation of the ATP-

dependent effects reported so far is the concept that phosphorylation of the LHC leads to its dissociation from PS II and its migration from the appressed regions of the membrane to the non-appressed regions, where it associates with PS I (Fig. 2). This concept explains the occurrence of α-transfer and is consistent with the apparent preferential loss of chlorophyll *b* from stacked membranes after phosphorylation. (Chow *et al.*, 1981) and with the ATP-dependent movement of putative LHC particles (as seen by freeze–fracture electron microscopy) from appressed to non-appressed regions (Staehelin *et al.*, 1982). It is also consistent with a higher concentration of phosphorylated LHC in non-appressed regions compared with appressed regions (Andersson *et al.*, 1982*a*).

What is the force that drives phosphorylated LHC out into the non-appressed regions? As mentioned earlier, the electrostatic model of LHC organization emphasizes the balance between hydrophobic attraction and coulombic repulsion. The LHC is phosphorylated on the surface-exposed *N*-terminal segment that is accessible to trypsin (Bennett, 1980). The phosphorylation site consists of one or both of the adjacent threonyl residues found in the tryptic hexapeptide. Thus, phosphorylation will greatly enhance the surface negative charge on LHC and antagonize the screening effect of actions in its vicinity. The enhanced coulombic repulsion experienced by individual phosphorylated LHC units may be sufficient to drive them out of the appressed regions. Phosphorylation of PS II units may enhance the repulsive force experienced by phosphorylated LHC units. It is not clear at present whether PS II units, on phosphorylation, also experience a repulsive force that ejects them from the appressed regions. Perhaps the ATP-dependent spillover recorded by Horton & Black (1982) at 1 mM-$MgCl_2$ and by Kyle *et al.* (1982) and Haworth *et al.* (1982) at 5 mM-$MgCl_2$ could be due to the movement of PS II from the edge of appressed regions into the non-appressed regions, where they would be able to interact with PS I. A model similar to this has been proposed by Barber (1982).

Another question concerns the locations of the kinase and phosphatase. I suggest that the kinase is located in the appressed regions and that the phosphatase is located in the non-appressed regions. This distribution would minimize the tendency of the kinase and the phosphatase to act in concert as an ATPase, but, more importantly, it would be consistent with the movement of phosphorylated LHC out of the appressed regions and the return of dephosphorylated LHC. Even so such a mechanism constitutes a 'futile cycle', but I calculate that the cycle would consume ATP at less than 1% of the rate at which a mature chloroplast could synthesize ATP at a light intensity only 10% of full sunlight.

Physiological significance of LHC phosphorylation

Plants must continually adjust the organization of their light-harvesting apparatus to make best use of the light energy that they receive, especially under limiting light intensities. The foregoing discussion has outlined the evidence that the physiological mechanism that permits this adjustment involves the reversible phosphorylation of the LHC. An imbalance in the rates of excitation of PS I and PS II is detected and corrected as a result of the sensitivity of the thylakoid protein kinase to the redox state of the PQ pool.

However, it is unlikely that the spectral quality of light is the only parameter to which the photosynthetic membrane adapts by this mechanism. Because of the inhibitory effect of ADP on the kinase (Markwell *et al.*, 1982), the system is sensitive to the ADP/ATP ratio and the energy charge of the chloroplast. Furthermore, the redox state of the PQ pool is sensitive to the availability of electron acceptors for PS I (Allen *et al.*, 1981; Allen & Horton, 1981), including the entire Calvin cycle (Allen & Bennett, 1981). Thus, the kinase will be responsive to the NADP/NADPH ratio of the chloroplast. Allen & Bennett (1981) have suggested that LHC phosphorylation serves to regulate the relative rates of cyclic and non-cyclic electron transport. This is an example of the use of the kinase/phosphatase system to produce an *imbalance* in the rates of excitation of PS I and PS II To promote cyclic electron transport (which is associated with PS I) at the expense of non-cyclic electron transport (which requires both photosystems), it is necessary to phosphorylate LHC. An effect of exactly this sort is seen during the lag phase that precedes rapid CO_2 fixation in isolated, intact, dark-adapted chloroplasts. During the lag phase non-cyclic electron transport is limited by the availability of Calvin cycle intermediates which is in turn limited by the ATP/ADP ratio (Walker & Robinson, 1978). Under these circumstances the PQ pool is reduced, the kinase is active and the LHC is phosphorylated (Allen & Bennett, 1981). These workers suggest that ATP generated by cyclic photophosphorylation allows the Calvin cycle intermediates to accumulate and so initiate the phase of rapid CO_2 fixation. As a necessary concomitant, the increased availability of intermediates leads to the oxidation of the PQ pool and the net dephosphorylation of the LHC. In this way, the system moves back to a position in which the rates of excitation of PS I and PS II are more evenly balanced, as befits chloroplasts undertaking non-cyclic electron tranport and CO_2 fixation.

Concluding remarks

The regulation of photosynthetic electron transport by reversible protein phosphorylation is well

established, but a great deal remains to be learned about the system and its full potentiality for regulating photosynthesis. Adaptation to changes in the spectral quality of ambient light has been well explored but further work is needed in relation to adaptation to changes in the ADP/ATP ratio, in the NADP/NADPH ratio, in the availability of CO_2 and in the demand for photosynthate by the rest of the cell and plant. There are also more specific questions concerning the system itself. How is the kinase activated by PQH_2? Where are the kinase and phosphatase located? Are there multiple forms of the two enzymes? What determines the extent to which the LHC is phosphorylated? What is the physiological role of the phosphorylation of the PS II polypeptides and what functions do these proteins play in PS II? Finally, it is known that State 1–State 2 transitions occur in algae which lack LHC (Murata, 1969*a*; Williams, 1977). Is there an analogous mechanism operating in these algae, involving the PQ-controlled phosphorylation of other thylakoid proteins?

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References

Adamson, H. & Hiller, R. G. (1981) in *Photosynthesis V: Chloroplast Development* (Akoyunoglou, G., ed.), pp. 213–221, Balaban Intern. Sci. Services, Philadelphia

Alfonzo, R., Nelson, N. & Racker, E. (1980) *Plant Physiol.* **65**, 730–734

Allen, J. F. & Bennett, J. (1981) *FEBS Lett.* **123**, 67–70

Allen, J. F. & Horton, P. (1981) *Biochim. Biophys. Acta* **638**, 290–295

Allen, J. F., Bennett, J., Steinback, K. E. & Arntzen, C. J. (1981) *Nature (London)* **291**, 21–25

Anderson, J. M. & Andersson, B. (1982) *Trends Biochem. Sci.* **7**, 288–292

Anderson, J. M. & Barrett, J. (1979) in *Chlorophyll Organization and Energy Transfer in Photosynthesis, Ciba Found. Symp.* **61**, 81–104

Anderson, J. M. & Levine, R. P. (1974) *Biochim. Biophys. Acta* **357**, 118–126

Anderson, J. M., Waldron, J. C. & Thorne, S. W. (1978) *FEBS Lett.* **92**, 227–233

Andersson, B., Åkerlund, H.-E., Jergil, B. & Larsson, C. (1982*a*) *FEBS Lett.* **149**, 181–185

Andersson, B., Anderson, J. M. & Ryrie, I. J. (1982*b*) *Eur. J. Biochem.* **123**, 465–472

Apel, K. (1977) *Biochim. Biophys. Acta* **462**, 390–402

Apel, K. (1979) *Eur. J. Biochem.* **97**, 183–188

Apel, K. & Kloppstech, K. (1978) *Eur. J. Biochem.* **85**, 581–588

Apel, K. & Kloppstech, K. (1980) *Planta* **150**, 426–430

Apel, K., Bogorad, L. & Woodcock, C. L. F. (1975) *Biochim. Biophys. Acta* **387**, 568–579

Arntzen, C. J., Armond, P. A., Briantais, J.-M., Burke, J. J. & Novitzky, W. P. (1977) *Brookhaven Symp. Biol.* **28**, 316–337

Barber, J. (1980) *FEBS Lett.* **118**, 1–10

Barber, J. (1982) *Annu. Rev. Plant Physiol.* **32**, 261–295

Barber, J., Horler, D. N. H. & Chapman, D. J. (1981) in *Plants and the Daylight Spectrum* (Smith, H., ed.), pp. 341–354, Academic Press, London

Bellemare, G., Bartlett, S. & Chua, N.-H. (1982) *J. Biol. Chem.* **257**, 7762–7767

Ben-Hayyim, G. & Krause, G. H. (1980) *Arch. Biochem. Biophys.* **202**, 546–557

Bennett, J. (1977) *Nature (London)* **269**, 344–346

Bennett, J. (1979*a*) *Eur. J. Biochem.* **99**, 133–137

Bennett, J. (1979*b*) *FEBS Lett.* **103**, 342–344

Bennett, J. (1980) *Eur. J. Biochem.* **104**, 85–89

Bennett, J. (1981) *Eur. J. Biochem.* **118**, 61–70

Bennett, J., Steinback, K. E. & Arntzen, C. J. (1980) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 5253–5257

Bennett, J., Markwell, J. P., Skrdla, M. & Thornber, J. P. (1981) *FEBS Lett.* **131**, 325–329

Benz, J., Wolf, C. & Rüdiger, W. (1980) *Plant Sci. Lett.* **19**, 225–230

Biggins, J. (1982) *Biochim. Biophys. Acta* **679**, 479–482

Block, M. A., Joyard, J. & Douce, R. (1980) *Biochim. Biophys. Acta* **631**, 210–219

Bonaventura, C. & Myers, J. (1969) *Biochim. Biophys. Acta* **189**, 366–383

Broglie, R., Bellemare, G., Bartlett, S. G., Chua, N.-H. & Cashmore, A. R. (1981) *Proc. Natl. Acad. Sci. U.S.A.* **78**, 7304–7308

Burke, J. J., Ditto, C. L. & Arntzen, C. J. (1978) *Arch. Biochem. Biophys.* **187**, 252–263

Butler, W. L. (1977) in *Encyclopedia of Plant Physiology (New Series). Volume 5, Photosynthesis I* (Trebst, A. & Avron, M., eds.), pp. 149–167, Springer-Verlag, Berlin

Butler, W. L. (1978) *Annu. Rev. Plant. Physiol.* **29**, 345–378

Butler, W. L. (1980) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 4697–4701

Camm, E. L. & Green, B. R. (1980) *Plant Physiol.* **66**, 428–432

Camm, E. L. & Green, B. R. (1981) *Plant Physiol.* **67**, 1061–1064

Cashmore, A. R. (1976) *J. Biol. Chem.* **251**, 2848–2853

Chow, W. S., Telfer, A., Chapman, D. J. & Barber, J. (1981) *Biochim. Biophys. Acta* **638**, 60–68

Chua, N.-H. & Gillham, N. (1977) *J. Cell Biol.* **74**, 441–452

Chua, N.-H. & Blomberg, F. (1979) *J. Biol. Chem.* **254**, 215–223

Coruzzi, G., Broglie, R., Cashmore, A. & Chua, N.-H. (1983) *J. Biol. Chem.*, in the press

Cuming, A. C. & Bennett, J. (1981) *Eur. J. Biochem.* **118**, 71–80

Delepelaire, P. & Chua, N.-H. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 111–115

Ellis, R. J. (1975) *Phytochemistry* **14**, 89–93

Ellis, R. J. (1981) *Annu. Rev. Plant Physiol.* **32**, 111–137

Farchaus, J. W., Widger, W. R., Cramer, W. A. & Dilley, R. A. (1982) *Arch. Biochem. Biophys.* **217**, 362–367

Fradkin, L. L., Chkanikova, R. A. & Shylk, A. A. (1981) *Plant Physiol.* **67**, 555–559

Gallagher, T. F. & Ellis, R. J. (1982) *EMBO J.* **1**, 1493–1498

Gantt, E. (1981) *Annu. Rev. Plant Physiol.* **32**, 327–347

Gough, S. P., Girnth, C. & Kannangara, C. G. (1981) in *Photosynthesis V. Chloroplast Development* (Akoyunoglou, G., ed.), pp. 107–116, Balaban Intern. Sci. Services, Philadelphia
Green, B. R. & Camm, E. L. (1982) *Biochim. Biophys. Acta* **681**, 256–262
Griffiths, W. T. (1978) *Biochem. J.* **174**, 681–692
Grossman, A. R., Bartlett, S. G. & Chua, N.-H. (1980) *Nature (London)* **285**, 625–628
Grossman, A. R., Bartlett, S. G., Schmidt, G. W., Mullet, J. E. & Chua, N.-H. (1982) *J. Biol. Chem.* **257**, 1558–1563
Haworth, P., Kyle, D. J. & Arntzen, C. J. (1982) *Biochim. Biophys. Acta* **680**, 343–351
Henriques, F. & Park, R. (1977) *Plant Physiol.* **60**, 64–68
Henriques, F. & Park, R. (1978) *Biochem. Biophys. Res. Commun.* **81**, 1113–1118
Hoober, J. K., Millington, R. H. & D'Angelo, L. P. (1980) *Arch. Biochem. Biophys.* **202**, 221–234
Horton, P. & Black, M. T. (1980) *FEBS Lett.* **119**, 141–144
Horton, P. & Black, M. T. (1981) *Biochim. Biophys. Acta.* **635**, 53–62
Horton, P. & Black, M. T. (1982) *Biochim. Biophys. Acta* **680**, 22–27
Horton, P., Allen, J. F., Black, M. T. & Bennett, J. (1981) *FEBS Lett.* **125**, 193–196
Kannangara, C. G. & Gough, S. P. (1979) *Carlsberg Res. Commun.* **44**, 11–20
Kannangara, C. G., Gough, S. P. & von Wettstein, D. (1978) in *Chloroplast Development* (Akoyunoglou, G., ed.), pp. 147–160, Elsevier/North-Holland Biomedical Press, Amsterdam
Klein, S., Katz, E. & Neeman, E. (1977) *Plant Physiol.* **60**, 335–338
Kung, S. D., Thornber, J. P. & Wildman, S. G. (1972) *FEBS Lett.* **24**, 185–188
Kyle, D. J., Haworth, P. & Arntzen, C. J. (1982) *Biochim. Biophys. Acta* **680**, 336–342
Li, J. & Hollingshead, C. (1982) *Biophys. J.* **37**, 363–370
Lichtenthaler, H. K., Burkard, G., Kuhn, G. & Prenzel, U. (1981) *Z. Naturforsch.* **36c**, 421–430
Lichtenthaler, H. K., Prenzel, U. & Kuhn, G. (1982) *Z. Naturforsch.* **37c**, 10–12
Lütz, C., Röper, U., Beer, N. S. & Griffiths, W. T. (1981) *Eur. J. Biochem.* **118**, 347–353
Machold, O. & Aurich, O. (1972) *Biochim. Biophys. Acta* **281**, 103–112
Machold, O. & Meister, A. (1979) *Biochim. Biophys. Acta* **546**, 472–480
Machold, O., Meister, A., Sagromsky, H., Hoyer-Hansen, G. & von Wettstein, D. (1977) *Photosynthetica* **11**, 200–206
Machold, O., Simpson, D. J. & Møller, B. L. (1979) *Carlsberg Res. Commun.* **44**, 235–254
Malkin, R. (1982) *Annu. Rev. Plant Physiol.* **33**, 455–479
Markwell, J. P., Reinman, S. & Thornber, J. P. (1978) *Arch. Biochem. Biophys.* **190**, 136–141
Markwell, J. P., Baker, N. & Thornber, J. P. (1982) *FEBS Lett.* **142**, 171–175
Masoner, M. & Kasemir, H. (1975) *Planta* **126**, 111–117
Melin, C., Axelsson, L., Ryberg, H. & Virgin, H. (1981) in *Photosynthesis V. Chloroplast Development* (Akoyunoglou, G., ed.), pp. 233–241, Balaban Intern. Sci. Services, Philadelphia
Millner, P. A., Widger, W. R., Abbott, M. S. & Cramer, W. A. (1982) *J. Biol. Chem.* **257**, 1736–1742
Mohr, H. (1977) *Endeavour (New Ser.)* **1**, 107–114
Mullet, J. E. & Arntzen, C. J. (1980) *Biochim. Biophys. Acta* **589**, 100–117
Mullet, J. E., Burke, J. J. & Arntzen, C. J. (1980) *Plant Physiol.* **65**, 814–822
Mullet, J. E., Baldwin, T. O. & Arntzen, C. J. (1981) in *Photosynthesis III. Structure and Molecular Organization of the Photosynthetic Membrane* (Akoyunoglou, G., ed.), pp. 577–582, Balaban Intern. Sci. Services, Philadelphia
Murata, N. (1969*a*) *Biochim. Biophys. Acta* **172**, 242–251
Murata, N., (1969*b*) *Biochim. Biophys. Acta* **189**, 171–181
Myers, J. (1971) *Annu. Rev. Plant. Physiol.* **22**, 289–312
Oelze-Karow, H., Kasemir, H. & Mohr, H. (1978) in *Chloroplast Development* (Akoyunoglou, G., ed.), pp. 787–792, Elsevier/North-Holland, Amsterdam
Ogawa, T., Obata, F. & Shibata, K. (1966) *Biochim. Biophys. Acta* **112**, 223–234
Owens, G. C. & Ohad, I. (1982) *J. Cell Biol.* **93**, 712–718
Portis, A. R. (1981) *Plant Physiol.* **67**, 985–989
Rebeiz, C. A. & Castelfranco, P. A. (1973) *Annu. Rev. Plant. Physiol.* **24**, 129–172
Rebeiz, C., Belanger, F. C., McCarthy, S. A., Freyssinet, G., Duggan, J. X., Wu, S. & Mattheis, J. R. (1981) in *Photosynthesis V. Chloroplast Development* (Akoyunoglou, G., ed.), pp. 197–212, Balaban Intern. Sci. Services, Philadelphia
Rüdiger, W., Benz, J. & Guthoff, C. (1980) *Eur. J. Biochem.* **109**, 193–200
Ryrie, I. J., Anderson, J. M. & Goodchild, D. J. (1980) *Eur. J. Biochem.* **107**, 345–354
Schmidt, G., Bartlett, S. G., Grossman, A. R., Cashmore, A. R. & Chua, N.-H. (1981) *J. Cell Biol.* **91**, 468–478
Schoch, S., Lempert, U. & Rüdiger, W. (1977) *Z. Pflanzenphysiol.* **83**, 427–436
Shlyk, A. A. (1971) *Annu. Rev. Plant. Physiol.* **22**, 169–184
Slovin, J. P. & Tobin, E. M. (1982) *Planta* **154**, 465–472
Soll, J. & Schultz, G. (1981) *Biochim. Biophys. Res. Commun.* **99**, 907–912
Staehelin, L. A. (1976) *J. Cell Biol.* **71**, 136–158
Staehelin, L. A. & Arntzen, C. J. (1979) *Chlorophyll Organization and Energy Transfer in Photosynthesis, Ciba Found. Symp.* **61**, 145–175
Staehelin, L. A., Kyle, D. J. & Arntzen, C. J. (1982) *Plant Physiol.* **69**, s69
Steinback, K. E., Burke, J. J. & Arntzen, C. J. (1979) *Arch. Biochem. Biophys.* **195**, 540–557
Steinback, K. E., Bose, S. & Kyle, D. J. (1982) *Arch. Biochem. Biophys.* **216**, 356–361
Süss, K.-H. & Brecht, E. (1980) *Biochim. Biophys. Acta* **592**, 369–374
Süss, K.-H., Schmidt, O. & Machold, O. (1976) *Biochim. Biophys. Acta* **448**, 103–113
Tanaka, A. & Tsuji, H. (1982) *Biochim. Biophys. Acta* **680**, 265–270
Telfer, A. & Barber, J. (1981) *FEBS Lett.* **129**, 161–165
Thornber, J. P. (1975) *Annu. Rev. Plant. Physiol.* **26**, 127–158
Thornber, J. P. & Highkin, H. R. (1974) *Eur. J. Biochem.* **41**, 109–116

Thornber, J. P., Gregory, R. P. F., Smith, C. A. & Bailey, J. L. (1967) *Biochemistry* **6**, 391–396
Thornber, J. P., Alberte, R. S., Hunter, F. A., Shiozawa, J. A. & Kan, K.-S. (1977) *Brookhaven Symp. Biol.* **28**, 132–148
Thornber, J. P., Markwell, J. P. & Reinman, S. (1979) *Photochem. Photobiol.* **29**, 1205–1216
Tobin, E. M. (1981) *Plant Mol. Biol.* **1**, 35–51
Velthuys, B. (1980) *Annu. Rev. Plant Physiol.* **31**, 545–567
Viro, M. & Kloppstech, K. (1982) *Planta* **154**, 18–23
Walker, D. A. & Robinson, S. P. (1978) in *Photosynthetic Carbon Assimilation* (Siegelman, H. W. & Hind, G., eds.), pp. 43–57, Plenum, New York
Wheeler, A. M. & Hartley, M. R. (1975) *Nature (London)* **257**, 66–67
Williams, W. P. (1977) in *Primary Processes in Photosynthesis* (Barber, J., ed.), pp. 94–147, Elsevier, Amsterdam