Review

Role of subunits in eukaryotic Photosystem I

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Abstract

Photosystem I (PSI) of eukaryotes has a number of features that distinguishes it from PSI of cyanobacteria. In plants, the PSI core has three subunits that are not found in cyanobacterial PSI. The remaining 11 subunits of the core are conserved but several of the subunits have a different role in eukaryotic PSI. A distinguishing feature of eukaryotic PSI is the membrane-imbedded peripheral antenna. Light-harvesting complex I is composed of four different subunits and is specific for PSI. Light-harvesting complex II can be associated with both PSI and PSII. Several of the core subunits interact with the peripheral antenna proteins and are important for proper function of the peripheral antenna. The review describes the role of the different subunits in eukaryotic PSI. The emphasis is on features that are different from cyanobacterial PSI. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Photosynthesis; Photosystem I; Light harvesting complex; Plant; Topology; Electron transport

1. Function and composition of eukaryotic Photosystem I

The Photosystem I (PSI) found in plants and algae has the same basic function as PSI in cyanobacteria, i.e., it mediates the light-driven electron transport from plastocyanin (or cytochrome *c* in some species) to ferredoxin (or flavodoxin).

Although eukaryotic PSI has many similarities to cyanobacterial PSI both in structure and in function, there are also important differences. Eukaryotic PSI is composed of a core complex and a light-harvesting complex (LHC). The core complex is composed of 13 different subunits, denoted PSI-A (or PsaA) to PSI-N (or PsaN). The light-harvesting complex I (LHCI) in plants is composed of four different subunits denoted Lhca1 to Lhca4. The subunit composition of plant PSI is summarized in Table 1 and a schematic model for the organization of the subunits is shown in Fig. 1. The holo-complex consisting of both the PSI core and the LHCI subunits can be isolated (Fig. 2). Light-harvesting complex II (LHCII) can be functionally connected with PSI but is not normally retained in solubilized and isolated PSI.

Whereas the PSI core complex in eukaryotes is relatively similar to the cyanobacterial PSI, the LHC is completely different from the phycobilisomes that serve as peripheral antennae in cyanobacteria. LHC is composed of transmembrane proteins, which belong to the large class of Chl *a*/*b* binding proteins [1–3]. Obviously, the core complex must have different structural and functional features that allows is

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Table 1
Composition of PSI in higher plants

Gene and subunit	Location of the gene	Molecular mass (kDa)[a]	Cofactors	Function
PsaA, PSI-A	C	83.2 ⎫	≈ 96 Chl a, ≈ 22 β-carotene, P700, A_0	Light-harvesting
PsaB, PSI-B	C	82.4 ⎭	A_1, F_X	Charge separation
				Electron transport
PsaC, PSI-C	C	8.8	F_A, F_B	Electron transport
PsaD, PSI-D	N	17.6		Binding of ferredoxin
				Binding of PSI-C
PsaE, PSI-E	N	10.8		Binding of ferredoxin and FNR
				Involved in cyclic electron transport
PsaF, PSI-F	N	17.5	Chl a?	Binding of plastocyanin
				Binding of LHCI-730
PsaG, PSI-G	N	10.8	Chl a?	Binding of LHCI-680
PsaH, PSI-H	N	10.2	Chl a?	Binding of LHCII (state transitions)
				Stabilization of PSI-D
PsaI, PSI-I	C	4.0		Stabilization of PSI-L
PsaJ, PSI-J	C	5.0		Stabilization of PSI-F
PsaK, PSI-K	N	9.0	Chl a?	Binding of LHCI-680
PsaL, PSI-L	N	18.0	Chl a?	Stabilization of PSI-H
PsaM, PSI-M	C	3.3		(Absent in angiosperms)
PsaN, PSI-N	N	9.8		Docking of plastocyanin
Lhca1, Lhca1	N	22	≈ 10 Chl a, ≈ 2 Chl b, ≈ 3 carotenoids	Light-harvesting, LHCI-730
Lhca2, Lhca2	N	23	≈ 10 Chl a, ≈ 2 Chl b, ≈ 3 carotenoids	Light-harvesting, LHCI-680B
Lhca3, Lhca3	N	25	≈ 10 Chl a, ≈ 2 Chl b, ≈ 3 carotenoids	Light-harvesting, LHCI-680A
Lhca4, Lhca4	N	22	≈ 10 Chl a, ≈ 2 Chl b, ≈ 3 carotenoids	Light-harvesting, LHCI-730

The location of the gene in plants in the chloroplasts (C) or nucleus (N) is indicated. In algae other than green algae, more genes are located in the chloroplast or cyanelle genome.
[a] Molecular masses of PSI-A and PSI-B are from maize and molecular mass of PSI-M from black pine. For all other core subunits, the molecular masses are from barley. Molecular masses of Lhca polypeptides are from Jansson [3].

to accommodate and interact with LHC and phycobilisomes in eukaryotes and cyanobacteria, respectively. Another major difference between cyanobacterial PSI and eukaryotic PSI is the supercomplex organization of the complexes. Cyanobacterial PSI can be present as trimers whereas there is no evidence that plant PSI assembles into larger structures than monomers. Thus, the special features of cyanobacterial PSI involved in the interaction between monomers can be expected to be absent in plant PSI. Most of the subunits in the core complex have homologues in cyanobacterial PSI and vice versa. Exceptions are the subunits PSI-G, PSI-H, and PSI-N, which are only found in eukaryotes, and PSI-M, which has not been found in angiosperms[1]. The newly reported PSI-X subunit in *Synechococcus* PSI [155] has also not been found in eukaryotes.

Photosynthetic eukaryotes are a diverse group of organisms. Chlorophytes, rhodophytes and glaucophytes have evolved from primary endosymbiosis involving a eukaryotic host and a prokaryotic endosymbiont. All other algae groups have evolved by secondary (or higher order) endosymbiosis between a simple eukaryotic alga and a nonphotosynthetic eukaryotic host [4]. Although the basic photosynthetic machinery is conserved in all these organisms, it should be emphasized that PSI does not necessarily have the same composition and fine-tuning in all of them. The subunits that have only been found in eukaryotes, i.e., PSI-G, PSI-H, and PSI-N, have actually only been found in plants and green algae, i.e., in *Chlorophyta*. Other groups of algae appear to have

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[1] Note added in proof. An additional subunit specific to plants, PSI-O of 10.1 kDa, has recently been found (J. Knoetzel and H.V. Scheller, unpublished data).
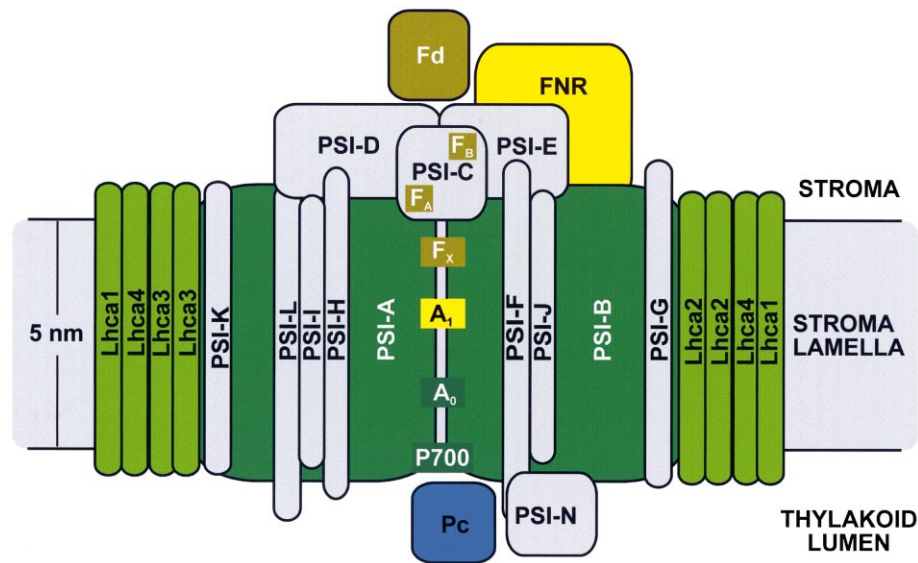
Fig. 1. Schematic model of PSI from plants.

a more cyanobacteria-like PSI. PSI-M is also peculiar since it has been found in several groups of algae including green algae, in mosses and in gymnosperms. Thus, the PSI-M subunit appears to be absent only in angiosperms. With respect to the peripheral antenna proteins, algae are in fact very divergent (see [1] for a recent review). All photosynthetic eukaryotes have LHC proteins that belong to the same class of proteins. However, the LHCs associated with PSI appear to have diverged relatively early and the stoichiometry and interaction with PSI may well differ significantly between species. Even the green algae do not possess the same set of four Lhca subunits that is found in plants [1]. The situation is furthermore complicated by the presence of phycobilisomes in red algae and glaucophytes. Almost all biochemical studies of PSI have been carried out with cyanobacteria, green algae, and angiosperms. Our knowledge about PSI in other groups of *Chlorophyta* (e.g., mosses and even gymnosperms) as well as in algae apart from green algae is very limited. The little information about PSI in these groups is largely based on nucleotide sequences. In this review, we summarize the current knowledge about PSI in plants and green algae. PSI in other eukaryotes will only be covered to a very limited extent. Furthermore, the emphasis on the role of subunits will be on the features that are specific for plants as compared to cyanobacteria.
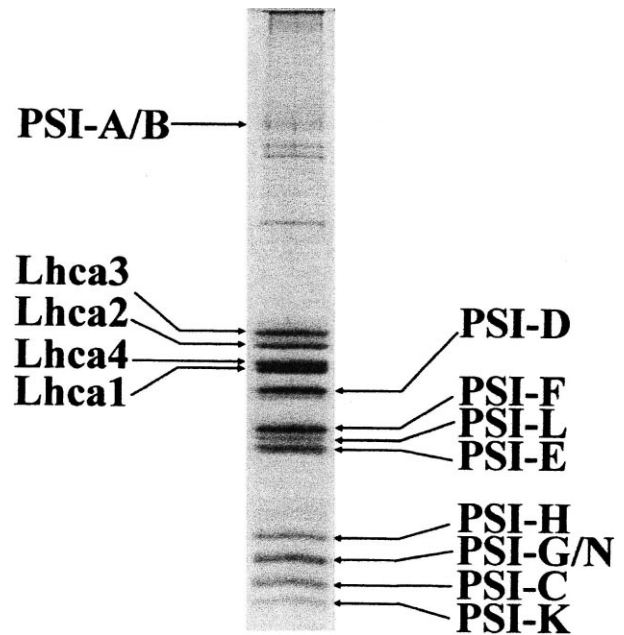

Fig. 2. The polypeptide composition of *Arabidopsis* PSI as determined by SDS–polyacrylamide gel electrophoresis. PSI complexes were prepared by mild solubilization of thylakoid membranes with dodecyl-β-D-maltoside followed by sucrose-gradient ultracentrifugation as described [80]. Proteins were separated by 16–23% gradient gels [82]. Identification of the individual proteins is based on immunodetection.

2. Role of PSI core subunits

2.1. The acceptor binding subunits PSI-A, PSI-B, and PSI-C

The most essential part of PSI is the three subunits that bind the electron acceptors. These subunits are highly conserved in all organisms and one may expect that their functions will be very similar between plants and other organisms. The subunits are all encoded in the chloroplast genome. The conservation of the central subunits goes beyond PSI as also green sulfur bacteria and heliobacteria have reaction center complexes that have homologues of PSI-A and PSI-B [5,6]. In fact, structural analyses have revealed that the central part of PSI, which is composed of PSI-A and PSI-B, is structurally similar to the central part of PSII composed of D1, D2, CP47 and CP43 [7]. For recent reviews on electron transport in PSI see [8] and reviews in this issue.

2.1.1. PSI-A and PSI-B

The heart of PSI is the reaction center P700. This is a chlorophyll dimer and spectroscopic evidence indicates little difference from the cyanobacterial counterpart. The same observation holds for the electron acceptors A_0 (a chlorophyll *a* molecule), A_1 (phylloquinone) and F_X (a [4Fe–4S] iron–sulfur cluster). Based on the sequence conservation of PSI-A and PSI-B of different species and the spectroscopic evidence it can be assumed that the electron transport components are bound to the PSI-A/B heterodimer and function essentially in the same way in all organisms. The PSI-A/B heterodimer can be isolated as a complex known as chlorophyll *a*-complex 1 (CP1) and has long been known to contain most if not all of the pigments in PSI. However, the recent structural information obtained by X-ray crystallography has led to a modification of this view. Some of the pigment molecules in *Synechococcus* PSI have turned out to be bound to the small subunits rather than to PSI-A/B [9]. This situation is likely also the case in plant PSI. However, there is no direct evidence for pigment binding to small subunits in plant PSI (but see below under PSI-F, PSI-G, PSI-K, and PSI-L).

Because the *psaA* and *psaB* genes are located in the chloroplast genome, it has been possible to make site-directed mutagenesis of these genes in the green alga *Chlamydomonas* [10–17]. These investigations have been helpful in defining the amino acid residues that bind F_X [14,17] and interact with PSI-C [13] as well as the ligands to P700 [12,18]. However, the conclusions from these investigations do not indicate any differences in the structure or function of the electron transport pathway between eukaryotes and cyanobacteria.

The rate of forward electron transport from A_1 to F_X is still a subject of controversy [8]. In addition, while it is mostly assumed that only one of the two quinones functions as A1, this has not been unambiguously shown [8]. Studies of cyanobacterial PSI have generally supported the view that only one phylloquinone is active (see, e.g., [19]). However, a recent investigation of *Chlorella* PSI led to the suggestion that both phylloquinones are active in electron transfer [20]. Interestingly, although the PSI-A and PSI-B subunits are highly conserved, it has been demonstrated that nuclear encoded subunits of PSI have a large effect on the rate of electron transfer from A_1 to F_X in *Chlamydomonas* [11]. In our opinion, the possibility remains that some of the discrepancies are related to differences between cyanobacterial and eukaryotic PSI. However, no comparative investigations have been performed.

2.1.2. PSI-C

The small PSI-C protein binds the terminal electron acceptors F_A and F_B, which are both [4Fe–4S] clusters. The protein is highly conserved and no doubt has a very similar function in all organisms. The location of *psaC* gene in the chloroplast genome is somewhat surprising. In plants and green algae, most of the PSI subunits have had their genes transferred to the nucleus in the course of evolution. The exceptions seem to be characterized primarily by high hydrophobicity, which may be presumed to pose an obstacle to the import of precursor proteins into the chloroplasts. However, PSI-C is a very hydrophilic protein and is perfectly soluble. The isolated PSI-C holoprotein is highly unstable in the presence of oxygen. However, chloroplast proteins that are encoded in the nucleus normally have their cofactors incorporated after they have been imported into the chloroplast. It has been hypothesized that genes may be retained in the chloroplast to ensure

proper redox regulation [21] and indeed the *psaA* and *psaB* genes are redox regulated [22]. However, the transcription and translation of *psaC* are constitutive and have no or little correlation with redox conditions [23].

F_A and F_B in plant PSI are similar to the cyanobacterial counterparts both in spectral properties and in midpoint potential. F_A and F_B in plants are more stable in the presence of chaotropic reagents. Treatment with for example urea is much more efficient in dissociating the PSI-C subunit from cyanobacterial PSI than from plant PSI [24,25]. However, this difference appears to be related to the different structure of PSI-D rather than to PSI-C itself or to the PSI-A/B heterodimer [24] (see below in Section 2.2.1).

The orientation of the PSI-C protein has been subject to some controversy. Reconstitution experiments carried out with PSI from barley [26] [27]and *Synechococcus* [28,29] showed that a central region of PSI-C of about eight amino acid residues could mediate binding to an F_X-containing core stripped of peripheral subunits. PSI-C bound to such a core was able to accept electrons from F_X and efficiently outcompete the backreaction from F_X to $P700^+$ [26,27]. This suggested that the PSI-C protein was oriented with the eight residues facing the PSI-A/B heterodimer. Unfortunately, reconstitution in the absence of PSI-D resulted in a relatively weak binding of PSI-C and thorough spectroscopic characterization of the complex was not possible [26,27]. Nevertheless, according to the contemporary structural model of *Synechococcus* PSI, the reconstitution experiments suggested that F_A should be the terminal acceptor. However, more refined structural analysis of *Synechococcus* PSI has shown that PSI-C is oriented differently with the eight residues facing away from the heterodimer and with F_B as the terminal acceptor [30]. Spectroscopic data and mutagenesis studies with both cyanobacteria and *Chlamydomonas* are in good agreement with this conclusion [31, 32, 32–35]. Thus, in view of the more recent evidence it appears that the binding of PSI-C observed in reconstitution experiments carried out in the absence of PSI-D was different from the normal binding. This would also explain the observation with *Synechocystis* that F_B rather than F_A is reduced at cryogenic temperatures when PSI-D is absent [36].

Knock-out of *psaC* has demonstrated an interest-ing difference between cyanobacteria and green algae. *Anabaena* lacking PSI-C assembled and accumulated a PSI complex with functional P700 [37]. In contrast, lack of PSI-C in *Chlamydomonas* resulted in complete destabilization of the PSI complex, which did not accumulate [38].

2.2. The extrinsic subunits at the stromal side of PSI

2.2.1. PSI-D

PSI-D is a hydrophilic subunit of about 18 kDa, which is exposed to the stroma. PSI-D is encoded in the nuclear genome and is therefore synthesized as a preprotein with a transit peptide. Once inside the chloroplast, the transit peptide is removed yielding the mature subunit. Compared to cyanobacteria, the mature eukaryotic PSI-D has an N-terminal extension of about 30 amino acid residues. In *Odontella* and *Porphyra* the protein is encoded in the chloroplast and the N-terminal extension is lacking [39,40].

PSI-D is known to interact strongly with ferredoxin. Chemical cross-linking of PSI and ferredoxin consistently yield a product consisting of PSI-D and ferredoxin [41–43], and recently the interaction has been shown even with isolated PSI-D and ferredoxin [44]. These observations clearly point to an important function of PSI-D in docking of ferredoxin in both eukaryotes and cyanobacteria. The stromal subunits PSI-C, PSI-D and PSI-E can be dissociated from the PSI-F_X core by treatment with urea. However, as discussed above (Section 2.1.2), a much harsher treatment is required to dissociate the proteins from eukaryotic PSI than from cyanobacterial PSI. Functional PSI-C can be rebound to the plant PSI complex, but the reconstitution only yields stable assembly when PSI-D is also present [24]. The same is true for cyanobacterial PSI [45]. Reconstitution of barley PSI with *Nostoc* PSI-D was possible, but PSI-C was not as tightly bound as in the presence of barley PSI-D [24]. Reconstitution in the presence of truncated barley PSI-D lacking the N-terminal extension was equivalent to using *Nostoc* PSI-D. Thus, the N-terminal domain of eukaryotic PSI-D is responsible for the tight binding of PSI-C that is characteristic for plant PSI. The terminals of PSI-D in the structural model of *Synechococcus* PSI are not in contact with other subunits [30]. However, the

stabilizing effect of the extended N-terminal indicates that it is in contact with intrinsic subunits. PSI-D in barley has been found to cross-link to PSI-H [41], which is an integral membrane protein located near PSI-I and PSI-L. Therefore, we have suggested that the N-terminal domain of PSI-D exerts its stabilizing effect through interaction with PSI-H [24]. Recent data showing a destabilization in the absence of PSI-H is in good agreement with this [46] (see below under PSI-H, Section 2.3.1).

2.2.2. PSI-E

PSI-E is like PSI-D a hydrophilic subunit exposed to the stroma. PSI-E is encoded in the nucleus and the mature protein is about 11 kDa. Just like PSI-D, the mature PSI-E in plants has an extended N-terminal region. The extension is variable from 30–40 amino acid residues. As was the case for PSI-D, there is no extension in the chloroplast encoded PSI-E in *Odontella* [39], *Porphyra* [40], and *Cyanidium*, nor in the cyanelle-encoded PSI-E in *Cyanophora*. *Chlamydomonas* is peculiar in having PSI-E with a short extension of only about ten residues compared to the cyanobacterial PSI-E. PSI-E has been shown by cross-linking to be in contact with PSI-D and PSI-F [47], and this is confirmed by the *Synechococcus* model of PSI [30]. PSI-E in barley has also been found to be associated with ferredoxin:NADP$^+$ oxidoreductase (FNR) [48]. In cyanobacteria, FNR has a domain linking it to the phycobilisomes [49]. However, recent observations have shown that in spite of this domain, FNR does appear to interact with PSI-E [50]. The significance of the interaction between PSI-E and FNR is not understood.

The function of PSI-E in plants has been investigated by chemical treatments that dissociate the subunit from PSI. Weber and Strotmann dissociated PSI-E from spinach PSI and showed an effect on electron transport to the terminal acceptors in PSI-C [51]. However, in reconstitution experiments with barley PSI-cores and PSI-E there was no effect of PSI-E on electron transport to the terminal acceptors [24]. A possible explanation is that the dissociation of PSI-E led to other changes in the PSI complex. However, the recent improvement of the structural model of *Synechococcus* PSI showed that part of PSI-E is sandwiched between the PSI-A/B heterodimer and PSI-C [30]. Thus, it seems possible that PSI-E could

affect intracomplex electron transport under some conditions. Reconstitution experiments with barley PSI-E showed that NADP$^+$ reduction was more efficient in the presence of PSI-E although PSI-E was not essential [24]. This function of PSI-E in mediating efficient electron transport from PSI to ferredoxin (or flavodoxin) is in good agreement with studies of cyanobacteria lacking PSI-E. These studies showed that PSI-E was required for optimal electron transport to ferredoxin and flavodoxin although photoautotrophic growth was barely affected [52,53]. The report of Yu et al. [54] that PSI-E has a role in cyclic electron transport in *Synechococcus* PCC 7002 has not been investigated in parallel studies in plants. Recent investigations have shown that PSI-E has a very significant role in plants. *Arabidopsis* plants in which one of the two *psaE* genes were inactivated had significantly reduced amounts of PSI-E and showed several changes in phenotype [55]. The plants were pale green, high fluorescing and susceptible to photoinhibition. Growth of the plants was reduced about 50%. Similar results have been obtained in our laboratory with *Arabidopsis* plants where PSI-E was downregulated by antisense suppression (C. Lunde, H.V. Scheller, unpublished results). Our plants exhibited severe effects especially in the later stages and many plants did not survive to set seeds. The moderate importance of PSI-E for ferredoxin reduction would not appear to explain the pronounced phenotypic effect of the reduced amounts of PSI-E. More likely, PSI-E is important for the stability of PSI. Possibly, the lack of PSI-E in plants leads to a high susceptibility to photodamage of the PSI complex.

2.3. Integral subunits: the PSI-H, PSI-I, PSI-L cluster

Chemical cross-linking studies, X-ray crystallography, and functional studies of mutants have placed three integral subunits, PSI-H, PSI-I, and PSI-L, in contact with each other on one side of the complex. The subunits are located in the region that in cyanobacterial PSI mediates interaction between monomers in a trimer. All three subunits are directly or indirectly in contact with PSI-D [41,47]. A schematic model of PSI viewed from the stromal side is shown in Fig. 3. The figure illustrates the two clusters of
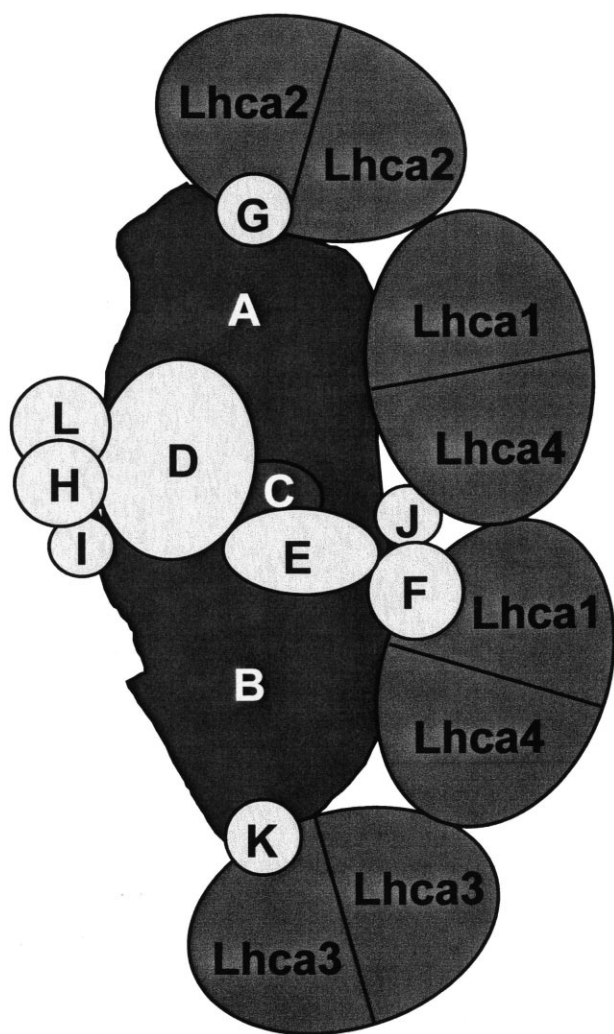
Fig. 3. Schematic view of PSI from the stroma. The position of the different subunits is deduced from cross-linking experiments, functional studies and X-ray crystallography. The location of the LHCI subunits on one side of the complex has been revealed by electron microscopy [81].

integral subunits, i.e., the H/I/L cluster and the F/J cluster.

2.3.1. PSI-H: the LHCII binding subunit

PSI-H is a 10 kDa protein with one predicted transmembrane helix. The subunit can be chemically cross-linked to PSI-D, PSI-I and PSI-L [41,47]. Thus, PSI-H must be located near the region that constitutes the domain of interaction between monomers in *Synechococcus* PSI [9]. PSI-H has only been found in plants and green algae. The orientation of PSI-H is not known but based on the positive-inside-rule [56], the N-terminal region is predicted to be in the stroma. Thus, PSI-H appears to have about 6 kDa of N-terminal region on the stromal side of the membrane and only about 2 kDa facing the lumen. PSI-H is encoded in the nuclear genome. Apparently, PSI-H was a late addition to PSI since it has not been found outside *Chlorophyta*.

Knowledge about the role of PSI-H comes from investigation of transgenic *Arabidopsis* plants lacking the subunit [46]. Surprisingly, plants lacking PSI-H are completely unable to perform state 1–state 2 transitions in response to changes in the spectral composition of light [57]. In the absence of PSI-H the antenna cross section of PSI remains identical to the wild type in state 1, irrespective of the illumination. In contrast, the antenna cross-section of the wild type in state 2 is significantly increased compared to state 1. These observations show that LHCII is associated with PSI in state 2, and most likely PSI-H forms the binding site for the interaction. In the absence of PSI-H, the redox-regulated phosphorylation of LHCII is unaffected. Surprisingly, LHCII appears to remain attached to PSII even in PSII (blue) light. In addition to the effect on state transitions, a number of other changes took place in the absence of PSI-H. In vitro photoreduction of $NADP^+$ was decreased about 40% under saturating ferredoxin concentration, but with limiting ferredoxin concentration there was no difference in electron transport rate. These observations indicate that the diffusion-limited reaction between PSI and soluble ferredoxin is not affected in the absence of PSI-H and therefore earlier steps in electron transport must be affected. In the absence of PSI-H, the PSI complex is less stable than in the wild type. Thus, in the presence of 6.5 M urea, dissociation of PSI-C was twice as fast as in the wild type. Furthermore, subsequent damage to F_X was seen only in PSI lacking PSI-H. The stabilizing effect of PSI-H is likely to be mediated through PSI-D. If the extended N-terminal of PSI-D is in contact with PSI-H, these data can explain the importance of the N-terminal for stability of the PSI complex (see above under PSI-D, Section 2.2.1).

Under optimal and constant conditions, plants lacking PSI-H are essentially indistinguishable from wild-type plants. The deficiency in PSI is compensated by an increase in the content of PSI. Under

standard growth conditions, the instability of PSI does not lead to dissociation of the complex in vivo. Hence, the compensation is sufficient to allow the plants to grow as well as the wild type and regulate electron transport.

2.3.2. PSI-L

PSI-L is an integral membrane protein with a mature size of about 18 kDa. The eukaryotic subunit does not contain any remarkable distinguishing features. An extended N-terminal of about 10 residues is present in plants but not in *Odontella* or *Porphyra*. PSI-L has not yet been reported from green algae. Based on the primary structure, the protein is predicted to have two membrane spanning regions connected by a short loop on the lumenal side. A large N-terminal region of about 10 kDa is predicted from the sequence to be on the stromal side of the membrane. In view of the recent high-resolution structural model of cyanobacterial PSI [155] it is however more likely that PSI-L also in plants form three transmembrane helices.

In cyanobacteria, a role of PSI-L in trimer interaction has been established [58]. However, PSI in plants probably does not form trimers. Transgenic *Arabidopsis* plants lacking PSI-L show many similarities to plants lacking PSI-H with respect to electron transport and regulation of photosynthesis [57], (P.E. Jensen, H.V. Scheller, unpublished). Transgenic plants where PSI-L is downregulated have a secondary loss of PSI-H and therefore the role of PSI-L in plants seems to be mediated in an interaction with PSI-H. However, since PSI-L is quite conserved between plants and cyanobacteria it is difficult to reconcile a role for PSI-L in interaction with PSI-H with the fact that PSI-H is not present in cyanobacteria. Most likely PSI-L plays an additional role in both cyanobacteria and plants, which is not yet understood.

2.3.3. PSI-I

The PSI-I subunit is a 4 kDa hydrophobic protein containing one transmembrane helix. PSI-I is encoded in the chloroplast genome and is quite highly conserved. The protein must be located near PSI-L and PSI-H since it has been possible to cross-link PSI-I to these two subunits [47]. In good agreement with the cross-linking studies, PSI-I has been located

in the structural model of cyanobacterial PSI near the domain for interaction between PSI trimers [9]. In cyanobacteria, knock-out of *psaI* leads to a substantial decrease in the amount of PSI-L and to a destabilization of trimers [59]. Similar experiments have not yet been carried out in plants or algae.

2.4. The lumenal subunits, PSI-F and PSI-N

2.4.1. PSI-F

PSI-F is an integral membrane protein of about 18 kDa. The protein is quite conserved but the eukaryotic PSI-F has two extra sequence segments in the N-terminal part. The 18 extra residues in the eukaryotic PSI-F appear to form an amphipathic helix located on the lumenal side of the thylakoid membrane [60]. The divergence of eukaryotic PSI-F has taken place much earlier than appears to be the case for the other subunits. Thus, the eukaryote-specific features of PSI-F are present also in *Odontella*, *Porphyra*, and *Cyanophora*.

PSI-F is the only integral membrane protein in PSI that is synthesized as a precursor protein with a lumenal-targeting sequence. Probably this targeting signal is necessary to transfer the positively charged N-terminal domain of about 9 kDa across the thylakoid membrane. PSI-F is predicted from the sequence to have just one transmembrane helix and hence it would represent a significant amount of protein (about 7 kDa) also on the stromal side of the membrane. However, the structural model of *Synechococcus* PSI suggests that PSI-F has additional segments buried in the membrane [30].

The role of PSI-F was suggested already in 1977 to be the docking of plastocyanin [61]. This suggestion was based on the observation that dissociation of PSI-F from PSI in the presence of nonionic detergents lead to a loss of interaction with plastocyanin. Considering how little was known about the organization of PSI at that time this suggestion was quite remarkable and it should take 18 years before it was confirmed [62]. Inactivation of the *psaF* gene in *Synechocystis* failed to confirm the role of PSI-F in plastocyanin docking [63]. However, the *psaF* gene was successfully inactivated in *Chlamydomonas* and this revealed that in eukaryotes PSI-F has indeed a role in docking of plastocyanin [62]. In the absence of PSI-F there was no stable complex formed between

PSI and plastocyanin, and the second order reaction rate was decreased by a factor of 20–100 depending on the conditions of the measurement [62,64]. Apparently, the amphipathic helix in the N-terminal region of eukaryotic PSI-F mediates the efficient binding of plastocyanin and fast kinetics that are characteristic of eukaryotic PSI. Indeed, introduction into *Synechococcus elongatus* of a modified PSI-F containing the N-terminal part of *Chlamydomonas* PSI-F led to a large increase in the rate of reaction with plastocyanin and cytochrome c_6 [65].

Although PSI-F had a significant effect on plastocyanin docking in *Chlamydomonas*, the algae devoid of PSI-F were almost not affected in photoautotrophic growth under normal light conditions. However, in high light, the *Chlamydomonas* lacking PSI-F became lethally photoinhibited [66]. In contrast to this, *Arabidopsis* plants devoid of PSI-F are barely able to survive even at low light intensity [67]. The plants are tiny and grow very slowly (Fig. 4). In plants lacking PSI-F, the second order rate constant for electron donation from plastocyanin to P700 is reduced about 13-fold. This effect is similar to the effect known from *Chlamydomonas*. Furthermore, plants lacking PSI-F are severely affected in the energy transfer from LHCI. Thus, although Lhca1 and Lhca4 remain in the PSI complex, they cannot transfer energy to the reaction center in the absence of PSI-F. This suggests that PSI-F in LHCI-containing plants and green algae should have regions optimized for interaction with LHCI. However, PSI-F proteins of plants and green algae do not contain a common motif that differs from PSI-F of other species. A possible explanation is that the involvement of PSI-F in LHCI function is specific for plants. As mentioned above, LHCI in *Chlamydomonas* has a somewhat different composition than in plants [1,68]. Unfortunately, no data are available on the energy transfer of PSI in *Chlamydomonas* lacking PSI-F. The inefficiency in the peripheral antenna in plants lacking PSI-F does not explain the severe change in phenotype. Under optimal growth conditions with sufficient light, plants lacking LHCI function would still be expected to grow reasonably well. A clue to the function of PSI-F is the secondary reduction in the stromal subunits PSI-C, -D, and -E. Presumably, the lack of PSI-F leads to a loss of stromal subunits, which is accelerated by the generation of active oxy-
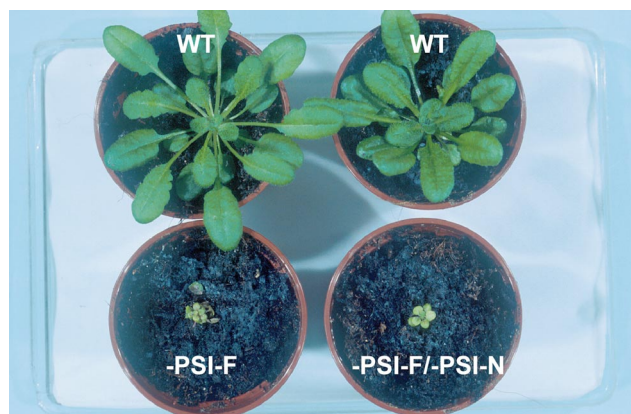

Fig. 4. *Arabidopsis* plants transformed with an antisense-*psaF* construct have very low levels of PSI-F (2–10%). The plants grow much more slowly than the wild type and are severely dwarfed. A double mutant lacking both PSI-F and PSI-N shows the same phenotype as the plants lacking only PSI-F.

gen at the reducing side of PSI. This in turn leads to more inactivation of PSI. This cascade of events may be similar to the effects taking place in plants lacking PSI-E. In *Synechococcus*, the absence of PSI-F affects the environment of A_1 [19]. Although, a destabilization was only seen in the presence of Triton X-100, this result suggests that lack of PSI-F could influence the quinone binding or function in plants.

2.4.2. PSI-N

PSI-N is a small extrinsic subunit of about 10 kDa. PSI-N is synthesized with a presequence directing it to the lumen and is the only subunit located exclusively on the lumenal side of PSI [69]. Unlike other lumenal proteins, the presequence is not cleaved by the stromal processing peptidase to an intermediate form prior to translocation across the thylakoid membrane. So far, PSI-N has only been identified in PSI from plants but genes encoding homologues of PSI-N are present in both *Chlamydomonas* and *Volvox*. PSI-N is quite easily dissociated from PSI [70]. Dissociation of PSI-N from spinach PSI was reported not to have effect on electron transport [70]. However, recent investigations of transgenic *Arabidopsis* devoid of PSI-N have shown an involvement of PSI-N in the docking of plastocyanin [71]. In the absence of PSI-N the second order rate constant for the reduction of P700$^+$ by plastocyanin was decreased about 40%. Steady state photoreduction of NADP$^+$ was lowered to a similar extent.

Thus, PSI-N plays a role in the efficient interaction with plastocyanin. It is not known if this role of PSI-N is mediated through a direct interaction with plastocyanin or through a modifying effect on PSI-F. Transgenic *Arabidopsis* plants lacking PSI-F also have no PSI-N [67].

Arabidopsis plants devoid of PSI-N are not affected in growth under optimal conditions [71]. The plants compensate for the deficiency in PSI by increasing the content of PSI. With this compensation, the plants are able to utilize light almost as efficiently as the wild type and to maintain normal redox conditions in the intersystem chain. However, under conditions that are more adverse, the lack of PSI-N does lead to increased photoinhibition (A. Haldrup, H.V. Scheller, unpublished).

2.5. Integral subunits: PSI-J and PSI-M

2.5.1. PSI-J

PSI-J is a hydrophobic subunit of 4–5 kDa. The protein is chloroplast encoded as is the case also for PSI-I, which has a similar size and hydrophobicity. PSI-J is located near PSI-F as evidenced by crosslinking [47]. The protein has been thought to be membrane spanning, however, the structural model of cyanobacterial PSI suggest that PSI-J may form an unusual bend helix in the plane of the membrane [30].

In *Synechocystis* PCC 6803, deletion of the *psaJ* gene resulted in PSI particles containing only 20% of the normal level of PSI-F [72]. A recent study has shown a significance of PSI-J in stabilizing PSI-F also in *Chlamydomonas* [73]. However, in this case there was no decrease in the content of PSI-F. Instead, the lack of PSI-J in *Chlamydomonas* resulted in a functional heterogeneity where only 30% of PSI exhibited the typical fast kinetics of plastocyanin and cytochrome c_6 oxidation. In the remaining 70% of the PSI complexes, the oxidation of plastocyanin was as slow as in PSI devoid of PSI-F [73]. The double mutant lacking both PSI-J and PSI-F was similar to the mutant lacking only PSI-F. Thus, PSI-J in eukaryotes has a function in maintaining PSI-F in a conformation that enables efficient electron transfer from plastocyanin.

In view of the known importance of PSI-F for antenna function in plants, it is likely that deletion of PSI-J would also have implications for this. However, no data on the antenna function in *Chlamydomonas* lacking PSI-J or PSI-F has been reported.

2.5.2. PSI-M

The PSI-M subunit is found in cyanobacteria and was tentatively localized in the structural model of *Synechococcus* PSI near PSI-J and PSI-F [30]. However, the recent improvement of the structural model has shown that PSI-M is located near PSI-I and PSI-L [155]. The PSI-M protein is very small (3–4 kDa) comprising little more than a transmembrane helix. In most plants, PSI-M has not been found. Considering the large amount of work with plant PSI and with sequencing of plant genomes and cDNAs, it seems highly unlikely that the PSI-M protein is present in angiosperms. Indeed, no open reading frame encoding a protein with similarity to PSI-M exists in *Arabidopsis*. Nevertheless, an open reading frame that appears to encode PSI-M has been found in the chloroplast genome of several gymnosperms, in *Marchantia*, and in different algae taxa including green algae. The role of PSI-M is unknown in both cyanobacteria and eukaryotes. A possible role in cyclic electron transport has been suggested but no data has so far been reported [74].

2.6. The LHCI-binding subunits, PSI-G and PSI-K

PSI-G and PSI-K are integral membrane proteins of about 11 and 9 kDa, respectively. On SDS-polyacrylamide gels, both proteins tend to run faster than expected. The two subunits are treated together here because of their sequence similarity. The nomenclature is somewhat confusing since the two eukaryotic subunits are equally similar to the cyanobacterial subunit known as PSI-K [75]. The phylogenetic tree of PSI-G and PSI-K (Fig. 5) shows that two different PSI-G and PSI-K subunits have only been found in the chlorophytes. In the red algae *Cyanidium* and *Porphyra*, a PSI-K subunit is present which is similar to the cyanobacterial PSI-K, and no additional PSI-G subunit has been found in these organisms. The same is true for the cryptomonad *Guellardia*. Cryptomonads appear to have evolved from secondary endosymbiosis of a red alga [1,4]. In green algae, PSI-G and PSI-K are clearly homologous to the corresponding plant proteins (Fig. 5).
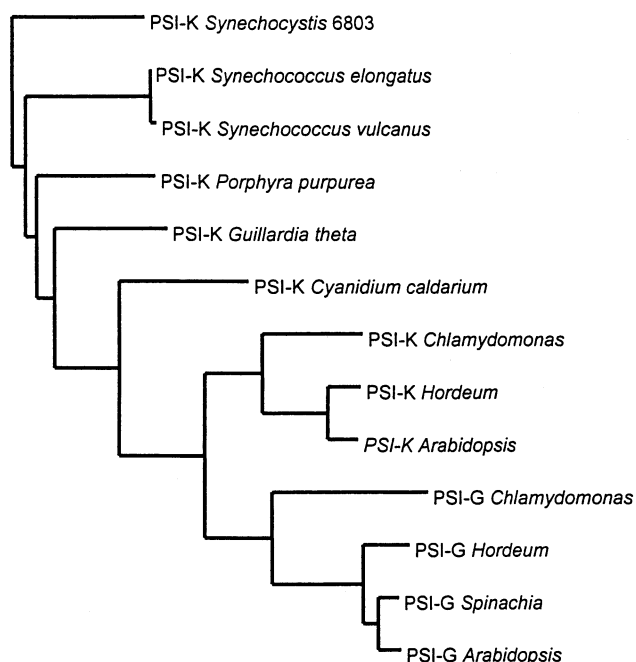
Fig. 5. Phylogenetic analysis of the PSI-G/PSI-K family of proteins. The amino acid sequences of the mature proteins were aligned, and the distances were calculated with the ClustalW program available at European Bioinformatics Institute (http://www.ebi.ac.uk). The tree was displayed using the Treeview program [154]. The following sequences from the Swissprot database were used: *Synechocystis* PCC 6803 (P72712), *Synechococcus elongatus* (P20453), *S. vulcanus* (P23318), *Porphyra purpurea* (P51370), *G. theta* (O78444), *Cyanidium caldarium* (P31567), *Chlamydomonas reinhardtii* PSI-K (P14225), *C. reinhardtii* PSI-G (P14224), *Hordeum vulgare* PSI-K (P36886), *H. vulgare* PSI-G (Q00327), *Spinachia oleracia* PSI-G (P12357). The sequences of *Arabidopsis* PSI-G and PSI-K are from M. Gilpin and P.E. Jensen (unpublished).

The improved structural model has shown that PSI-K is located away from the symmetry axis of PSI on the 'northern pole' [155]. Chemical cross-linking of plant PSI showed that PSI-G and PSI-K differed from all the other small subunits by not forming cross-linking products with other small subunits in the PSI core [47]. This suggested, in agreement with the refined model of the cyanobacterial PSI, that both subunits should be located away from the two-fold symmetry axis. PSI-K in plants is dissociated from PSI relatively easily in the presence of nonionic detergents such as Triton X-100, and PSI-G is clearly substoichiometric in PSI isolated in the presence of such detergents. This observation also points to a rather external location of the two subunits.

Cyanobacteria lacking PSI-K have been generated by several workers but no major change in the function of PSI has been reported [76,77]. A number of observations have provided circumstantial evidence for an involvement of PSI-G and PSI-K in interaction with LHCI in plants. Thus, the treatments that remove PSI-K from isolated PSI simultaneously remove LHCI [75]. Furthermore, a cross-linking product between PSI-K and Lhca3 was identified in barley and spinach, and a product between PSI-G and Lhca2 was tentatively identified [47]. Finally, the barley mutant *viridis-zb*[63], which is almost devoid of PSI, retains all the LHCI proteins in normal amounts [78]. In this mutant, all PSI core subunits except for PSI-G are essentially absent [79].

Very recently, the involvement of PSI-K in light-harvesting has been unambiguously shown in transgenic *Arabidopsis* plants. In the absence of PSI-K, the energy transfer from long wavelength chlorophylls was impaired and the content of Lhca2 and Lhca3 was decreased [80]. Electron transport was not affected in the transgenic plants lacking PSI-K, but state 1–state 2 transitions were somewhat reduced and during electrophoresis under mildly denaturing conditions, all four Lhca subunits were partially dissociated from PSI lacking PSI-K. Thus, PSI-K is not essential for attachment of LHCI to the core but is important for stable interaction and proper function of the antenna. Under standard conditions, the plants grew and developed almost as wild-type plants. Preliminary investigations with plants devoid of PSI-G have revealed a more dramatic reduction in state-transitions (nearly 50%) but less severe loss of antenna proteins compared to plants devoid of PSI-K. However, when analyzed by electrophoresis under mildly denaturing conditions, a more unstable interaction between core and the LHCI antenna was observed (P.E. Jensen, H.V. Scheller, unpublished results).

Recently, electron microscopy of single PSI particles has revealed that LHCI only binds to the core complex at the side of the PSI-F/J subunits [81]. In this study, it was further found that the PSI monomers had a tendency to associate into artificial dimers in which the monomers were oppositely oriented. Preliminary investigation of single PSI particles from plants lacking PSI-G or PSI-K have shown that in the absence of PSI-K no PSI

dimers are formed (P.E. Jensen, J. Dekker, E. Boekema, H.V. Scheller, unpublished data). This suggests that the PSI-K subunit in plants is actually located on the 'south pole' of the complex, i.e., opposite of the position of PSI-K in cyanobacteria. Furthermore, the absence of PSI-G is correlated with loss of protein density in the region corresponding to the 'north pole', i.e., the location of cyanobacterial PSI-K (Fig. 3).

3. The LHC subunits

3.1. LHCI

3.1.1. Protein composition

The Chl a/b-binding peripheral antenna of plant PSI (LHCI) is composed of the products of four nuclear genes, $Lhca1$–4, with molecular mass of 20–24 kDa (Table 1). [3,82]. In $Arabidopsis$, two additional genes were identified and named $Lhca5$ and $Lhca6$ ('second $Lhca2$') [83], but their expression is very low, so the proteins may not be normal subunits of LHCI. A number of $Lhca$ sequences have been determined from red algae ($Porphyridium$, $Galdiera$) and from green algae ($Chlamydomonas$, $Volvox$, $Euglena$) (for review see [1]). These sequences could not be assigned to higher plant $Lhca1$–4 types, reflecting a large divergence within LHCI composition.

PSI from plants can be isolated in an intact form with a Chl/P700 ratio of about 160–210. Assuming that 96 Chl are bound to the PSI core, LHCI should bind about 70–110 Chl $a+b$ molecules. LHCI can be subfractionated into antenna complexes that have been designated according to their 77 K fluorescence emission maxima [82,84,85]. The LHCI-680 subcomplex can be further fractionated into LHCI-680A (Lhca3) and LHCI-680B (Lhca2) [82]. The Lhca1 and Lhca4 subunits form the heterodimer LHCI-730, a subcomplex characterized by its 77 K fluorescence emission peak at 730 nm [86]. Lhca1/4 heterodimers can be assembled in vitro and, in contrast to the corresponding homodimers, they closely resemble the native LHCI-730 with respect to composition and spectroscopic properties [86,87]. In certain Chl b-free mutants of barley ($chlorina$-$f2^{f2}$, -$f2^{101}$ and -$f2^{108}$), the Lhca1 protein accumulates in the absence of Lhca4 [88]. Similarly, Lhca1 accumulates in transgenic $Arabidopsis$ plants lacking Lhca4 [89].

3.1.2. Biogenesis of LHCI

The biogenesis of the nuclear encoded LHC proteins involves their post-translational insertion into the thylakoid membrane. For the main light-harvesting protein in plants, Lhcb1, it has been shown that this integration proceeds in two steps. First, Lhcb1 forms a transit complex by interacting with chloroplast signal recognition particle (cpSRP) composed of cpSRP 54 and cpSRP 43 [90–92]. A chloroplast homologue of the SRP receptor α-subunit, cpFtsY, is the second soluble factor to reconstitute the soluble phase of Lhcb1 transport [93,94]. Second, Lhcb1 integrates into the thylakoid membrane in the presence of GTP, and the Lhcb translocon composed of or containing the chloroplast homologue of Oxa1p, the albino3 protein (ALB3) [95]. $Arabidopsis$ mutants lacking either cpSRP54 or cpSRP43 led to the conclusion that the three Lhca proteins Lhca1, Lhca3, and Lhca4 (and Lhcb1, 2, 3 and 5) are dependent on the cpSRP pathway [96]. Lhca2 (as well as Lhcb4, 6 and PsbS) is less dependent or independent of cpSRP. Nevertheless, incorporation of Lhca2 is dependent on GTP and stroma factors [97,98].

3.1.3. Pigment composition

The pigment compositions of the different Lhca proteins have not been quantitatively determined. Estimates have been based on comparison of the pigment content of PSI-200 versus LHCI-depleted PSI core, and on analysis of preparations of total LHCI [99,100]. Assuming that eight Lhca proteins build up plant LHCI, and including a recent LHCI pigment pattern [101], a single Lhca protein could bind about 1.3 mol β-carotene, 10.4 mol Chl a, 2.6 mol Chl b, 1.3 mol lutein, and 0.8 mol violaxanthin (Table 1). According to this estimate, the total number of bound chlorophylls is almost the same as in pea LHCII, where 7 Chl a, 5 Chl b, 2 lutein, and 1 neoxanthin are bound per monomer [102]. The number of about three carotenoids in each Lhca protein would implicate that the central cross, presumably consisting of two lutein molecules in LHCII, harbors two different carotenoids. Comparison with a Chl a-binding LHCI protein from the red alga $Porphyridium$ $cruentum$ enabled the identification of six amino

acid residues that bind Chl *a* in LHCI proteins [103]. Mutational analyses reached the same conclusion and identified one additional Chl *a*-binding site in LHCII [104–106]. Thus, it seems likely that in these sites seven Chl *a* can be found in Lhca proteins as well. This calculation gives higher numbers than found associated with in vitro reconstituted Lhca1/Lhca4 heterodimer, which binds 10 molecules Chl *a* and 4 molecules Chl *b* per dimer [86].

As mentioned above, PSI-F, PSI-G and PSI-K are involved in the interaction between LHCI and the PSI core, and chlorophyll molecules are bound to PSI-F and PSI-K in the *Synechococcus* PSI [9]. Furthermore, a considerable amount of Chl and β-carotene is selectively lost during preparation of LHCI from PSI-200 [100]. Together, these observations suggest that a considerable number of chlorophylls could function in connecting LHCI proteins and the reaction center. Xanthophyll cycle carotenoids are associated with Lhca proteins and the xanthophyll cycle operates in PSI as well. However, it is not known whether all or a subset of Lhca proteins are sites for the cycle [107–109].

3.1.4. Structure and topology

To obtain structural information of LHCI organization, electron microscopy with single particle analysis, chemical cross-linking, and pigment stoichiometries were used. Initial studies on negatively stained PSI preparations led to the proposal that the reaction center is surrounded by a monolayer of eight LHCI proteins [110]. Chemical cross-linking studies of PSI preparations from barley and spinach revealed that the LHCI subunits are organized as dimers, and associate to the PSI-A/B-proteins independently of each other [47]. However, the Lhca proteins could not be localized with confidence. As mentioned above (Section 2.6) recent data reveal that LHCI binds to the core complex at the side of the PSI-F/J subunits [81]. The binding of LHCI to the PSI-F/J-side could explain the copurification of PSI-F with LHCI from barley and maize [111], and with LHCI-680 from *Chlamydomonas* [68]. Similarly, the isolation of PSI-E together with LHCI-680B (Lhca2) from barley can be understood [82]. Furthermore, *Arabidopsis* plants lacking PSI-F are strongly affected in LHCI-730 antenna binding and excitation energy transfer to the reaction center indicating a functional association between PSI-F and LHCI-730 [67].

PSI complexes are usually assumed to have a homogeneous composition and in the recent study by Boekema and coworkers [81] it was estimated that the PSI antenna contained maximally eight monomeric units of LHCI, which is in agreement with earlier estimations [47,110]. However, if nonoptimal packing of the LHCI complexes and/or a detergent contribution to the LHCI area in the PSI-200 complex is taken into account, it is possible that each PSI-200 only contains six LHCI monomers [81]. Judged from electrophoretic patterns, all the Lhca proteins are present in about equal amounts (Fig. 2). However, it should be emphasized that it is possible that there are subpopulations of PSI with a different set of antenna proteins. Experimental evidence for a different antenna composition of PSI in different domains of the thylakoid membrane has been presented [112].

A working model of PSI-200 is proposed in Fig. 3. Cross-linking studies have shown that Lhca3, Lhca2, and Lhca1/4 dimers are in contact with other Lhca subunits, i.e., each Lhca protein is in contact with two others [47]. Thus, in view of the recent structural evidence [81], all Lhca dimers are placed next to each other on one side of the complex. The two Lhca1/Lhca4 heterodimers are located in contact with PSI-F/J near the interface between PSI-A and PSI-B. Whether Lhca2 and Lhca3 exclusively form homodimers or also can form heterodimers is still uncertain. In *Arabidopsis* plants devoid of PSI-K, the amount of both Lhca2 and Lhca3 is reduced and this seems to support the presence of Lhca2/Lhca3 heterodimers. Alternatively, Lhca2 or Lhca3 homodimers can associate both to the PSI-G and PSI-K end of PSI.

3.1.5. Far-red absorbing pigments in LHCI

A remarkable feature of LHCI is the presence of pigments with energy levels significantly lower than the reaction center itself. The 77 K fluorescence emission spectrum obtained from intact PSI shows a characteristic long-wavelength band around 735 nm (F735), which originates from LHCI [82,84,85]. Removal of LHCI leads to PSI reaction center complexes that exhibit 77 K emission of core antenna Chl at 720 nm (F720) [82,84]. In cyanobacterial

PSI lacking LHCI proteins, F720 emission could be attributed to a small number of Chl *a* molecules that connect the bulk of core antenna chlorophyll through excitation energy transfer to P700 [113–115]. These Chl molecules may act as a sink to focus excitons near P700.

Using both steady-state and time-resolved techniques, the red Chl forms, accounting for 5–7% of the total PSI absorption, were shown to dominate the fluorescence emission at room temperature [114,116]. For maize PSI [116] and for *Synechococcus* PSI core [117], the room-temperature fluorescence spectrum can be almost exactly calculated from the absorption spectrum [118]. Thus, thermal equilibration of excited states between all energy levels in the system can be assumed. About 85–90% of the excited states at room temperature are associated with the red Chl forms indicating their importance in energy transfer to the reaction center [116,118].

The possible physiological functions for F735 have been discussed. Stahl et al. [119] argued that the pericentral long-wavelength chlorophylls are not essential for excitation energy transfer, but have a protective role against overexcitation. Mukerji and Sauer [120] suggested that F735 concentrates excitons near the reaction center, interacting with the long wavelength chlorophylls of the PSI core antenna. However, PSI has been shown to form a thermally equilibrated system at room temperature [116]. The red Chl slow down the excitation energy transfer [121,122] but this disadvantage is outweighed by the increase in absorption cross-section [121]. Model calculations demonstrated that the red Chl forms increase the light absorption in leaves exposed to 'shade light' [123,124]. Due to the transmission and reflectance properties of vegetation, shade light is enriched in wavelengths between 600 and 700 nm.

Despite the great interest in the long-wavelength chlorophylls responsible for F735, and a number of steady-state and time-resolved spectroscopic studies on isolated PSI-200 [125], on isolated dimers consisting of all LHCI proteins [118], or the isolated [126] or reconstituted Lhca1/Lhca4-heterodimer [87], the picture of the spectroscopic states of LHCI and their location remains unclear. Antenna subfractions isolated from spinach PSI using anion-exchange perfusion chromatography led to the localization of a Chl *b*-pool on Lhca4 responsible for an enhancement of

77 K fluorescence emission at 730 nm [85]. This is consistent with the conclusion that Chl *b* must be specifically associated with emission around 730 nm at 77 K [120]. The authors concluded that this long-wavelength emission originates from Lhca4 or emanates from an interaction between Lhca1 and Lhca4 subunits [85]. Recently, LHCI from maize PSI-200 complexes has been described as a mixture of two types of dimers [127]. In the LHCI-730-heterodimer consisting of Lhca1 and Lhca4, two Chls absorbing at about 711 nm and emitting at 733 nm could be identified. Two Chls with absorption and emission maxima at 693 and 702 nm, respectively, were attributed to homo- or heterodimers of Lhca2 and Lhca3 [127]. In vitro reconstitution experiments showed that monomers of Lhca1 fluoresce around 685–687 nm, whereas Lhca4 monomers had a 77 K fluorescence maximum at 730–732 nm [86,128]. Thus, in vitro the Lhca4 monomer has a long-wavelength fluorescence peak similar to the isolated LHCI-730 heterodimer. Most of this long wavelength 77 K fluorescence emission is shifted to 685–686 nm in the in vitro reconstituted Lhca4 monomers when the N-terminal 38 amino acids are removed indicating a direct or indirect implication of the N-terminal domain in the generation of this Chl form [129].

Croce et al. [116] identified three Chl spectral forms in native PSI from maize thylakoids with 77 K fluorescence maxima at 720, 730 and 742 nm. For each PSI complex, 10–12 Chl molecules seem to be associated with the three red forms. Two red Chl emitting at 720 nm are present in the isolated core [118]. The two other emission maxima could be assigned to individual Lhca proteins by analyzing *chlorina* and *viridis* mutants of barley with 77 K fluorescence measurements combined with immunoblotting. The two emission peaks at 730 and 742 nm emanate from Lhca1 and Lhca4, respectively, when these proteins are bound to the reaction center of PSI [78], in contrast to the emission at 685–687 and 730–732 in the isolated Lhca1 and Lhca4 proteins. This represents a remarkable example of the influence of protein environment on spectral properties of Chl. The red-most fluorescence at 742 nm has to be explained by an interaction between Lhca4 and the PSI core. In excellent agreement with this, *Arabidopsis* mutants lacking Lhca4 [89] or PSI-F [67] show a large blue-shift. Further evidence for a spe-

cific interaction of Lhca4 with chlorophyll(s) of the reaction center in producing a 742-nm emission comes from the fluorescence characteristics of the barley mutant *viridis-zb*[63]. Despite having less than 5% of wild-type levels of PSI-A/B [79], thylakoid membranes from *viridis-zb*[63] contain wild-type levels of all four Lhca proteins [78]. The 77 K fluorescence has an emission maximum at 730 nm in this mutant like in isolated LHCI-730, and the red Chl form emitting at 742 nm is absent.

In our opinion, the finding that 77 K fluorescence emission at 730 and 742 nm is only found when Lhca1 and Lhca4 are associated with the PSI reaction center, suggests that the long wavelength chlorophylls are located on Lhca1 and Lhca4. However, Kochubey et al. [130] interpreted the finding as due to the creation of conditions for energy transfer to Chl forms with 730 and 742 nm fluorescence bands in a location close to the reaction center.

3.2. Lhcb1 and Lhcb2

Lhcb1 and Lhcb2 are the most abundant proteins in LHCII trimers. The majority of LHCII trimers are bound to PSII [131]. However, a fraction of LHCII is able to dissociate from PSII thereby decreasing the antenna size of PSII. The reversible dissociation of LHCII is responsible for the state transitions that serve to balance electron flow in PSI and PSII. Apparently, the dissociation is controlled by a protein kinase that is regulated by the redox level of the plastoquinone pool. Kinase activation requires interaction of plastoquinol with cytochrome b_6/f-complex in which the high potential path is reduced (for a review see [132]). There has been some controversy whether the mobile pool of LHCII in fact docks onto PSI and increases PSI cross section. However, Lhcb1 and Lhcb2 were associated with PSIα and with PSIβ in stroma lamellae [112] and PSIβ seems to have a full complement of eight Lhca proteins plus one LHCII trimer (or three LHCII monomers) attached. Furthermore, an increase in PSI cross section upon state 1 to state 2 transition has been observed in both plants [57] and green algae [133]. Phosphorylation of the N-terminal region of LHCII leads to intramolecular conformational changes that may initiate movement of LHCII [134,135]. However, phosphorylation seems to take place after a light-induced conforma-

tional change of LHCII [129]. In any case, the conformational change could affect the trimerization motif in the N-terminal segment thereby allowing the trimers to dissociate from PSII and associate with PSI as monomers of Lhcb1/2 [135]. The site of docking of LHCII on PSI seems to be PSI-H (see above in Section 2.3.1). In the absence of PSI-H, there is no change in nonphotochemical fluorescence quenching in state 2-light [57]. This suggests that dissociation of LHCII from PSII is not taking place unless LHCII can associate with PSI-H. We suggest that state transitions represent a change in the binding equilibrium of LHCII with PSI and PSII. Thus, when PSI-H is absent, LHCII cannot bind to PSI and effectively remains associated with PSII. In agreement with this view, LHCII remained functionally associated with PSII in *Chlamydomonas* lacking PSI complexes irrespective of the degree of LHCII phosphorylation [133].

The correlation between state transitions and phosphorylation of LHCII has been demonstrated in numerous studies and no other regulatory mechanism has been proposed. Nevertheless, a demonstration that LHCII phosphorylation causes or is required for state transitions has in fact not been presented. Phosphorylation of LHCII could be coincidental or could have a function for example in preventing proteolytic breakdown of exposed LHCII.

4. Assembly and turnover of eukaryotic PSI

4.1. Assembly

A detailed review of assembly is beyond the scope of this review. The assembly of PSI and other thylakoid protein complexes has recently been reviewed extensively [136]. Below only a short reference is given to some recent advances in the field.

A number of known factors are involved in the assembly of eukaryotic PSI. A chloroplast gene, *ycf3*, has been disrupted in both *Chlamydomonas* [137] and tobacco [138]. In both cases, the result was a severe deficiency in PSI. Transcription of PSI genes was however not affected. The Ycf3 protein was localized to thylakoid membranes but was not part of the PSI complex. Apparently the Ycf3 protein participates in the assembly of the PSI complex

but the mechanism is still not understood. A second open reading frame in the *Chlamydomonas* chloroplast genome, *ycf4*, was also disrupted [137]. The results were similar to the results with *ycf3*.

Recently, two maize mutants, hcf47 and hcf44, that are deficient in PSI have been described [139]. In hcf47, the defect was more general since also PSII was deficient. However, the hcf44 mutant is specifically deficient in PSI and the results point to a defect in the synthesis or assembly of PSI-C.

The barley mutant *viridis-zb*[63] was originally described as deficient in PSI [140]. The mutation is seedling lethal. A careful analysis has shown that the plants contain about 2% of active PSI, which appears to contain all subunits [79]. Transcript levels of all the PSI genes are normal in the mutants and the essential subunits PSI-A, PSI-B, and PSI-C are synthesized in normal amounts. Apparently, assembly of PSI is affected by an unknown mechanism.

4.2. Photoinhibition of PSI

Photoinhibition has traditionally been thought of as a process that primarily affects PSII. However, it is now clear that under conditions of moderate light and chilling temperatures PSI and not PSII is the primary target of photoinhibition in chilling-sensitive plants [141–144]. In chilling-tolerant plants, PSI and PSII are about equally affected by such photoinhibitory treatments [144,145]. However, an important difference is the very slow recovery of PSI compared to PSII. Under optimal conditions, photodamaged PSII is fully rebuild in a few hours [146]. The repair process for PSII has been studied in considerable detail [147,148]. Damaged PSII is transferred from the grana stacks to exposed regions of the thylakoid membranes, partly disassembled, and reassociated with de novo synthesized D1. The damaged D1 protein is removed in the process and the repaired PSII is transferred back to the grana stacks. Thus, a damage to PSII will have little long-term effect on photosynthetic capacity. In contrast, for PSI the repair process is much slower taking several days [145] [149] (J. Knoetzel, H.V. Scheller, unpublished data). We believe that PSI damage is much more severe since a damage that takes place during a very short exposure to unfavorable conditions will affect the ability to photosynthesize in many days following

the exposure. In this view, PSII photodamage may in fact be a process optimized to protect PSI from damage.

Photodamage to PSI is associated with the formation of active oxygen species at the stromal side of PSI. Superoxide or hydrogen peroxide in combination with reduced PSI acceptors is required for the damage to occur [150]. The damage initially destroys the terminal iron–sulfur clusters in PSI-C and subsequently more severe damage to the earlier electron acceptors may take place. Simultaneously with the damage to the electron acceptors, some breakdown of proteins, particularly PSI-B, is seen [151]. PSI-A is also partly degraded during photoinhibition [144]. We do not know whether the protein breakdown is part of a repair cycle, or if it is coincidental to the damage to the electron acceptors. Whether damaged electron acceptors can be repaired without the complete disassembly and resynthesis of PSI is not known. However, it would seem feasible that damage restricted to PSI-C could be repaired in a process where only the stromal subunits are replaced. Unfortunately, there is almost no information available about the repair processes. Pulse-chase experiments would be the logical way to study turn over of proteins but the very slow process of PSI repair makes such experiments difficult. Immunological studies have indicated that substantial disassembly and resynthesis of the entire PSI complex is required for the repair process in cucumber (J. Knoetzel, H.V. Scheller, unpublished).

4.3. Regulation of PSI

The balance between PSI and PSII is maintained by mechanisms that are only partly understood. The regulation is beyond the scope of this review. However, it should be noted that plants with slightly inefficient PSI units, such as the *Arabidopsis* plants lacking subunits PSI-H, PSI-N, or PSI-K, all respond by increasing the PSI/PSII ratio [46,71,80]. We do not know how the imbalance is sensed and how the signal transduction is mediated. Apparently, plants regulate the redox state of the plastoquinone pool both in the short term by state transitions and in the long term by modulating transcription of key chloroplast genes [22]. The short-term regulation is thought to be mediated through the redox activated

LHCII kinase [135] (see above, Section 3.2). The long-term regulation is thought to be mediated also through a redox-activated kinase that triggers a cascade ultimately leading to regulation of gene expression [22,152].

5. Concluding remarks

The understanding of the role of eukaryotic PSI subunits has reached a high level. Investigation of function in transgenic plants has been a particularly important development and allowed studies of physiological significance in vivo. The steady improvement of the structure of *Synechococcus* PSI has inspired a tremendous amount of research. Clearly, a better structural model of plant PSI would enable the study of plant-specific issues to move on. With plant PSI, electron microscopy of two-dimensional crystals [153] or single particles [81] are promising although the resolution has so far been relatively low. A major unresolved question is the stoichiometry and organization of LHC proteins. Whereas the basic structure and function of the eukaryotic PSI is now relatively well understood, much remains to be learned in the area of assembly and turnover of PSI. The discovery that PSI plays a crucial role in the regulation of state transitions calls for renewed attention to the mechanism of state transitions and for investigations of their biological significance.

Acknowledgements

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