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Photosystem I and Regulatory Proteins for its Biogenesis

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ABSTRACT

Photosystem I (PSI) is a pigment-protein complex located in the thylakoid membrane of cyanobacteria and chloroplasts of algae and higher plants, which functions as a plastocyanin (or cytochrome c_6)-ferredoxin oxidoreductase. The reducing potential of ferredoxin is utilized for a variety of biochemical processes such as the reduction of $NADP^+$, or the assimilation of nitrate or sulfate. In higher plants the complex consits of 14 different polypeptide subunits, nine of them are encoded in the nucleus (PSI-D/E/F/G/H/K/L/N/O) and the residual five (PSI-A/B/C/I/J) are plastid-encoded. Most of the information about the function of PSI derives from mutants impared in one or more subunit genes or from biochemical studies. In 2003, the crystal structure of PSI from a higher plant was determined. In contrast to the trimeric cyanobacterial PSI, the plant PSI was purified as a monomer. Compared to the structural information, much less is known about the regulation and assembly of PSI and only a few regulatory factors have been identified so far. In this review we describe the structure of PSI and the role of the individual subunits and present an overview on those factors which are required for PSI accumulation in pro- and eukaryotic photosynthetic organisms.

Keywords: *Arabidopsis*, chloroplast, mutant, photosynthesis, PsaA/B

CONTENTS

THE PHOTOSYSTEM I COMPLEX

The photosystem I (PSI) is a pigment-protein complex located in the thylakoid membranes of cyanobacteria and chloroplasts of algae and higher plants, which functions as a plastocyanin (or cytochrome c_6)-ferredoxin oxidoreductase. The complex consists of a reaction center core and an associated light-harvesting antenna complex (LHC) which is composed of chlorophylls, carotenoids and chlorophyll *a*/*b*-binding proteins (LHCPs) required for capturing most of the light energy. Genes for six LHCPs have been identified in *Arabidopsis* (cf. Jansson 1999 and references therein) and at least one copy of four LHCPs, LhcaI-4, is present in a PSI-LHCI complex (Ben-Shem *et al*. 2003; Ballottari *et al*. 2004). The presence of a fifth LHC polypeptide, Lhc5, in the PSI antenna has also been reported (Ganeteg *et al*. 2004). The antenna size of PSI varies depending on the light intensity and spectral distribution as well as on other environmental factors (Bailey *et al*. 2001). Light is also captured by chlorophylls and β-carotenes associated with the reaction center core (Jordan *et al*. 2001), which function as additional inner antenna. The excitation energy captured by the pigments is delivered to a special chlorophyll-pair, P_{700}, in the reaction center (RC) of the core complex. P_{700} is responsible for charge-separation, which is followed by a series of redox reactions and ultimately the reduction of ferredoxin at the reducing site of PSI. The reducing potential of ferredoxin is utilized for a variety of biochemical processes, such as the reduction of $NADP^+$, or the assimilation of nitrate or sulfate (Ben-Shem *et al*. 2003; Nelson and Yocum 2006).

In 2003, the crystal structure of PSI from a higher plant was determined (Ben-Shem *et al*. 2003). In contrast to the trimeric cyanobacterial PSI (Chitnis 2001; Jordan *et al*. 2001), the plant PSI was purified as a monomer. At least 14 different polypeptide subunits (PSI-A-L, PSI-N and PSI-O) are required for the backbone of the PSI core of higher plants (Chitnis 1996, 2001; Jensen *et al*. 2004). In eukaryotes, five of them, PSI-A, PSI-B, PSI-C, PSI-I and PSI-J, are plastome-encoded, while the residual ones are encoded in the nucleus (Shinozaki *et al*. 1986; Hayashida *et al*. 1987; Sugiura 2003). Five subunits (PSI-G, -H, -N, -O and -P) have not been detected in cyanobacteria, while PSI-M is not present in the PSI of angiosperms (cf. below).

PSI-A and PSI-B, the two largest polypeptide subunits with 11 transmembrane helices each, form a heterodimer and bind the primary electron donor P_{700}, the electron acceptors A_0 (a chlorophyll *a* molecule), A_1 (a phylloquinone), F_X (a [4Fe-4S] cluster) and most of the remaining PSI-cofactors including chlorophyll *a* and β-carotene molecules. The terminal two cofactors involved in the electron transfer, the two [4Fe-4S] clusters F_A and F_B, are bound by PSI-C at the reducing site of the complex (Chitnis 2001).

The initial steps in PSI biogenesis are the formation of the heterodimer PSI-A/B. In *Chlamydomonas reinhardtii*, PSI-B seems to be required for stable PSI-A accumulation

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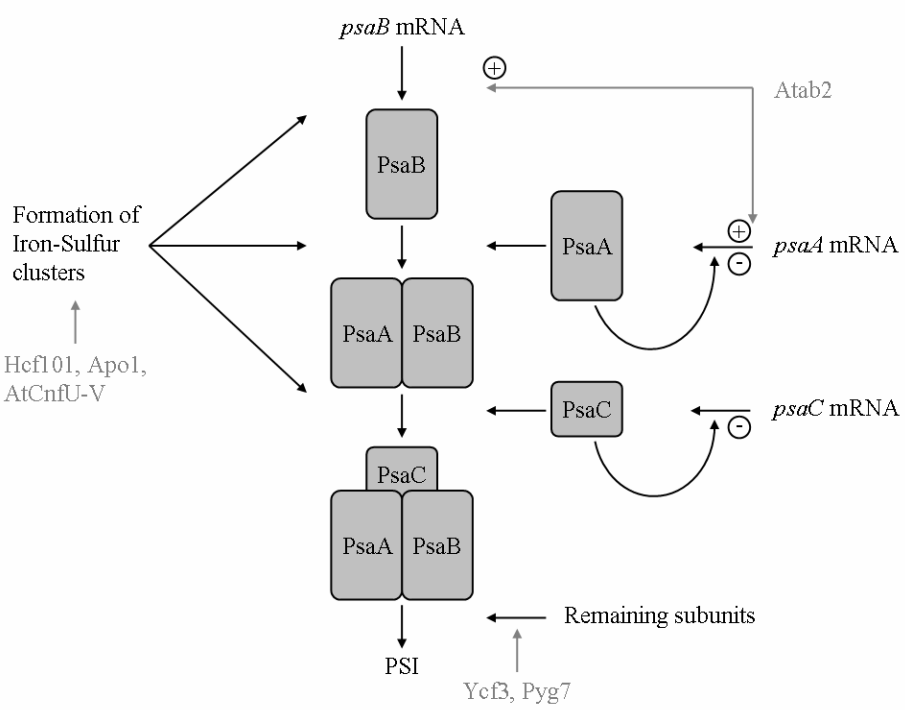

Fig. 1 Proposed model for the early steps in PSI biogenesis in *Arabidopsis*. In this model PsaB is the main trigger for PSI biogenesis. PsaB and PsaA assemble at an early time-point, followed by PsaC. PsaB is required for PsaA synthesis and thus PsaA/B is required for PsaC synthesis. Both, PsaA and PsaC repress the translation of their own messages in the absence of PsaB and PsaA, respectively (as shown for *Chlamydomonas*, cf. Wostrikoff *et al*. 2004). The remaining subunits assemble at a later time. PSI contains three iron-sulfur clusters, bound to PsaA/B and PsaC, respectively. The proteins Hcf101, Apo1 and AtCnfU-V have been identified to be involved in biogenesis and formation of iron-sulfur clusters. Atab2 is proposed to activate photosystem core protein translation. Ycf3 interacts with PsaA and PsaD, Pyg7 is a TPR protein that co-purifies with PSI.

and translation of its message (cf. below). Subsequently, the presence of PSI-A is required for stable PSI-C accumulation and association with the PSI-A/B dimer (Wostrikoff *et al*. 2004).

MUTANT ANALYSIS HELPED TO ELUCIDATE THE ROLE OF POLYPEPTIDE SUBUNITS IN PSI FUNCTION

Besides the crystal structure, most of the information about the function of the PSI subunits derives from mutants impaired in one or more subunit genes or from biochemical studies. The importance of the PSI-A/B dimer for the assembly of the complex has been demonstrated for a variety of pro- and eukaryotic mutants. Mutants lacking PSI-A/B generally fail to assemble the entire core complex, although some of the more peripheral subunits can accumulate in the thylakoid membranes. In a *Synechocystis psaA* mutant, generated by insertional mutagenesis (Smart *et al*. 1991), the *psaA*/*B* mRNA is truncated and the P_{700} apoproteins cannot be detected. Similarly, a *Synechocystis* strain lacking a functional *psaA*/*B* operon shows no accumulation of the PSI core, does not grow autotrophically and has dramatically reduced chlorophyll levels (Shen *et al*. 1993). This was also confirmed for the eukaryotic unicellular green algae *Chlamydomonas* (Cournac *et al*. 1997; Redding *et al*. 1999). In eukaryotes, the three cofactor-binding proteins PsaA, -B and -C are encoded by plastid genes and their dommain structures are highly conserved. PSI-A and PSI-B assemble at an early step of PSI biogenesis (Smart *et al*. 1993), forming the chlorophyll *a*-protein complex CPI, that binds most of the cofactors and pigments of PSI. PSI-B is required for significant PSI-A synthesis and in the absence of PSI-B, unassembled PSI-A represses its own translation (Wostrikoff *et al*. 2004; cf. **Fig. 1**). CPI is the template for PSI-C binding. PSI-C, a 9-kDa protein located at the reducing site of the complex, coordinates the Fe-S clusters F_A and F_B through two cysteine-rich domains. Again, in the absence of CPI, unassembled PSI-C represses its own translation. This suggests that significant accumulation of PSI-A and -C occurs only when the previous assembly steps are completed. Thus, PSI-B accumulation is the main control step in the biogenesis of PSI in *Chlamydomonas* (Wostrikoff *et al*. 2004).

Studies on PSI-C revealed differences between pro- and eukaryotic PSI. While the prokaryotic PSI assembles properly in PSI-C deficient mutants and is functional (Mannan *et al*. 1991), the absence of this protein in eukaryotes leads to an unassembled PSI (Takahashi *et al*. 1991). This indicates that besides evolutionary conservation, the importance of this subunit for the electron transfer and the assembly process differs.

The other, more peripheral subunits bind to the assembled PSI-A, -B and -C core complex. *Arabidopsis* mutants lacking PSI-D do not grow photoautotrophically, have reduced levels of all other subunits and do not assemble a functional PSI complex (Haldrup *et al*. 2003). PSI-D and -C provide a docking niche for ferredoxin and PSI-D is also involved in the stable association of PSI-C, -E and -L into the PSI complex (Kruip *et al*. 1997; Xu *et al*. 2001). A transposon insertion line of *Arabidopsis* with disrupted *PsaE1* (Varotto *et al*. 2000) showed a light sensitive phenotype and had increased levels of chlorophyll fluorescence and photoinhibition. Besides its structural role on the reducing site of the complex, PSI-E is also involved in the cyclic electron flow. The role of PSI-F as plastocyanin docking site was shown for *Chlamydomonas* and *Arabidopsis* (Farah *et al*. 1995; Haldrup *et al*. 2000). Inactivation of *PsaF* in *Chlamydomonas* results in impaired electron transport from plastocyanin to PSI, although PSI still assembles and mutants can still grow photoautotrophically (Farah *et al*. 1995). In contrast, PSI-F in *Arabidopsis* is important for photoautotrophic growth. Plants lacking PSI-F are chronically photoinhibited and show disturbed energy transfer from Lhc1 to P_{700} (Haldrup *et al*. 2000). In cyanobacteria, PSI-F provides the docking site for cytochrome c_6.

PSI-G and -H are only present in green algae and higher plants and *Arabidopsis* mutants lacking PSI-G or PSI-H grow like wild type plants under standard conditions (Naver *et al*. 1999; Jensen *et al*. 2002). Kjaerulff *et al*. (1993) suggest that PSI-G has the same ancestor as PSI-K. PSI-H is important for the adaptation of plants to different light conditions (Lunde *et al*. 2000). When plants are illuminated with light which is preferentially absorbed by either PSII or PSI, they redistribute excitation towards the light-limiting photosystem. If PSI is limited, plants phosphorylate and detach the mobile antenna LHCPII from PSII, which migrates to PSI. Lunde *et al*. (2000) have shown that LHCII cannot transfer energy to PSI in mutants lacking PSI-H. Thus PSI-H probably forms the docking site for the mobile antenna. Furthermore, PSI-H hinders the formation of contacts among PSI monomers, so that trimer formation does not oc-

cur in higher plants (Ben-Shem *et al*. 2003). Trimerization was probably lost in plants to facilitate re-allocation of phospohorylated LHCII to PSI under light conditions favoring PSII excitation (cf. Ben-Shen *et al*. 2003).

In contrast to PsaD-H, the two small subunits PSI-I and -J of 4-kDa and 5-kDa, respectively, are plastome-encoded. Whether translation of their messages is also downregulated, when the translation products are not stabilized at the assembling PSI complex, is unknown at present. PSI-I is required for a proper assembly of PSI-L and inactivation of *psaI* in cyanobacterial mutants decreases the PSI-L protein levels to 80% (Xu *et al*. 1995). In addition, deletion of the cyanobacterial PSI-J reduces both *PsaF* mRNA and the amount of PSI-F (Xu *et al*. 1994).

PSI-K is crucial for the interaction of the PSI core with the LHCI antenna. However, *Arabidopsis* mutants lacking PSI-K appear to grow like wild type plants under normal conditions. In several cyanobacteria, more than one PSI-K is present. *Synechocystis* contains two PSI-K subunits, K1 and K2. PSI-K2 is involved in the transfer of excitation energy from the phycobilisomes to the reaction center under high-light conditions. Thus, PSI-K2 is involved in high light acclimation (Fujimori *et al*. 2005, cf. below).

PSI-L is thought to be responsible for trimerization of PSI in *Synechocystis* through the C-terminal part of the polypeptide which protrudes in the reaction center (Chitnis *et al*. 1993; Ben-Shem *et al*. 2003). The C-terminal extension is missing in the plant PSI-L. Apparently, PSI-L in *Synechocystis* is not required for photoautotrophic growth and PSI assembly (Chitnis *et al*. 1993). *Arabidopsis* plants lacking PSI-L also have reduced levels of PSI-H (and PSI-O), and therefore PSI-L can be grouped into state transitions mutants (Lunde *et al*. 2000). PSI-M is a cyanobacterial subunit of PSI. *PsaM* genes are also present on the plastid genome of several algae and gymnosperms, but have not been found in angiosperms. In *Synechocystis* lacking PSI-M, trimerization of PSI is impaired, although photoautotrophic growth and photosynthetic activity appear to be unaffected (Naithani *et al*. 2000).

PSI-N is only present in higher plants. It is involved in docking of plastocyanin to PSI. Mutant analysis in *Arabidopsis* revealed impaired electron transfer from plastocyanin to P_{700}^{+} (Haldrup *et al*. 1999). However, absence of PSI-N does not affect PSI assembly and photoautotrophic growth in *Arabidopsis* (Haldrup *et al*. 1999).

Recently, two new PSI subunits, PSI-O (Knoetzel *et al*. 2002; Jensen *et al*. 2004) and PSI-P (Khrouchtchova *et al*. 2005), have been described. PSI-O is restricted to eukaryotes. Mutant analysis in *Arabidopsis* revealed that PSI-O requires the presence of PSI-H and PSI-L, and together with them might form a structure within PSI being responsible for state transition (Jensen *et al*. 2004). The exact role of PSI-P is still unknown.

Regulatory proteins

Only a few regulatory proteins controlling the expression of the plastid-encoded genes, translation of their messages on plastid ribosomes, the assembly of the complex with the proper association of all cofactors and the integration of the complex into the thylakoid membranes have been identified. The correct assembly of the pigment-protein-complex in eukaryotes requires the interplay between nucleus and chloroplasts. Genes for the nuclear-encoded subunits including most of the regulatory proteins are transcribed and the messages translated on cytosolic ribosomes. The synthesized precursor polypeptides in the cytosol are recognized by the plastid import machinery at the surface of the organelle and subsequently imported into plastids, cleaved to the mature protein and targeted to their correct location. Transcription and translation of the plastid-encoded proteins occur by the prokaryotic expression machinery located in the plastids, thus formation of the PSI complex must be highly regulated.

Mutants which are unable to perform photosynthesis can be isolated easily by their inability to grow photoautotrophically, or by a high chlorophyll fluorescence phenotype, or both. If the light energy captured by the photosynthetic pigments cannot be utilized for photochemical reactions, it is emitted from the pigments as fluorescence. This can be detected as blue light under UV irradiation. Among the photosynthesis mutants, those are specifically affected in PSI, which show a relatively normal PSII activity, but lack the P_{700} redox function. Many such mutants have been isolated from *Synechocystis*, *Synechococcus*, *Chlamydomonas*, *Arabidopsis* or maize, to mention a few well studied model systems for PSI research. Here we describe some of the regulatory factors identified and characterized in those mutants.

Trans-*splicing mutants from* Chlamydomonas

About one fourth of the PSI mutants, which were isolated from *Chlamydomonas*, are specifically affected in the splicing of the *psaA* precursor transcripts (Goldschmidt-Clermont *et al*. 1990). The maturation of the *Chlamydomonas psaA* transcript requires two *trans*-splicing steps. Raa1 is required for *trans*-splicing of both *psaA* introns (Merendino *et al*. 2006). Raa2 is involved in *trans* splicing of the second *psaA* intron (Perron *et al*. 1999). Interestingly, Raa2 shares sequence similarity to pseudouridine synthases, however, exchange of several amino acid residues that are essential for pseudouridine synthase activity did not alter *trans* splicing. This indicates that this enzyme activity is not required for *trans* splicing (Perron *et al*. 1999; Rochaix *et al*. 2004). Both proteins are membrane-associated. In contrast, Raa3 is a soluble protein and part of a high-molecular weight complex. Raa3 is required for *trans* splicing of the first intron of *psaA* (Rivier *et al*. 2001). It has been proposed that the splicing of the first intron occurs first in the chloroplast stroma, followed by splicing reactions at the thylakoid membrane.

Factors involved in translation of PSI messages

Two proteins, translation of *psaB* (TAB)1 and TAB2, are required for *psaB* translation in *Chlamydomonas*. In both mutants no PSI-A and PSI-B accumulation can be detected (Stampacchia *et al*. 1997). Transgenic *Chlamydomonas* cells with the 5′-UTR of *psaB*, fused to a resistance gene, demonstrated that the reporter gene is not expressed in the TAB1 and TAB2 mutant background. This indicates that the target sites for TAB1 and TAB2 are located in the 5′-UTR of *psaB* and that these proteins are most likely required for translation initiation of this mRNA (Dauvillee *et al*. 2003). Interestingly, polyribosome-loading of the *psaB* message, but not of the *psaA* message was severely reduced in the TAB2 mutant, indicating that the reduced protein levels of PSI-A and PSI-B are caused by different mechanisms: PSI-B is reduced because of the lower translation rate of its message, while PSI-A is most likely reduced, because the synthesized protein is turned over in the absence of PSI-B (Rochaix *et al*. 2004). While no obvious homologs could be identified for TAB1 in other species, TAB2 is conserved in photosynthetic organisms including *Arabidopsis*. Recently, an *Arabidopsis* knockout line of the homologous *Chlamydomonas* Tab2, Atab2, was described and Atab2 was found to be a RNA binding protein that might activate photosystem protein translation (Barneche *et al*. 2006).

Protein accumulation and/or assembly mutants

Targeted inactivation of *ycf3*, a hypothetical chloroplast open reading frame in higher plants, uncovered a gene involved in assembly of PSI (Ruf *et al*. 1997). Plants lacking Ycf3 specifically lack PSI subunits, whereas transcripts of plastid encoded PSI genes accumulate to wild type levels. The same observations were made in *Chlamydomonas* mutants lacking either Ycf3 or Ycf4: both proteins are neces-

sary for the accumulation of the entire complex (Boudreau *et al*. 1997). Later on, it was shown that Ycf3 specifically interacts with PSI-A and PSI-D (Naver *et al*. 2001). Ycf4 is associated with a high-molecular mass complex which contains also subunits of PSI (Rochaix *et al*. 2004). Inactivation of *Ycf4* in *Synechocystis* still allows PSI accumulation, although to a lower extent (Wilde *et al*. 1995, cf. below).

Inactivation of the *Synechocystis* open reading frame (ORF) *slr0171* decreases the PSI/PSII ratio, and slr0171 is further suggested to be an assembly or stability factor of PSI (Wilde *et al*. 2001). This ORF shows similarity to the conserved chloroplast ORF *ycf37* of several algae and encodes a TPR protein (Wilde *et al*. 2001). More recently, Dühring *et al*. (2007) have shown that Ycf37 is involved in late assembly steps in the cyanobacterial PSI. The authors propose that a PSI-L/PSI-K less monomeric PSI complex, called PSI**, is first formed. Integration of PSI-L results in the formation of the PSI* intermediate. These two monomeric complexes are missing in the *ycf37* mutant, indicating that Ycf37 is either required for the synthesis or the stabilization of the intermediates. Finally, integration of one of the two PSI-K subunits leads to the formation of the complete PSI monomer in *Synechocystis*, prior to trimerization. PSI-K2 is assembled into PSI* at high light intensities, while PSI-K1 is assembled under normal light conditions (Dühring *et al*. 2007). The presence of PSI* intermediates, in which different PSI-K subunits can be integrated, allows a rapid re-adaptation of PSI to different light conditions. In *Arabidopsis*, the homologous gene, named *Pale yellow green7* (*Pyg7*), is encoded in the nucleus, suggesting a gene transfer during evolution. The *pyg7* mutant is deficient in PSI. PSI subunits are synthesized, but do not assemble into a stable complex. The mutant fails to grow autotrophically and shows alterations in leaf coloration and plastid ultrastructure. Pyg7 was isolated by map-based cloning and encodes a TPR protein (Stöckel *et al*. 2006). Immunological studies with antisera raised against Pyg7 revealed that the protein is present in thylakoid membrane fractions of *Arabidopsis* leaf extracts. In addition, when analysed in sucrose gradients, Pyg7 co-purifies with PSI. While cyanobacteria can still grow photoautotrophically in the absence of functional Ycf37 (Wilde *et al*. 2001), Pyg7 is absolutely necessary for photoautotrophic growth and proper PSI function. To this end, *ycf37* and *Pyg7* provide an interesting system to compare genes and their function in prokaryotes and eukaryotes.

The *cyanobacterial* BtpA protein was found to stabilise the PSI reaction center (Bartsevich and Pakrasi 1997; Zak *et al*. 1999; Zak and Pakrasi 2000). BtpA is associated with thylakoid membranes and required for stability of PsaA and the PSI reaction center, respectively, at low temperature.

Many photosynthetic mutants with nuclear lesions were also isolated from maize (Barkan *et al*. 1986; Heck *et al*. 1999). *Hcf44*, for instance, was isolated from a variety of ethyl-methanesulfonate-induced (EMS) mutants (Heck *et al*. 1999). PSI-C, PSI-D and PSI-E subunits are missing in *hcf44* mutants, while PSI-A/B accumulation was not impaired (Heck *et al*. 1999). Since PSI-C is required for the assembly of PSI-D and PSI-E in *Synechocystis* (Yu *et al*. 1995), it is reasonable to assume that either PSI-C synthesis or PSI-C integration into the PSI complex is defective in *hcf44* mutants of maize (Heck *et al*. 1999).

Synthesis of the Fe-S cluster

Fe-S clusters are believed to be among the oldest structures found in living organisms. PSI contains three 4[Fe-S] clusters and the electron acceptor ferredoxin one 2[Fe-S] cluster. Biochemical and genetic studies suggest that chloroplasts have their own Fe-S biosynthesis machinery. However little is known about the enzymes involved in the biogenesis and the assembly of the cluster into their target protein (complexes). Cystein is the sulphur source and formation of the cluster involves a plastid-localized NifS protein (Leon *et al*. 2002; Pilon-Smits *et al*. 2002). At least 10 genes have been identified which might have functions in Fe-S cluster biogenesis in *Arabidopsis*, however their roles is mainly unknown.

In *Synechococcus*, inactivation of *rubA* revealed a novel PSI mutant (Shen *et al*. 2002a), which does not grow photoautotrophically and lacks PSI activity, although all membrane intrinsic PSI subunits are present. The three iron-sulfur clusters, F_X, F_A and F_B, are not present in the monomeric PSI complexes and the loss of F_A and F_B may be a secondary effect due to the absence of F_X (Shen *et al*. 2002b). Based on these observations, it has been proposed that RubA is required for the assembly of the F_X Fe-S cluster. The protein is present in purified thylakoid membranes of *Synechocystis*, but not found in the plasma membrane preparations (Shen *et al*. 2002a). *RubA* homologs are found in cyanobacteria, prochlorophytes, cryptomonads, green algae and higher plants, and thus most likely in all oxygen-evolving photosynthetic organisms. The presence of RubA in spinach and *Chlamydomonas* was confirmed immunologically (Shen *et al*. 2002a).

AtCnfU-V and AtCnfU-IVb are two recently described chloroplast-localized NifU-like proteins in *Arabidopsis* (Yabe *et al*. 2004). *Arabidopsis* AtCnfU-V mutants have a pale-green and dwarf phenotype, reduced amounts of PSI and ferredoxin, as well as lower activity of stromal iron-sulfur cluster insertion activity (Yabe *et al*. 2004). AtCnfu is proposed to function as a scaffold protein for Fe-S clustering in chloroplast and thus is required for biogenesis of ferredoxin and PSI (Yabe *et al*. 2004).

The hcf mutant *101* is a novel protein required for PSI biogenesis (Lezhneva *et al*. 2004; Stöckel and Oelmüller 2004). The gene *Hcf101* encodes an MRP-like protein with a nucleotide-binding domain. PSI subunits are synthesized in *Arabidopsis* mutants lacking Hcf101 but do not assemble into a stable complex. Hcf101 is a soluble protein and only loosely associated with membranes and *hcf101* mutants have lesions in Fe-S cluster biogenesis, although the exact function of the protein is unknown at present. Most likely, Hcf101 is involved in delivering components from the stroma to the assembly site of PSI in the stroma thylakoids. *Synechocystis* mutants with a deletion in the *Hcf101* homolog, *slr0067*, can still grow photoautotrophically, but are severely impaired in their Fe/S metabolism (Stöckel and Oelmüller, submitted). Thus, this couple of regulatory proteins provides another interesting system to study homologous genes and their function in prokaryotes and eukaryotes. Comparison of the roles of PSI-C (and other structural components), *ycf4*, *Hcf101*/*slr0067* and *Pyg7*/*ycf37* for cyanobacterial and eukaryotic PSI clearly demonstrates that deletion of any of these components in cyanobacterial causes an impairment in PSI accumulation or function, while comparable mutants in plants are unable to assemble the entire complex.

The regulatory protein "accumulation of photosystem I" (APO1) is specifically required for [4Fe-4S] cluster assembly in chloroplasts of *Arabidopsis* (Amann *et al*. 2004). *Apo1* mutants do not grow photoautotrophically and PSI core subunits are barely detectable (Amann *et al*. 2004). Several [4Fe-4S] cluster-containing complexes in plastids, such as the ferredoxin-thioredoxin reductase and the NADP(H)-dependent dehydrogenase are also missing in the mutant, while the [2Fe-2S] cluster containing ferredoxin is present. Thus, based on a 100 amino acid containing motif, APO1 is thought to provide ligands for [4Fe-4S] clusters (Amann *et al*. 2004). Four APO1 homologs with unknown function are present in the *Arabidopsis* genome and similar genes are only present in vascular plants. Because the different phenotypes of *hcf101* and *apo1* mutants with *apo1* mutants having a stronger phenotype, both proteins might be required for different steps in the Fe/S cluster biogenesis in plastids.

REFERENCES

Amann K, Lezhneva L, Wanner G, Herrmann RG, Meurer J (2004) ACCUMULATION OF PHOTOSYSTEM ONE1, a member of a novel gene family, is required for accumulation of [4Fe-4S] cluster-containing chloroplast complexes and antenna proteins. *Plant Cell* **16**, 3084-3097

Bailey S, Walters RG, Jansson S, Horton P (2001) Acclimation of *Arabidopsis thaliana* to the light environment: the existence of separate low light and high light responses. *Planta* **213**, 794-801

Ballottari M, Govoni C, Caffarri S, Morosinotto T (2004) Stoichiometry of LHCI antenna polypeptides and characterization of gap and linker pigments in higher plants Photosystem I. *The European Journal of Biochemistry* **271**, 4659-4665

Barkan A, Miles D, Taylor WC (1986) Chloroplast gene-expression in nuclear, photosynthetic mutants of maize. *The EMBO Journal* **5**, 1421-1427

Barneche F, Winter V, Crevecoeur M, Rochaix JD (2006) ATAB2 is a novel factor in the signalling pathway of light-controlled synthesis of photosystem proteins. *The EMBO Journal* **25**, 5907-5918

Bartsevich VV, Pakrasi HB (1997) Molecular identification of a novel protein that regulates biogenesis of photosystem I, a membrane protein complex. *The Journal of Biological Chemistry* **272**, 6382-6387

Ben-Shem A, Frolow F, Nelson N (2003) Crystal structure of plant photosystem I. *Nature* **426**, 630-635

Boudreau E, Takahashi Y, Lemieux C, Turmel M, Rochaix JD (1997) The chloroplast *ycf3* and *ycf4* open reading frames of *Chlamydomonas reinhardtii* are required for the accumulation of the photosystem I complex. *The EMBO Journal* **16**, 6095-6104

Chitnis PR (1996) Photosystem I. *Plant Physiology* **111**, 661-669

Chitnis PR (2001) Photosystem I: Function and physiology. *Annual Review of Plant Physiology and Plant Molecular Biology* **52**, 593-626

Chitnis VP, Xu Q, Yu L, Golbeck JH, Nakamoto H, Xie DL, Chitnis PR (1993) Targeted inactivation of the gene psaI encoding a subunit of photosystem-I of the cyanobacterium *Synechocystis sp PCC 6803*. *The Journal of Biological Chemistry* **268**, 11678-11684

Cournac L, Redding K, Bennoun P, Peltier G (1997) Limited photosynthetic electron flow but no CO_2 fixation in *Chlamydomonas* mutants lacking photosystem I. *FEBS Letters* **416**, 65-68

Dauvillee D, Stampacchia O, Girard-Bascou J, Rochaix JD (2003) Tab2 is a novel conserved RNA binding protein required for translation of the chloroplast psaB mRNA. *The EMBO Journal* **22**, 6378-6388

Dühring U, Ossenbühl F, Wilde A (2007) Late assembly steps and dynamics of the cyanobacterial photosystem I. *The Journal of Biological Chemistry* **282**, 10915-10921

Farah J, Rappaport F, Choquet Y, Joliot P, Rochaix JD (1995) Isolation of a *psaF*-deficient mutant of *Chlamydomonas reinhardtii* – efficient interaction of plastocyanin with the photosystem-I reaction-center is mediated by the PsaF subunit. *The EMBO Journal* **14**, 4976-4984

Fujimori T, Hihara Y, Sonoike K (2005) PsaK2 subunit in photosystem I is involved in state transition under high light condition in the cyanobacterium *Synechocystis* sp. *PCC 6803*. *The Journal of Biological Chemistry* **280**, 22191-22197

Ganeteg U, Klimmek F, Jansson S (2004) Lhca5 - an LHC-type protein associated with photosystem I. *Plant Molecular Biology* **54**, 641-651

Goldschmidt-Clermont M, Girard-Bascou J, Choquet Y RJ (1990) *Trans*-splicing mutants of *Chlamydomonas reinhardtii*. *Molecular and General Genetics* **223**, 417-425

Haldrup A, Lunde C, Scheller HV (2003) *Arabidopsis thaliana* plants lacking the PSI-D subunit of photosystem I suffer severe photoinhibition, have unstable photosystem I complexes, and altered redox homeostasis in the chloroplast stroma. *The Journal of Biological Chemistry* **278**, 33276-33283

Haldrup A, Naver H, Scheller HV (1999) The interaction between plastocyanin and photosystem I is inefficient in transgenic *Arabidopsis* plants lacking the PSI-N subunit of photosystem I. *Plant Journal* **17**, 689-698

Haldrup A, Simpson DJ, Scheller HV (2000) Down-regulation of the PSI-F subunit of photosystem I (PSI) in *Arabidopsis thaliana* – The PSI-F subunit is essential for photoautotrophic growth and contributes to antenna function. *The Journal of Biological Chemistry* **275**, 31211-31218

Hayashida N, Matsubayashi T, Shinozaki K, Sugiura M, Inoue K, Hiyama T (1987) The gene for the 9 kd polypeptide, a possible apoprotein for the iron-sulfur center-A and center-B of the photosystem-I complex, in tobacco chloroplast DNA. *Current Genetics* **12**, 247-250

Heck DA, Miles D, Chitnis PR (1999) Characterization of two photosynthetic mutants of maize. *Plant Physiology* **120**, 1129-1136

Jansson S (1999) A guide to the Lhc genes and their relatives in *Arabidopsis*. *Trends in Plant Science* **4**, 236-240

Jensen PE, Haldrup A, Zhang SP, Scheller HV (2004) The PSI-O subunit of plant photosystem I is involved in balancing the excitation pressure between the two photosystems. *The Journal of Biological Chemistry* **279**, 24212-24217

Jensen PE, Rosgaard L, Knoetzel J, Scheller HV (2002) Photosystem I activity is increased in the absence of the PSI-G subunit. *The Journal of Biological Chemistry* **277**, 2798-2803

Jordan P, Fromme P, Witt HT, Klukas O, Saenger W, Krauss N (2001) Three-dimensional structure of cyanobacterial photosystem I at 2.5 Å resolution. *Nature* **411**, 909-917

Khrouchtchova A, Hansson M, Paakkarinen V, Vainonen JP, Zhang SP, Jensen PE, Scheller HV, Vener AV, Aro EM, Haldrup A (2005) A previously found thylakoid membrane protein of 14 kDa (TMP14) is a novel subunit of plant photosystem I and is designated PSI-P. *FEBS Letters* **579**, 4808-4812

Kjaerulff S, Andersen B, Nielsen VS, Møller BL, Okkels JS (1993) The PSI-K subunit of photosystem-I from barley (*Hordeum-Vulgare* L.) – Evidence for a gene duplication of an ancestral PSI-G/K gene. *The Journal of Biological Chemistry* **268**, 18912-18916

Knoetzel H, Mant A, Haldrup A, Jensen PE, Scheller HV (2002) PSI-O, a new 10-kDa subunit of eukaryotic photosystem I. *FEBS Letters* **510**, 145-148

Kruip J, Chitnis PR, Lagoutte B, Rögner M, Boekema EJ (1997) Structural organization of the major subunits in cyanobacterial photosystem I – Localization of subunits PsaC, -D, -E, -F, and -J. *The Journal of Biological Chemistry* **272**, 17061-17069

Leon S, Touraine B, Briat JF, Lobreaux S (2002) The AtNFS2 gene from *Arabidopsis thaliana* encodes a NifS-like plastidial cysteine desulphurase. *Biochemical Journal* **366**, 557-564

Lezhneva L, Amann K, Meurer J (2004) The universally conserved HCF101 protein is involved in assembly of [4Fe-4S]-cluster-containing complexes in *Arabidopsis thaliana* chloroplasts. *Plant Journal* **37**, 174-185

Lunde C, Jensen PE, Haldrup A, Knoetzel J, Scheller HV (2000) The PSI-H subunit of photosystem I is essential for state transitions in plant photosynthesis. *Nature* **408**, 613-615

Mannan RM, Whitmarsh J, Nyman P, Pakrasi HB (1991) Directed mutagenesis of an iron-sulfur protein of the photosystem-I complex in the filamenttous cyanobacterium *Anabaena-variabilis ATCC-29413*. *Proceedings of the National Academy of Sciences USA* **88**, 10168-10172

Merendino L, Perron K, Rahire M, Howald I, Rochaix JD, Goldschmidt-Clermont M (2006) A novel multifunctional factor involved in *trans*-splicing of chloroplast introns in *Chlamydomonas*. *Nucleic Acids Research* **34**, 262-274

Naithani S, Hou JM, Chitnis PR (2000) Targeted inactivation of the *psaK1*, *psaK2* and *psaM* genes encoding subunits of Photosystem I in the cyanobacterium *Synechocystis* sp. *PCC 6803*. *Photosynthesis Research* **63**, 225-236

Naver H, Boudreau E, Rochaix JD (2001) Functional studies of Ycf3: its role in assembly of photosystem I and interactions with some of its subunits. *Plant Cell* **13**, 2731-2745

Naver H, Haldrup A, Scheller HV (1999) Cosuppression of photosystem I subunit PSI-H in *Arabidopsis thaliana* – Efficient electron transfer and stability of photosystem I is dependent upon the PSI-H subunit. *The Journal of Biological Chemistry* **274**, 10784-10789

Nelson N, Yocum CF (2006) Structure and function of photosystems I and II. *Annual Review of Plant Biology* **57**, 521-565

Perron K, Goldschmidt-Clermont M, Rochaix JD (1999) A factor related to pseudouridine synthases is required for chloroplast group II intron *trans*-splicing in *Chlamydomonas reinhardtii*. *The EMBO Journal* **18**, 6481-6490

Pilon-Smits EAH, Garifullina GF, Abdel-Ghany S, Kato SI, Mihara H, Hale KL, Burkhead JL, Esaki N, Kurihara T, Pilon M (2002) Characterization of a NifS-like chloroplast protein from *Arabidopsis*. Implications for its role in sulfur and selenium metabolism. *Plant Physiology* **130**, 1309-1318

Redding K, Cournac L, Vassiliev IR, Golbeck JH, Peltier G, Rochaix JD (1999) Photosystem I is indispensable for photoautotrophic growth, CO_2 fixation, and H_2 photoproduction in *Chlamydomonas reinhardtii*. *The Journal of Biological Chemistry* **274**, 10466-10473

Rivier C, Goldschmidt-Clermont M, Rochaix JD (2001) Identification of an RNA-protein complex involved in chloroplast group II intron *trans*-splicing in *Chlamydomonas reinhardtii*. *The EMBO Journal* **20**, 1765-1773

Rochaix JD, Perron K, Dauvillee D, Laroche F, Takahashi Y, Goldschmidt-Clermont M (2004) Post-transcriptional steps involved in the assembly of photosystem I in *Chlamydomonas*. *Biochemical Society Transactions* **32**, 567-570

Ruf S, Kössel H, Bock R (1997) Targeted inactivation of a tobacco intron-containing open reading frame reveals a novel chloroplast-encoded photosystem I-related gene. *Journal of Cell Biology* **139**, 95-102

Shen G, Boussiba S, Vermaas WF (1993) *Synechocystis sp PCC 6803* strains lacking photosystem I and phycobilisome function. *Plant Cell* **5**, 1853-1863

Shen G, Antonkine ML, van der Est A, Vassiliev IR, Brettel K, Bittl R, Zech SG, Zhao J, Stehlik D, Bryant DA, Golbeck JH (2002a) Assembly of photosystem I. II. Rubredoxin is required for the *in vivo* assembly of F-X in *Synechococcus* sp. *PCC 7002* as shown by optical and EPR spectroscopy. *The Journal of Biological Chemistry* **277**, 20355-20366

Shen G, Zhao J, Reimer SK, Antonkine ML, Cai Q, Weiland SM, Golbeck JH, Bryant DA (2002b) Assembly of photosystem I. I. Inactivation of the *rubA* gene encoding a membrane-associated rubredoxin in the cyanobacterium *Synechococcus* sp. *PCC 7002* causes a loss of photosystem I activity. *The Journal of Biological Chemistry* **277**, 20343-20354

Shinozaki K, Ohme M, Tanaka M, Wakasugi T, Hayashida N, Matsubayashi T, Zaita N, Chunwongse J, Obokata J, Yamaguchi-Shinozaki K, Ohto C, Torazawa K, Meng BY, Sugita M, Deno H, Kamogashira T, Yamada K, Kusuda J, Takaiwa F, Kato A, Tohdoh N, Shimada H, Sugiura M (1986)

The complete nucleotide-sequence of the tobacco chloroplast genome - its gene organization and expression. *The EMBO Journal* **5**, 2043-2049

Smart LB, Anderson SL, McIntosh L (1991) Targeted genetic inactivation of the photosystem-I reaction center in the cyanobacterium *Synechocystis sp PCC 6803*. *The EMBO Journal* **10**, 3289-3296

Smart LB, Warren PV, Golbeck JH, McIntosh L (1993) Mutational analysis of the structure and biogenesis of the photosystem-I reaction center in the cyanobacterium *Synechocystis* sp. *PCC 6803*. *Proceedings of the National Academy of Sciences USA* **90**, 1132-1136

Stampacchia O, Girard-Bascou J, Zanasco JL, Zerges W, Bennoun P, Rochaix JD (1997) A nuclear-encoded function essential for translation of the chloroplast *psaB* mRNA in *Chlamydomonas*. *Plant Cell* **9**, 773-782

Stöckel J, Bennewitz S, Hein P, Oelmüller R (2006) The evolutionarily conserved tetratrico peptide repeat protein pale yellow green7 is required for photosystem I accumulation in *Arabidopsis* and copurifies with the complex. *Plant Physiology* **141**, 870-878

Stöckel J, Oelmüller R (2004) A novel protein for photosystem I biogenesis. *The Journal of Biological Chemistry* **279**, 10243-10251

Sugiura M (2003) History of chloroplast genomics. *Photosynthesis Research* **76**, 371-377

Takahashi Y, Goldschmidt-Clermont M, Soen SY, Franzen LG, Rochaix JD (1991) Directed chloroplast transformation in *Chlamydomonas reinhardtii* – insertional inactivation of the *psaC* gene encoding the iron sulfur protein destabilizes photosystem-I. *The EMBO Journal* **10**, 2033-2040

Varotto C, Pesaresi P, Meurer J, Oelmüller R, Steiner-Lange S, Salamini F, Leister D (2000) Disruption of the *Arabidopsis* photosystem I gene *psaE1* affects photosynthesis and impairs growth. *Plant Journal* **22**, 115-124

Wilde A, Härtel H, Hübschmann T, Hoffmann P, Shestakov SV, Börner T (1995) Inactivation of a *Synechocystis sp strain PCC 6803* gene with homology to conserved chloroplast open reading frame-184 increases the photosystem-II-to-photosystem-I ratio. *Plant Cell* **7**, 649-658

Wilde A, Lünser K, Ossenbühl F, Nickelsen J, Börner T (2001) Characterization of the cyanobacterial ycf37: mutation decreases the photosystem I content. *Biochemical Journal* **357**, 211-216

Wostrikoff K, Girard-Bascou J, Wollman FA, Choquet Y (2004) Biogenesis of PSI involves a cascade of translational autoregulation in the chloroplast of *Chlamydomonas*. *The EMBO Journal* **23**, 2696-2705

Xu Q, Hoppe D, Chitnis VP, Odom WR, Guikema JA, Chitnis PR (1995) Mutational analysis of photosystem-I polypeptides in the cyanobacterium *Synechocystis* sp., *PCC 6803* - targeted inactivation of PsaI reveals the function of PsaI in the structural organization of PsaL. *The Journal of Biological Chemistry* **270**, 16243-16250

Xu Q, Odom WR, Guikema JA, Chitnis VP, Chitnis PR (1994) Targeted deletion of *psaJ* from the cyanobacterium *Synechocystis sp PCC 6803* indicates structural interactions between the PsaJ and PsaF subunits of photosystem-I. *Plant Molecular Biology* **26**, 291-302

Xu W, Tang HD, Wang YC, Chitnis PR (2001) Proteins of the cyanobacterial photosystem I. *Biochimica et Biophysica Acta - Bioenergetics* **1507**, 32-40

Yabe T, Morimoto K, Kikuchi S, Nishio K, Terashima I, Nakai M (2004) The *Arabidopsis* chloroplastic NifU-like protein CnfU, which can act as an iron-sulfur cluster scaffold protein, is required for biogenesis of ferredoxin and photosystem I. *Plant Cell* **16**, 993-1007

Yu JP, Smart LB, Jung YS, Golbeck J, McIntosh L (1995) Absence of PsaC subunit allows assembly of photosystem-I core but prevents the binding of PsaD and PsaE in *Synechocystis* sp. *PCC 6803*. *Plant Molecular Biology* **29**, 331-342

Zak E, Norling B, Andersson B, Pakrasi HB (1999) Subcellular localization of the BtpA protein in the cyanobacterium *Synechocystis* sp. *PCC 6803*. *The European Journal of Biochemistry* **261**, 311-316

Zak E, Pakrasi HB (2000) The BtpA protein stabilizes the reaction center proteins of photosystem I in the cyanobacterium *Synechocystis* sp. *PCC 6803* at low temperature. *Plant Physiology* **123**, 215-222