Plant Physiol. (1994) 106: 841-848

# Organization of the Light-Harvesting Complex of Photosystem I and Its Assembly during Plastid Development[1]

**Beth Welty Dreyfuss[2]* and J. Philip Thornber**

Department of Biology, University of California, Los Angeles, Los Angeles, California 90024-1606

**Photosystem I (PSI) holocomplexes were fractionated to study the organization of the light-harvesting complex I (LHC I) pigment-proteins in barley (*Hordeum vulgare*) plastids. LHC Ia and LHC Ib can be isolated as oligomeric, presumably trimeric, pigment-protein complexes. The LHC Ia oligomeric complex contains both the 24- and the 21.5-kD apoproteins encoded by the *Lhca3* and *Lhca2* genes and is slightly larger than the oligomeric LHC Ib complex containing the *Lhca1* and *Lhca4* gene products of 21 and 20 kD. The synthesis and assembly of LHC I during light-driven development of intermittent light-grown plants occurs rapidly upon exposure to continuous illumination. Complete PSI complexes are detected by nondenaturing Deriphat (disodium *N*-dodecyl-β-iminodipropionate-160)-PAGE after 2 h of illumination, and their appearance correlates with that of the 730- to 740-nm emission characteristic of assembled LHC I. However, the majority of the newly synthesized LHC I apoproteins are present as monomeric complexes in the thylakoids during the early hours of greening. We propose that during development of the protochloroplast the LHC I apoproteins are first assembled into monomeric pigmented complexes that then aggregate into trimers before becoming attached to the pre-existing core complex to form a complete PSI holocomplex.**

PSI in higher plants is composed of a pigmented multiprotein LHC I attached to a functional, evolutionarily conserved CC I that contains P700, other pigments, and multiple proteins. A comparison of isolated PSI particles with antenna-depleted PSI subcomplexes provided the first evidence for the existence of an LHC I. The native PSI complex contained polypeptides of 20 to 25 kD, 17 kD, and approximately 11 kD that were not found in its subfractions (Mullet et al., 1980a, 1980b; Haworth et al., 1983; Peter et al., 1988; Anandan et al., 1989). Later, isolation of an intact, green LHC I showed that it contained Chl *a* and Chl *b*, which represented about 20% of the total Chl of the thylakoid membranes (Thornber et al., 1993). The LHC I complex contains the pigment(s) responsible for the characteristic 77 K fluorescence emission maximum at 730 nm observed from intact leaves and the PSI holocomplex (Haworth et al., 1983; Kuang et al., 1984).

At least three pigmented subcomplexes of LHC I have been fractionated from a PSI preparation by either nondenaturing gel electrophoresis or Suc gradient ultracentrifugation (Lam et al., 1984a, 1984b; Bassi et al., 1985; Bassi and Simpson, 1987; Anandan et al., 1989; Vainstein et al., 1989; Welty and Thornber, 1992; Preiss et al., 1993). LHC Ia (LHC I-680; LHCPIa) has a fluorescence maximum between 680 and 690 nm at 77 K and a Chl *a/b* ratio of 2.0 to 3.1 and, in barley, contains primarily two apoproteins of 24 and 21.5 kD, the products of the *Lhca3* (*Cab8*) and *Lhca2* (*Cab7*) genes, respectively (Lam et al., 1984a, 1984b; Bassi and Simpson, 1987; Ikeuchi et al., 1991; Knoetzel et al., 1992; Welty and Thornber, 1992). LHC Ib (LHC I-730; LHCPIb) has a slightly higher Chl *a/b* ratio of 2.2 to 4.4 and consists of a doublet of apoproteins of about 20 kD in barley (Lam et al., 1984a, 1984b; Bassi and Simpson, 1987; Knoetzel et al., 1992; Welty and Thornber, 1992) that are encoded by the *Lhca1* (*Cab6*) and *Lhca4* (*Cab11/12*) genes (Knoetzel et al., 1992; Anandan et al., 1993). Isolated LHC Ib exhibits a fluorescence maximum at 730 nm at 77 K and consequently contains the long-wavelength-emitting Chl molecule(s) characteristic of LHC I and the PSI holocomplex (Lam et al., 1984a, 1984b; Bassi and Simpson, 1987; Ikeuchi et al., 1992; Knoetzel et al., 1992; Welty and Thornber, 1992). LHC Ib has been reported to occur as a pigmented complex that is larger than LHC Ia when isolated by nondenaturing PAGE or by Suc gradient ultracentrifugation (Bassi and Simpson, 1987; Knoetzel et al., 1992; Welty and Thornber, 1992; Preiss et al., 1993). Therefore, it has been surmised that LHC Ib occurs as oligomers in situ, whereas LHC Ia occurs as monomeric pigment-proteins. A third, minor LHC I pigment-protein complex, LHC Ic, has been isolated from the bundle sheath cells of maize (Vainstein et al., 1989) and the stromal lamellae of barley (Preiss et al., 1993). LHC Ic contains the 17-kD apoprotein encoded by the *psaF* gene (Anandan et al., 1989; Preiss et al., 1993).

Owing to the relatively recent identification of LHC I and isolation of the genes encoding its apoproteins, information on its structure and biogenesis is limited in comparison to the knowledge available about the major LHC IIb pigment-pro-

[1] This research was supported by U.S. Department of Agriculture/Cooperative State Research Services award 93-37306-9577 and National Science Foundation award 91-18942 to J.P.T. B.W.D. was supported in part by a U.S. Department of Agriculture National Needs Fellowship and a University of California President's Dissertation Year Fellowship.

[2] Present address: Department of Chemistry and Biochemistry, University of California, Los Angeles, Los Angeles, CA 90024-1569.

* Corresponding author; fax 1-310-206-1035.

Abbreviations: CC, core complex; CF1, coupling factor 1; Deriphat, disodium *N*-dodecyl-β-iminodipropionate-160; IML, intermittent light; LHC, light-harvesting complex.

tein complex of PSII. The *Lhca* genes exhibit similar phytochrome-mediated light-induced expression (Anandan et al., 1993; Morishige et al., 1993) as well as diurnal and circadian oscillations, as do the *Lhcb* genes (Kellman et al., 1993). Accumulation of the LHC I apoproteins is affected when the availability of Chl *b* is limited, e.g. in plants grown under IML (Mullet et al., 1980b; White and Green, 1988). Studies of light-driven development of the etioplast have revealed a lag in the accumulation in the thylakoid membrane of LHC Ib and LHC IIb apoproteins relative to the accumulation of their message levels (Anandan et al., 1993). A delay has been reported in the accumulation of LHC I polypeptides relative to the LHC II polypeptides in both greening etioplasts and chloroplasts of basal sections of wheat leaves where the gradient of plastid development characteristic of monocotyledonous leaves is initiated (Bredenkamp and Baker, 1987; Anandan et al., 1993).

The biogenesis of LHC I has been studied primarily by following the appearance of the low-temperature long-wavelength fluorescence emission emanating from LHC I, specifically LHC Ib (Mullet et al., 1980b; Bredenkamp and Baker, 1987; Anandan et al., 1993). Biochemical studies utilizing mildly denaturing or nondenaturing PAGE have followed the assembly of LHC I by simply examining the formation of a complete PSI unit during greening (Kalosakas et al., 1981; Jaing et al., 1992). The significance of the various pigmented subcomplexes of LHC I in the assembly process for the PSI holocomplex remained to be examined. Examination in this paper of the LHC I subcomplexes that have been isolated upon fractionation of PSI holocomplexes has shown that LHC Ia as well as LHC Ib occurs in a trimeric form. The implication of this oligomeric subcomplex for the organization of LHC I within the PSI holocomplex is discussed. The assembly of LHC I as a pigmented multiprotein complex and attachment to CC I has been followed during the light-driven maturation of developing protochloroplasts from barley seedlings grown in IML. LHC I appears to assemble first as monomeric and then oligomeric pigment-protein complexes prior to attachment to CC I.

## MATERIALS AND METHODS

Unless stated otherwise, all procedures were performed exactly as described in the preceding paper (Dreyfuss and Thornber, 1994).

### Isolation and Fractionation of PSI

Thylakoid membranes (1 mg Chl/mL) prepared from seedlings grown in the greenhouse under standard conditions were solubilized with 0.9% *n*-nonyl-β-D-glucoside/0.1% SDS for 2 to 5 min on ice. Pigment-protein complexes were fractionated by Deriphat-PAGE. Excised PSI complex was electroeluted with 12.4 mM Tris, 48 mM Gly, pH 8.3, 20% glycerol, 0.02% *n*-dodecyl-β-D-maltoside in the elution cups and 24.8 mM Tris, 96 mM Gly, pH 8.3, reservoir buffer at 4°C and 1 W for at least 8 h and then concentrated using Centricon-30 concentrators (Amicon, Inc., Beverly, MA). Electroeluted PSI was fractionated into its pigmented subcomplexes by treatment with 0.5% nonyl-glucoside for 15 min at room temperature, followed by separation by Deriphat-PAGE at 50 V, 4°C for 2 to 3 h.

### Western Blot Analysis

Western blots were incubated with either P2 polyclonal antibodies (Williams and Ellis, 1986) or the monoclonal antibody CMpLHCI:1 (Høyer-Hansen et al., 1988) and visualized by color precipitation using alkaline phosphatase-conjugated secondary antibodies.

### Spectral Analysis

Steady-state fluorescence emissions at 77 K were recorded on an Aminco SPF-500 spectrophotometer with an exciting wavelength of 436 nm. Spectra of LHC I complexes were taken of excised pigmented bands from Deriphat-PAGE. Whole leaf sections from IML-grown seedlings exposed to various periods of continuous light were used. Fluorescence emission spectra for leaf sections were normalized to equivalent emission values at 687 nm.

## RESULTS

PSI was initially isolated after solubilization of thylakoids with 0.9% nonyl-glucoside/0.1% SDS by electrophoresis on nondenaturing Deriphat gels. Such treatment dissociates most multimeric pigmented complexes, except that of PSI, allowing excision from the gel of an almost pure green band. After elution, PSI was further fractionated by treatment with 0.5% nonyl-glucoside, resulting in the liberation of at least seven pigmented subcomplexes on Deriphat-PAGE (Fig. 1A). However, a relatively large portion of the PSI complex remains intact after this secondary solubilization with nonyl-glucoside (Fig. 1A, band 1. Use of increased surfactant concentrations results in poorer separation of the subcomplexes and increases the amount of free pigment, without an appreciable gain in the number of subcomplexes fractionated. The two largest subcomplexes (Fig. 1A, bands 2 and 3) are composed of the P700-containing heterodimer of the *psa* A/B apoproteins and are devoid of LHC I (Fig. 1B). Band 2 also possesses the nonpigmented subunits of CC I, whereas band 3 lacks the colorless CC I subunits but has the co-migrating subunits of the nonpigmented ATPase-CF1 complex. The absorption and fluorescence spectra of these two bands reveal the absence of Chl *b* and the 730- to 740-nm emission component (data not shown), both of which are characteristic of the presence of LHC I. The third largest subcomplex (Fig. 1A, band 4) is identified from its polypeptide content as LHC IIb in its trimeric form (Fig. 1B). Its presence in PSI material results from the co-migration of an even larger oligomeric form of LHC IIb with the PSI holocomplex on Deriphat-PAGE (see Dreyfuss and Thornber, 1994).

The smaller subcomplexes consist of various LHC I-containing complexes (Fig. 1). The two closely migrating green bands (bands 5 and 6) that electrophorese just faster than the trimeric LHC IIb (band 4) represent oligomeric forms of LHC I. The slower-migrating green band of this doublet, band 5, has subunits of 24 and approximately 21 kD (Fig. 1B) and a single fluorescence peak at 690 nm (Fig. 2). The apoproteins

of CP47 and CP43 are also contained within this band and represent a small fraction of CC II contamination in the PSI isolated from Deriphat-PAGE. The faster-migrating green band of this doublet, band 6, is composed primarily of the 21- and 20.5-kD LHC Ib apoproteins (Fig. 1B). These two apoproteins have been identified as the gene products of the *Lhca*1 (*Cab*6) and *Lhca*4 (*Cab*11/12), respectively (Anandan et al., 1993). This LHC Ib band fluoresces at 730 nm (Fig. 2), and its absorption spectrum shows appreciable amounts of Chl *b* (data not shown). Band 7 contains LHC IIb apoproteins of 27 and 28 kD, derived from some LHC IIb material that migrates as a monomeric pigment-protein complex on Deriphat gels under the solubilization conditions used, and apparently the same 24- and approximately 21-kD apoproteins that are contained in band 5. Band 7 also emits a 691-nm fluorescence, similar to band 5 (Fig. 2). The absorption spectra of bands 5 and 7 show the presence of Chl *b* in the complex, but in a lesser proportion with relation to Chl *a* than is seen in band 6 (data not shown). The 24- and 21.5-kD apoproteins of this pigmented complex (band 7) have been determined to be the products of the *Lhca*3 (*Cab*8) and *Lhca*2 (*Cab*7) genes, respectively (Knoetzel et al., 1992; Welty and Thornber, 1992). The fastest migrating and hence the smallest green band appears to be devoid of apoproteins (Fig. 1B), fluoresces at 679 nm (data not shown), and presumably represents the free pigment fraction.

It appears from the foregoing observations that the LHC Ia complex occurs as an oligomeric structure, most likely trimeric as judged by its apparent size. However, it was necessary to confirm that the approximately 21-kD apoprotein of band 5 was not one of those associated with the LHC Ib band (band 6), since all of these apoproteins have similar sizes. Western blots of the LHC I-containing subcomplexes were probed with the monoclonal antibody CMpLHCI:1 (Høyer-Hansen et al., 1988). This antibody reacts specifically with the 21.5-kD gene product of *Lhca*2; it also has some minor reaction with the LHC IIa (CP29) apoprotein (Knoetzel and Simpson, 1991). The 21.5-kD LHC Ia apoprotein that is

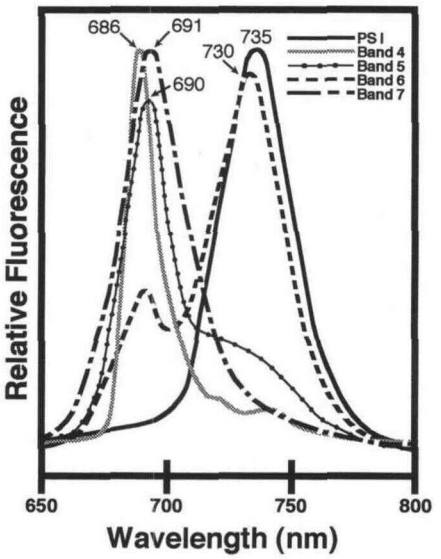


**Figure 2.** Fluorescence emission spectra at 77 K excited at 436 nm obtained from band 1 (PSI), band 4 (LHC IIb trimer), band 5 (oligomeric LHC Ia), band 6 (LHC Ib), and band 7 (monomeric LHC Ia).

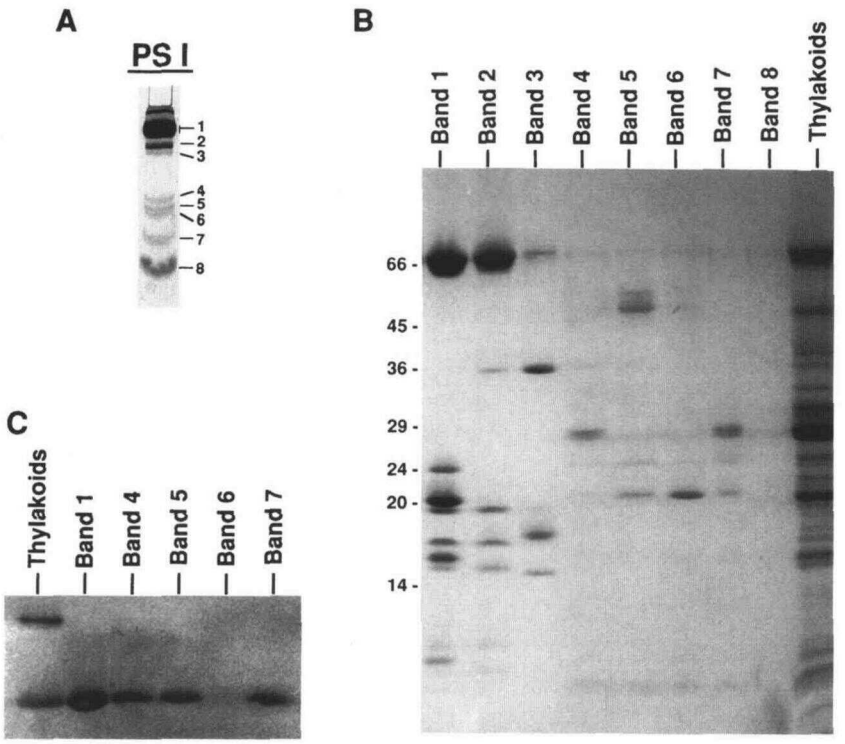


**Figure 1.** Fractionation of PSI pigment-protein complexes and analysis of their polypeptide content. A, Nondenaturing Deriphat-PAGE of isolated PSI treated with 0.5% nonyl-glucoside (gel is unstained). The various green bands are numbered (1–8). B, Denaturing SDS-PAGE of thylakoid membranes and excised bands of pigment-protein complexes (bands 1–8 from A) stained with Coomassie blue. Molecular mass standards (kD) are marked at left. C, Western blot analysis of thylakoid membranes and green bands 1, 4, 5, 6, and 7 from Deriphat-PAGE (A) probed with CMpLHCI:1 antibodies.

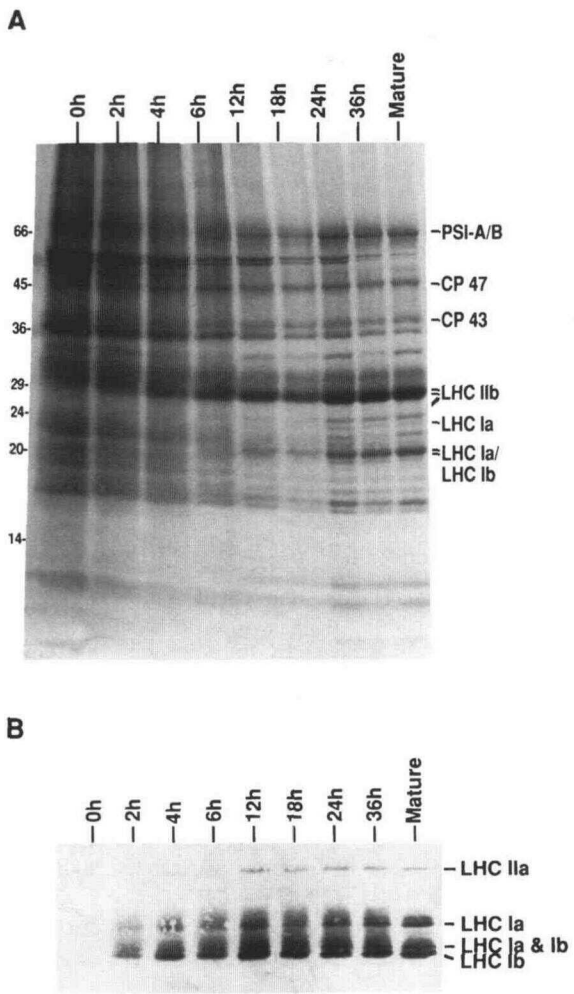


**Figure 3.** SDS-PAGE and western blot analysis of thylakoid membranes (15 μg of Chl) from IML-grown seedlings exposed to continuous illumination for various times (0–36 h) and from mature greenhouse-grown seedlings. A, SDS-PAGE stained with Coomassie blue. The location of Chl *a*-binding proteins of CC II and CC I (CP47, CP43, and PSI-A/B) and apoproteins of the LHC of PSII (LHC IIb) and PSI (LHC Ia and LHC Ib) are marked. B, Protein blots probed with P2 anti-LHC I antiserum. Molecular mass standards (kD) are marked at left.

encoded by the *Lhca2* gene is detected in PSI, band 5 and band 7, but not in band 6 (Fig. 1C). Therefore, band 5 represents an oligomeric form of the LHC Ia monomeric pigment-proteins in band 7. A minor amount of the 21.5-kD apoprotein is also detected in band 4 containing the trimeric LHC IIb.

The assembly of LHC I was followed during the biogenesis of the plastid. Thylakoid membranes isolated from barley grown in IML followed by continuous illumination for the indicated times were examined by SDS-PAGE to observe the changes in the apoprotein composition (cf. LHC IIb in Dreyfuss and Thornber, 1994). The LHC apoproteins are not evident in the thylakoid membranes after IML treatment (zero time point) but increase rapidly during constant illumination (Fig. 3A); however, the apoproteins of CC I, CC II, and CF1 complexes are present at zero time, as was expected (Argyroudi-Akoyunoglou et al., 1971, 1979a, 1079b; Armond et al., 1976; Hiller et al., 1978). Western blot analysis using the polyclonal antibody P2 (Williams and Ellis, 1986) confirms that all four of the major LHC I apoproteins are absent prior to continuous illumination (Fig. 3B). All four increase gradually during light-driven development of the protochloroplast and by 12 h reach levels comparable to those of mature chloroplasts (Fig. 3B). The 24-kD LHC Ia apoprotein accumulation appears to be slightly delayed compared with the LHC Ib apoproteins but parallels the rate of LHC Ib apoprotein accumulation (cf. Anandan et al., 1993). The absence of all LHC I apoproteins after IML treatment and the timing of their appearance contrast sharply with what was observed for the accumulation of the LHC IIb apoproteins in thylakoid membranes of IML-grown plants exposed to continuous light (see Dreyfuss and Thornber, 1994).

The status of assembly of LHC I as pigment-protein complexes was followed by nondenaturing Deriphat-PAGE. The thylakoid membranes from plants grown in IML (0-h time point) could not be completely solubilized and thus the complexes did not fully enter the Deriphat gel (Fig. 4, cf. Dreyfuss and Thornber, 1994). However, the 2-h sample is well resolved into several pigmented bands. At 2 h, the dominant pigmented bands are those of CC I and CC II. A faint band with a migration rate similar to that of the PSI holocomplex can be observed, as well as monomeric LHC I and/or LHC II pigment-protein complexes (Fig. 4). The amount of total Chl present in this PSI holocomplex band increases steadily upon further exposure to light. The increase in abundance of PSI correlates with the decrease in preva-

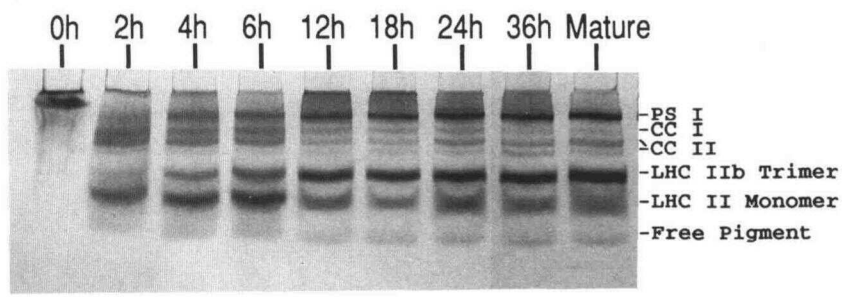


**Figure 4.** Separation of pigmented complexes of thylakoids by nondenaturing Deriphat-PAGE. Thylakoid membranes (12.5 μg of Chl) from IML-grown seedlings exposed to continuous illumination for various times up to 36 h and mature greenhouse-grown seedlings were solubilized with decylmaltoside. (Gel is unstained; see Dreyfuss and Thornber, 1994, for color photograph.) Pigmented complexes of mature thylakoids are marked at right.

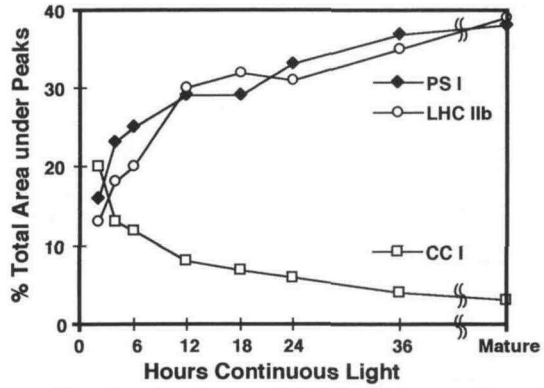


**Figure 5.** Changing abundances of thylakoid pigmented complexes during greening of IML-grown seedlings. Percentage of total Chl in thylakoid membranes associated PSI, CC I, and trimeric LHC IIb were determined by densitometric scanning of nondenaturing gels as in Figure 4.

lence of the CC I pigmented complex and parallels the increase in abundance of the trimeric LHC IIb complex (Fig. 5). This suggests, and quantification substantiates (Fig. 5), that CC I units are being utilized in the formation of the PSI holocomplex by the addition to them of LHC I units.

SDS-PAGE (Fig. 6) of some of the lanes obtained in Figure 4 reveals that the subunits of the ATPase-CF1 complex are the dominant components of thylakoid membranes prior to exposure to continuous light. They decrease in proportion to the abundantly synthesized photosystem components during greening (see Dreyfuss and Thornber, 1994). The largest pigmented band present at 2 h, the aforementioned PSI holocomplex, appears to contain LHC I apoproteins along with all of the subunits of CC I (Fig. 6). The LHC I apoproteins are faint; however, the western blot confirms their presence in the PSI band. By 4 h of illumination, it is increasingly apparent, even from the Coomassie blue-stained gel, that a complete holocomplex of PSI (i.e. one containing LHC I) is formed. Western blot analysis of the two-dimensional gel for the 4-h time point shows that an increased proportion of the total LHC I apoproteins is contained in the PSI complex. The PSI band observed after 4 h of greening contains the LHC Ia and LHC Ib apoproteins in a stoichiometry similar to that seen in PSI of mature thylakoid membranes (Fig. 6). Thus, the apoproteins of both LHC Ia and LHC Ib appear to coordinately assemble with CC I.

The western blots (Fig. 6) detect the presence of the LHC I apoproteins not only in the PSI holocomplex but also in the putative monomeric and trimeric pigmented LHC I complexes (equivalent to bands 5–7 in Fig. 1) on the nondenaturing gel. The distribution of cross-reacting polypeptides between PSI, the LHC I trimer, and the LHC I monomer bands changes during greening. At the early stages of greening the majority of LHC I apoproteins present within the thylakoid membranes are not complexed within PSI but are present in discrete pigment-protein complexes. After 2 h of greening the majority of the LHC Is are present as monomeric pigmented complexes, as evidenced by most of the cross-reaction with the antibody occurring in a region of the gel where proteins with a faster migration rate than that of monomeric LHC IIa would be located (Fig. 6). LHC IIa provides a useful

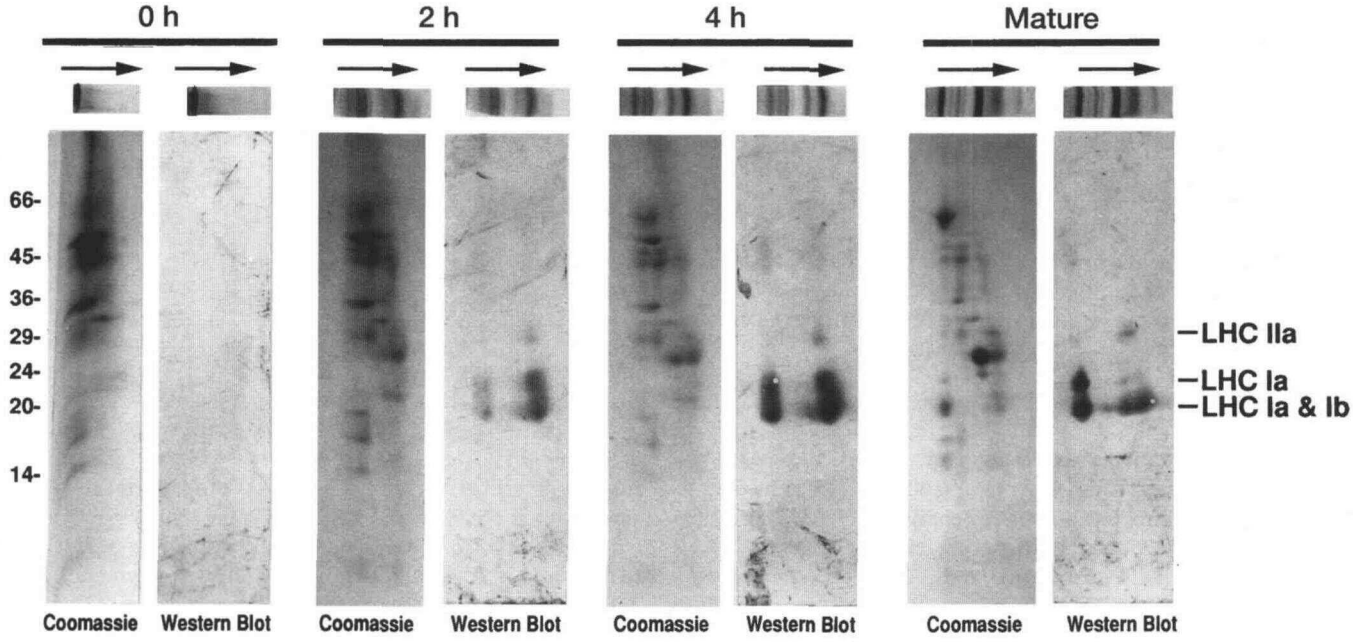


**Figure 6.** Analysis of apoprotein composition of pigmented complexes from IML-grown seedlings greened from 0, 2, and 4 h and mature light-grown seedlings by two-dimensional SDS-PAGE. Excised lanes from nondenaturing gel (Fig. 4) were denatured and placed on duplicate fully denaturing SDS-PAGE, and gels were either stained with Coomassie blue or transferred to membranes for western blot analysis using P2 anti-LHC I antiserum. Molecular mass standards (kD) are marked at left.

marker because it is the only other protein to react with this antibody and it is known to migrate at the lagging edge of the monomeric pigment-protein band on Deriphat gels (Peter and Thornber, 1991). Upon further greening (4 h), a portion of both LHC Ia and LHC Ib apoproteins can be observed in their presumed trimeric forms. In mature thylakoid membranes, the LHC I apoproteins are almost exclusively complexed within PSI holocomplexes (Fig. 6). A small portion of the LHC I apoproteins in mature thylakoids is observed in a location that indicates that they are derived from trimeric and monomeric complexes, but these most likely represent some breakdown of the PSI holocomplex during solubilization of the thylakoids. Therefore, we conclude that assembly of LHC I proceeds from the initial pigmentation of the apoprotein to the aggregation of pigmented monomers into discrete LHC I trimeric complexes that then become attached to CC I to form the complete PSI holocomplex. Results from a pulse-chase experiment in which the fate of proteins synthesized during the initial exposure to continuous light also suggest that monomeric LHC I is an intermediate in the assembly of LHC I into PSI, but these results are not as definitive owing to the poor labeling of the LHC I apoproteins (see Dreyfuss and Thornber, 1994).

The data on the appearance of the LHC I subunits during light-driven biogenesis of the plastid were correlated with the appearance of the long-wavelength fluorescence band at 77 K, characteristic of the biogenesis of LHC Ib and its assembly with CC I. Fluorescence spectra taken of leaf samples from IML-grown barley display a major emission at 687 nm and a minor one at 727 nm (Fig. 7), characteristic of the presence of only PSII and CC I, respectively (Mullet et al., 1980a, 1980b). As greening proceeds, the 727-nm peak gradually shifts to longer wavelengths and increases in intensity relative to the 687-nm emission. By 3 h, this peak is at 740 nm, which clearly demonstrates the presence of completely synthesized PSI units. The changes in the shape of the long-wavelength emission band probably represent the decreased presence of CC I in the thylakoid membrane, because CC I is utilized in the formation of PSI. Indeed, at 1 h the long-wavelength peak appears to be a doublet with overlapping peaks at 727 and 735 nm (Fig. 7). Therefore, the assembly of PSI observed utilizing nondenaturing Deriphat-PAGE (Fig. 4) clearly mirrors the in vivo assembly of PSI monitored by fluorescence that occurs upon exposure of IML-grown plants to continuous light.

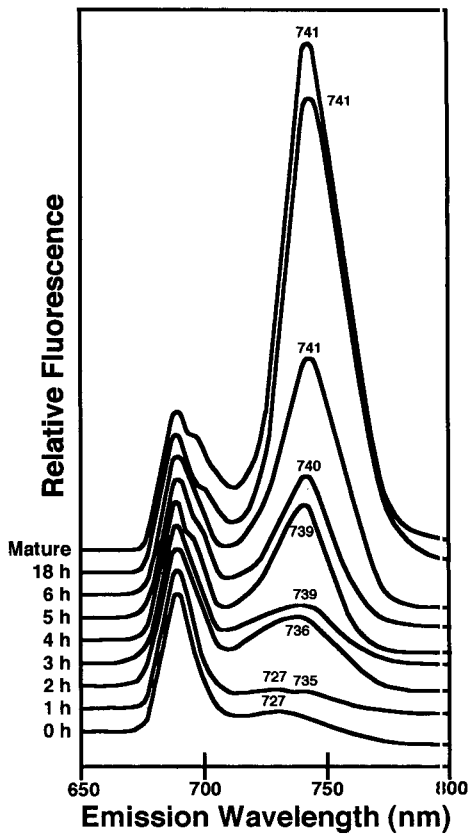


**Figure 7.** Fluorescence emission spectra at 77 K obtained from mid-leaf segments of IML-grown barley seedlings exposed to various periods of constant illumination and mature light-grown seedlings. Spectra were normalized to equivalent emissions at 687 nm.

## DISCUSSION

Historically, the major LHC I pigment-proteins have been isolated as a larger, presumably oligomeric (LHC Ib) complex and a smaller, monomeric (LHC Ia) complex. However, the polypeptide composition of the two fractions was not mutually exclusive, with the 24-kD LHC Ia apoprotein frequently observed in the LHC Ib pigmented fraction (Bassi and Simpson, 1987; Welty and Thornber, 1992). Hence, we decided to investigate whether LHC Ia also existed as an oligomeric complex, albeit less stable than its LHC Ib counterpart, which would explain why some of its apoproteins would be found together with those of LHC Ib in the oligomeric and monomeric complexes. An oligomeric LHC Ia pigmented complex was separated from the LHC Ib pigmented complex by extending the duration of nondenaturing electrophoresis (Welty and Thornber, 1992). At least half of the LHC Ia apoproteins fractionates in a colored band migrating between the trimeric LHC IIb and oligomeric LHC Ib (Fig. 2). The remaining LHC Ia apoproteins fractionate as monomeric pigment-protein complexes along with monomeric LHC IIb. Knoetzel et al. (1992) described an LHC I oligomeric form, termed LHC I-680A, that was slightly larger than the oligomeric form of LHC Ib (LHC I-730) and had a single fluorescence peak at 680 nm. However, their oligomeric form differed in its apoprotein composition from the oligomeric LHC Ia form isolated here in that theirs was composed predominantly of the 24-kD product of the *Lhca3* gene. In their study the LHC I apoprotein encoded by *Lhca2* is found mainly in the monomeric pigment-protein fraction (LHC I-680B). Thus, it is significant that the oligomeric form of LHC Ia that we isolated contains major amounts of the 21.5-kD apoprotein (Fig. 2), verifying that both types of LHC Ia apoproteins are complexed into higher-order aggregates.

The LHC Ia and LHC Ib oligomers are probably trimers and not dimers, as suggested by Knoetzel et al. (1992). It is well established that the related LHC IIb complex occurs as a trimer (Kühlbrandt and Wang, 1991). Because every *Lhc*

gene product shows strong sequence conservation, it is very likely that each has basically the same tertiary (Green et al., 1991) and quaternary structures, i.e. all of them have the propensity to form trimers. Furthermore, the rates of electrophoretic migration of LHC Ia and LHC Ib oligomers are those expected for trimers containing apoproteins of slightly smaller size than those of LHC IIb, and are not high enough for them to be dimers (Fig. 1B). Whether LHC Ia and LHC Ib are homo- or heterotrimers is debatable. The fact that some isolated LHC Ia fractions contain essentially only one of the two apoproteins is evidence in favor of homotrimers (Ikeuchi et al., 1991; Knoetzel and Simpson, 1992). Several models for the arrangement of LHC I within the native PSI holocomplex have been proposed (Bassi et al., 1990; Ikeuchi et al., 1991; Knoetzel et al., 1992; Thornber et al., 1993). The concerted synthesis and assembly of both LHC Ia and LHC Ib to form PSI suggests that all four LHC I apoproteins form a single LHC I holocomplex.

The stoichiometry of the four LHC I apoproteins within barley PSI is difficult to determine owing to the similar apparent sizes of the two LHC Ib apoproteins and the 21.5-kD LHC Ia apoprotein. However, in other plant species in which their apparent molecular masses show greater differences, it is evident that all four LHC I apoproteins are present in equal stoichiometry within PSI (Ikeuchi et al., 1991). It is probable that equal amounts of Chl are associated with LHC Ia and LHC Ib (Lam et al., 1984). Therefore, a model consisting of two sets of LHC Ia and two sets of LHC Ib trimers surrounding PSI must be evoked to establish a 1:1:1:1 ratio of the four LHC I apoproteins. Of the some 200 Chl molecules associated with PSI, approximately 100 to 120 are believed to be associated with the LHC I complex (Ortiz et al., 1984; Bassi et al., 1985). Four sets of LHC I trimers containing 12 apoprotein molecules would then bind an estimated 8 to 10 Chl molecules per apoprotein. This ratio is comparable to the 12 Chl molecules associated with each subunit of the LHC IIb trimer (Kühlbrandt et al., 1994), taking into account the 4- to 8-kD difference in size between the LHC I and the LHC IIb apoproteins. Confirmation of the pigment content and the trimeric structure of the LHC Is will probably have to await resolution of the PSI structure at the molecular level.

All four LHC I apoproteins are absent after 3 d of IML treatment but begin to accumulate at a steady rate within 2 h of exposure to continuous light (Fig. 3B). It has been suggested that the accumulation of LHC I within the thylakoids requires extended exposure to light as well as the presence of Chl *b* (White and Green, 1988). Nevertheless LHC I is present, although reduced, in the thylakoids of the Chl *b*-less barley *chlorina* f2 mutant (White and Green, 1988; Peter and Thornber, 1991; Harrison et al., 1993; S. Preiss and J.P. Thornber, unpublished data) or in maize exposed to 11 d of IML treatment (Marquardt and Bassi, 1993). The failure to accumulate LHC I in IML-grown plants may also be due to a hierarchy of association of Chl with the various LHC apoproteins when Chl *b* is limiting (Greene et al., 1988). The rate of accumulation of the LHC I apoproteins in relation to the accumulation observed for the LHC II apoproteins is delayed. This delayed synthesis of LHC I compared to LHC II has been observed during greening of etiolated plants (Anandan et al., 1993) and after the gradient of plastid development along a monocotyledonous leaf (Brendenkamp and Baker, 1987).

The newly synthesized LHC I apoproteins appear in the thylakoid membranes primarily as monomeric pigment-protein complexes during the early hours of greening (Fig. 6). This suggests that the newly integrated LHC I in the thylakoid membranes are first assembled with their respective pigments into a monomeric intermediate complex that later assembles into PSI. The detection of the faint presence of trimeric forms of both LHC Ia and LHC Ib after 4 h of greening further suggests that the monomeric LHC I pigment complexes may associate into their trimeric states prior to binding to CC I, adding an additional step in the assembly of LHC I into the PSI holocomplex. A similar sequential pathway of assembly has been demonstrated for the 21.5-kD *Lhca2* gene product of LHC Ia (Adam and Hoffman, 1993). The conversion of the monomeric LHC Ia intermediate into PSI did not require stroma or ATP (Adam and Hoffman, 1993). These results, taken together with the results we obtained, suggest that LHC I assembles in a sequential manner similar to that demonstrated for LHC IIb (see Dreyfuss and Thornber, 1994).

The addition of the LHC I to the CC I to yield a complete PSI pigmented complex during the greening of IML-grown seedlings has previously been followed by mildly denaturing or nondenaturing PAGE (Kalosakas et al., 1981; Jaing et al., 1992). Whereas complete PSI units were observed in the present work (Figs. 4 and 7) after 2 h of constant illumination, the previous studies detected complete formation of PSI only after 20 to 24 h of continuous illumination. This difference may be due to a slower assembly occurring in dicots versus monocots or to a maturation of PSI units over the 24-h period that made them more stable to the harsher solubilization conditions used earlier. Therefore, the assembly of LHC I into PSI occurs very rapidly after the initial synthesis of the LHC I apoproteins and follows the steady increase in the apoproteins' abundance. The gradual shift and enhancement in the long-wavelength fluorescence at 77 K (Fig. 7) correlated with the increase in the abundance of PSI observed by nondenaturing electrophoresis and verifies that the changes observed in the abundance of PSI closely resemble the in vivo status of assembly in the thylakoid membranes of the developing plastids.

## ACKNOWLEDGMENTS

The authors wish to thank James Jaing and Daryl T. Morishige for their contributions to this research, and recognize Shivanthi Anandan's seminal observations on PSI fractionation that triggered some experimental approaches used here.

Received April 26, 1994; accepted July 5, 1994.


## LITERATURE CITED

**Adam Z, Hoffman NE** (1993) Biogenesis of a photosystem I light-harvesting complex. Plant Physiol **102:** 35–43

**Anandan S, Morishige DT, Thornber JP** (1993) Light-induced biogenesis of light-harvesting complex I (LHC I) during chloroplast development in barley (*Hordeum vulgare*). Studies using cDNA clones of the 21-kilodalton and 20-kilodalton LHC I apoproteins. Plant Physiol **101:** 227–236

**Anandan S, Vainstein A, Thornber JP** (1989) Correlation of some published amino acid sequences for photosystem I polypeptides to a 17 kDa LHCI pigment-protein and to subunits III and IV of the core complex. FEBS Lett **256:** 150–154

**Argyroudi-Akoyunoglou JH, Akoyunoglou G** (1979a) The chlorophyll-protein complexes of the thylakoids in greening plastids of *Phaseolus vulgaris*. FEBS Lett **104:** 78–84

**Argyroudi-Akoyunoglou JH, Akoyunoglou G** (1979b) On the formation of photosynthetic membranes in bean plants. Photochem Photobiol **18:** 219–228

**Argyroudi-Akoyunoglou JH, Feleki Z, Akoyunoglou G** (1971) Formation of two chlorophyll-protein complexes during greening of etiolated bean leaves. Biochem Biophys Res Commun **45:** 606–613

**Armond PA, Arntzen CJ, Briantais JM, Vernotte C** (1976) Differentiation of chloroplast lamellae. Light harvesting efficiency and grana development. Arch Biochem Biophys **175:** 54–63

**Bassi R, Machold O, Simpson D** (1985) Chlorophyll-proteins of two photosystem I preparations from maize. Carlsberg Res Commun **50:** 145–162

**Bassi R, Rigoni F, Giacometti GM** (1990) Chlorophyll binding proteins with antenna function in higher plants and green algae. Photochem Photobiol **52:** 1187–1206

**Bassi R, Simpson D** (1987) Chlorophyll-protein complexes of barley photosystem I. Eur J Biochem **163:** 221–230

**Bredenkamp GJ, Baker NR** (1988) The changing contribution of LHC I to photosystem I activity during chloroplast biogenesis in wheat. Biochim Biophys Acta **934:** 14–21

**Dreyfuss BW, Thornber JP** (1994) Assembly of the light-harvesting complexes (LHCs) of photosystem II. Monomeric LHC IIb complexes are intermediates in the formation of oligomeric LHC IIb complexes. Plant Physiol **106:** 829-839

**Green BR, Pichersky E, Kloppstech K** (1991) Chlorophyll *a/b* binding proteins: an extended family. Trends Biochem Sci **16:** 181–186

**Greene BA, Allred DR, Morishige DT, Staehelin LA** (1988) Hierarchical response of light-harvesting chlorophyll-proteins in a light-sensitive chlorophyll *b* deficient mutant of maize. Plant Physiol **87:** 357–364

**Harrison MA, Nemson JA, Melis A** (1993) Assembly and composition of the chlorophyll *a-b* light-harvesting complex of barley (*Hordeum vulgare* L.): immunochemical analysis of chlorophyll *b*-less and chlorophyll *b*-deficient mutants. Photosynth Res **38:** 141–151

**Haworth P, Watson JL, Arntzen CJ** (1983) The detection, isolation and characterization of a light-harvesting complex which is specifically associated with photosystem I. Biochim Biophys Acta **724:** 151–158

**Hiller RG, Pilger TBG, Genge S** (1978) Formation of chlorophyll-protein complexes during greening of etiolated barley leaves. Dev Plant Biol **2:** 215–220

**Høyer-Hansen G, Bassi R, Hoenberg LS, Simpson DJ** (1988) Immunological characterization of chlorophyll *a/b*-binding proteins of barley thylakoids. Planta **173:** 12–21

**Ikeuchi M, Hirano A, Inoue Y** (1991) Correspondence of apoproteins of light-harvesting chlorophyll *a/b* complexes associated with photosystem I to *Cab* genes: evidence for a novel type-IV apoprotein. Plant Cell Physiol **32:** 103–112

**Jaing J, Welty BA, Morishige DT, Thornber JP** (1992) Assembly of the photosystem I multi-protein complex and the oligomeric form of the major light-harvesting chlorophyll *a/b*-protein in pea seedlings grown in flashed light followed by continuous illumination. *In* JH Argyroudi-Akoyunoglou, ed, Regulation of Chloroplast Biogenesis. Plenum Press, London, pp 282–294

**Kalosakas K, Argyroudi-Akoyunoglou JH, Akoyunoglou G** (1981) The formation of the pigment-protein complexes in thylakoids of *Phaseolus vulgaris* during chloroplast development. Prog Photosynth Res **5:** 569–580

**Kellmann JW, Merforth N, Wiese M, Pichersky E, Piechulla B** (1993) Concerted circadian oscillations in transcript levels of nineteen *Lha/b* (*Cab*) genes in *Lycopersicon esculentum* (tomato). Mol Gen Genet **237:** 439–448

**Knoetzel J, Simpson D** (1991) Expression and organisation of antenna proteins in the light-sensitive and temperature-sensitive barley mutant *chlorina*-104. Planta **185:** 111–123

**Knoetzel J, Svendsen I, Simpson DJ** (1992) Identification of the photosystem I antenna polypeptides in barley. Isolation of three pigment-binding antenna complexes. Eur J Biochem **206:** 209–215

**Kuang TY, Argyroudi-Akoyunoglou JH, Nakatani HY, Watson J, Arntzen CJ** (1984) The origin of the long-wavelength fluorescence emission band (77K) from photosystem I. Arch Biochem Biophys **235:** 618–627

**Kühlbrandt W, Wang DN** (1991) Three-dimensional structure of plant light-harvesting complex determined by electron crystallography. Nature **350:** 130–134

**Kühlbrandt W, Wang DN, Fujiyoshi Y** (1994) Atomic model of plant light-harvesting complex. Nature **367:** 614–621

**Lam E, Ortiz W, Malkin R** (1984a) Chlorophyll *a/b* proteins of photosystem I. FEBS Lett **168:** 10–14

**Lam E, Ortiz W, Mayfield S, Malkin R** (1984b) Isolation and characterization of a light-harvesting chlorophyll *a/b* protein complex associated with photosystem I. Plant Physiol **74:** 650–655

**Marquardt J, Bassi R** (1993) Chlorophyll-proteins from maize seedlings grown under intermittent light conditions: their stoichiometry and pigment content. Planta **191:** 265–273

**Morishige DT, Anandan S, Dreyfuss BW, Williams RS, Ellis RJ, Thornber JP** (1993) A pea cDNA clone (*Lhca3*) encoding the 24-kD light-harvesting protein of photosystem I. Plant Physiol **103:** 1461–1462

**Mullet JE, Burke JJ, Arntzen CJ** (1980a) Chlorophyll proteins of photosystem I. Plant Physiol **65:** 814–822

**Mullet JE, Burke JJ, Arntzen CJ** (1980b) A developmental study of photosystem I peripheral chlorophyll proteins. Plant Physiol **65:** 823–827

**Ortiz W, Lam E, Ghirardi M, Malkin R** (1984) Antenna function of a chlorophyll *a/b* protein complex of photosystem I. Biochim Biophys Acta **766:** 505–509

**Peter GF, Machold O, Thornber JP** (1988) Identification and isolation of photosystem I and photosystem II pigment-proteins from higher plants. *In* JL Harwood, TJ Walton, eds, Plant Membranes: Structure, Assembly and Function. The Biochemical Society, London, pp 17–31

**Peter GF, Thornber JP** (1991) Biochemical composition and organization of higher plant photosystem II light-harvesting pigment-proteins. J Biol Chem **266:** 16745–16754

**Preiss S, Peter GF, Anandan S, Thornber JP** (1993) The multiple pigment-proteins of the photosystem I antenna. Photochem Photobiol **57:** 152–157

**Thornber JP, Peter GF, Morishige DT, Gomez S, Anandan S, Welty BA, Lee A, Kerfeld C, Takeuchi T, Preiss S** (1993) Light harvesting in photosystems I and II. Biochem Soc Trans **21:** 15–18

**Vainstein A, Peterson CC, Thornber JP** (1989) Light-harvesting pigment-proteins of photosystem I in maize. Subunit composition and biogenesis. J Biol Chem **264:** 4058–4063

**Welty BA, Thornber JP** (1992) Correlation of the apoproteins of LHC I to their respective *Lhc* (*Cab*) genes in barley. *In* N Murata, ed, Research in Photosynthesis, Vol 1. Kluwer Academic, Dordrecht, The Netherlands, pp 323–326

**White MJ, Green BR** (1988) Intermittent-light chloroplasts are not developmentally equivalent to *chlorina* f2 chloroplasts in barley. Photosynth Res **15:** 195–203

**Williams RS, Ellis RJ** (1986) Immunological studies on the light-harvesting polypeptides of photosystem I and II. FEBS Lett **203:** 295–300