Photosynthesis Research **44:** 23–40, 1995.

Minireview

Function and organization of Photosystem I polypeptides*

Parag R. Chitnis[1], Qiang Xu[1], Vaishali P. Chitnis[1] & Rachel Nechushtai[2]
[1]*Division of Biology, Kansas State University, Manhattan, Kansas, USA;* [2]*Department of Botany, The Hebrew University of Jerusalem, Jerusalem, Israel*

Received 18 November 1994; accepted in revised form 27 December 1994

Key words: chloroplasts, cyanobacteria, ferredoxin, photosynthesis, plastocyanin, thylakoids

Abstract

Photosystem I functions as a plastocyanin:ferredoxin oxidoreductase in the thylakoid membranes of chloroplasts and cyanobacteria. The PS I complex contains the photosynthetic pigments, the reaction center P700, and five electron transfer centers (A_0, A_1, F_X, F_A, and F_B) that are bound to the PsaA, PsaB, and PsaC proteins. In addition, PS I complex contains at least eight other polypeptides that are accessory in their functions. Recent use of cyanobacterial molecular genetics has revealed functions of the accessory subunits of PS I. Site-directed mutagenesis is now being used to explore structure-function relations in PS I. The overall architecture of PSI complex has been revealed by X-ray crystallography, electron microscopy, and biochemical methods. The information obtained by different techniques can be used to propose a model for the organization of PS I. Spectroscopic and molecular genetic techniques have deciphered interaction of PS I proteins with the soluble electron transfer partners. This review focuses on the recent structural, biochemical and molecular genetic studies that decipher topology and functions of PS I proteins, and their interactions with soluble electron carriers.

Abbreviation: NHS – *N*-hydroxysuccinamide

Introduction

PS I is a heteromultimeric Chl-protein complex in the photosynthetic membranes that generate ATP and NADPH by oxygenic photosynthesis in cyanobacteria and chloroplasts. PS I is localized in the non-appressed thylakoid regions in chloroplasts (Anderson and Melis 1983). Similarly, the PS I in cyanobacterial membranes is spatially distributed in a radial asymmetric fashion, with higher concentration in the outermost thylakoids of a cyanobacterial cell (Sherman et al. 1994). PS I catalyzes the photooxidation of plastocyanin or cytochrome c_6 on the lumenal side and photoreduction of ferredoxin or flavodoxin on the stromal (in chloroplasts) or cytoplasmic (in cyanobacteria) side of photosynthetic membranes. PS I also participates in cyclic electron transport. PS I contains polypeptides and cofactors required to absorb photons, use their energy to cause the primary photochemical charge separation, and then to stabilize these charges by spatial displacement of electrons through a series of electron transfer centers. PS I can be isolated as monomers (~ 340 kDa) or as trimers. A monomer of PS I complex consists of at least eleven different polypeptides, approximately 100 Chl *a* molecules, several β-carotenes, and two phylloquinones. Cofactors of PS I participate in electron transport across the membrane from plastocyanin to ferredoxin in the following sequence: *Plastocyanin (Cu-protein) or cytochrome c_6 (heme)* → P700 (Chl *a* dimer) → A_0 (Chl *a*) → A_1 (phylloquinone) → F_X (a [4Fe-4S] center) → F_A or F_B ([4Fe-4S] centers) → *ferredoxin ([2Fe-2S] center) or flavodoxin (flavin)*. The cofactors of PS I are bound to the PsaA, PsaB and PsaC proteins. The remaining subunits of PS I do not bind electron transfer cen-

* This review is dedicated to Prof. J. Philip Thornber, in whose laboratory PRC was introduced to the green world of chlorophyll-proteins.

ters. Details of electron transfer kinetics have been recently described (Lagoutte and Mathis 1989; Golbeck 1992; Setif 1992). Similarly extensive reviews discuss structures of soluble electron-transfer partners of PS I (Gross 1993; Morand et al. 1994; Redinbo et al. 1994).

The primary structures of individual PS I subunits and the overall mechanism of PS I function are remarkably conserved among cyanobacteria, green algae and plants (Golbeck 1992). The major difference is the existence of membrane-embedded light-harvesting complexes associated with the PS I of green algae and higher plants. The light harvesting complexes serve as additional antenna to harvest the light and funnel its energy to the PS I reaction center. The biochemistry light harvesting complexes and biophysics of energy trapping in photosystem I have been recently reviewed (Setif 1992; Thornber et al. 1993, 1994; Van Grondelle et al. 1994). PS I, the P840 photosynthetic reaction center of green sulfur bacteria and P800 reaction center of Heliobacteria share structural and functional similarities, indicating a common evolutionary origin (see reviews by Golbeck 1993a; Lockau and Nitschke 1993).

Details of PS I architecture are currently being revealed by X-ray diffraction analysis of PS I crystals (Krauß et al. 1993; Fromme et al. 1994), biochemical characterization of PS I topology (Ortiz et al. 1985; Lagoutte and Vallon 1992; Vallon and Bogorad 1993; Xu et al. 1994a,b), and the success in generating cyanobacterial mutants that lack specific polypeptides of PS I (Chitnis and Nelson 1992a; Chitnis and Chitnis 1993; Cohen et al. 1993; Xu et al. 1994c,d,e, 1995). The targeted cyanobacterial and algal mutants have also revealed the roles of PS I proteins in mediating electron transfer through PS I and in regulating and stabilizing the quaternary structure of the complex. In this minireview, we describe the recent advances in our understanding of the organization and function of PS I polypeptides. We will discuss recent structural, biochemical and molecular genetic studies that decipher topology and functions of PS I proteins, and their interactions with soluble electron carriers.

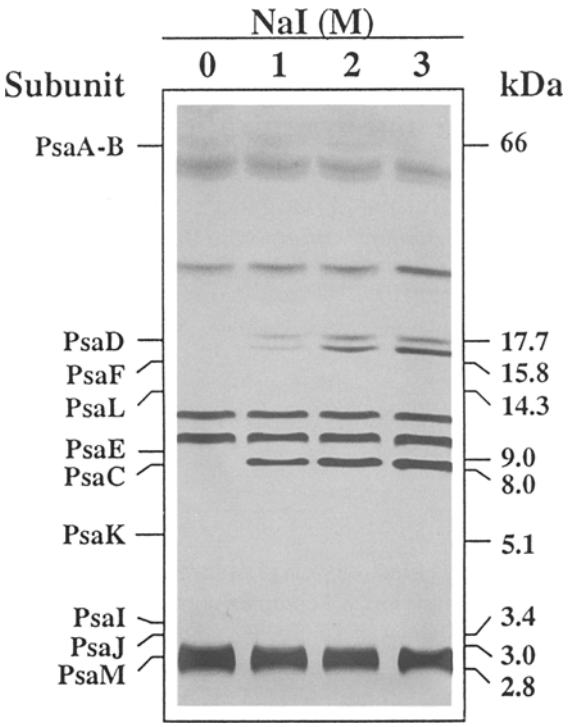

Fig. 1. Polypeptide composition of the PS I complex from *Synechocystis* sp. PCC 6803. PS I complexes were purified by solubilization of thylakoid membranes by Triton X-100, followed by DEAE-cellulose chromatography and sucrose gradient ultracentrifugation (Xu et al. 1994b). The complexes were treated with different concentrations NaI to identify the peripheral and transmembrane subunits of PS I. The polypeptides in PS I complexes containing 5 μg Chl were separated on a Tricine-urea-SDS-PAGE (Xu et al. 1994c) and visualized by silver staining. The subunits labeled in the figure have been identified using immunodetection and N-terminal amino acid sequencing, and their molecular masses were determined from migration of the following protein markers: insulin (2.9 kDa), bovine trypsin inhibitor (6.2 kDa), lysozyme (14.3 kDa), β-lactoglobulin (18.4 kDa), carbonic anhydrase (29 kDa), ovalbumin (43 kDa), and bovine serum albumin (68 kDa).

Polypeptides of Photosystem I

PS I is a heteromultimeric membrane-protein complex that is composed of at least eleven polypeptides in cyanobacteria (Fig. 1) and three additional ones in plants and algae. PS I subunits are believed to be present as one copy per P700 reaction center (Bruce and Malkin 1988a,b; Scheller et al. 1989a). They vary considerably in their molecular weights, hydrophobicity and their location with respect to the lipid bilayer. PsaC, PsaD, and PsaE are the only subunits of cyanobacterial PS I that can be removed by chaotropic treatment (Fig. 1; Ikeuchi 1992), thereby demonstrating their peripheral nature. All cofactors of PS I bind to PsaA, PsaB, and PsaC (Table 1). The remaining subunits of PS I are likely to be accessory in their function.

PS I proteins are encoded in both chloroplast and nuclear genomes of eukaryotes; the *psaA*, *psaB*, *psaC*,

Table 1. Polypetide subunits of PS I

Subunit	Gene	Mass (Predicted)	Cofactors	Functions	Properties
PsaA (Ia, PS I-A) PsaB (Ib, PS I-B)	*psaA* *psaB*	83.0 (66) 82.4 (66)	~ 100 chlorophyll *a* P700 chlrophyll *a* dimer 12–16 β carotenes 2 phylloquinones 1 [4Fe-4S] cluster (F_X)	Light-harvesting Charge separation Photoprotection Charge stabilization Charge stabilization	Transmembrane proteins with 8–11 putative helices
PsaC (VII, PS I-C)	*psaC*	8.9 (8)	2 [4Fe-4S] centers (F_A,F_B)	Terminal electron acceptor donate e^- to ferredoxin	Peripheral on stromal (cytoplasmic) side
PsaD (II, PS I-D)	*psaD*	15.6 (17.7)	None	Ferredoxin-docking; Stable assembly of PS I; Correct orientation of PsaC	Peripheral on stromal (cytoplasmic) side
PsaE (IV, PS I-E)	*psaE*	8.0 (8)	None	Facilitates interaction with ferredoxin; Essential for cyclic electron transport	Peripheral on stromal (cytoplasmic) side
PsaF (III, PS I-F)	*psaF*	15.7 (15.8)	Chlorophyll *a*[b]	Plastocyanin-docking for fast electron transfer	One putative transmembrane helix; large exposure on lumenal side
PsaG (?, PS I-G)	*psaG*	10–10.8	None[a]	Interaction with LHCI? *Only in chloroplasts*	Two putative transmembrane helices
PsaH (VI, PS I-H)	*psaH*	10.2–11	None	Interaction with LHCI? *Only in chloroplasts*	Peripheral on stromal side
PsaI (IX, PS I-I)	*psaI*	4.3 (3.4)	None[a]	Normal organization of PsaL; trimerization?	One putative transmembrane helix
PsaJ (VIII, PS I-J)	*psaJ*	4.4 (3.0)	None[a]	Normal organization of PsaF	One putative transmembrane helix
PsaK (PS I-K)	*psaK*	8.5 (5.1)	None[a]	?	Two putative transmembrane helices
PsaL (V, PS I-L)	*psaL*	16.6 (14.3)	None[a]	PS I trimerization	Two or three putative transmembrane helices
PsaM (PS I-M)	*psaM*	3.4 (2.8)	None[a]	Cyclic electron flow	One putative transmembrane helix
PsaN (PS I-N)	*psaN*		None	?	Peripheral on *p*-side

The subunit nomenclature used in this review is given in the beginning while the alternative names in the literature are given in the parentheses.

The molecular masses predicted from the deduced primary sequences of *Synechocystis* sp. PCC 6803 PS I are given; the apparent mass observed during PAGE is parenthesized. For PsaG, PsaH, and PsaN a ranges of molecular masses of proteins from higher plants are given. For PsaK, PsaI, and PsaM the predicted masses are calculated from *Synechococcus elongatus* sequences.

[a] Although not demonstrated, may potentially bind chlorophyll.

[b] PsaF from higher plants has been isolated as a chlorophyll-protein complex, termed LHCIc, (see Thornber et al. 1993).

psaI, *psaM*, and *psaJ* genes are localized in the chloroplast genome, while others are located in the nucleus (see review by Ikeuchi 1992). In many plants, the nuclear-encoded PS I subunits are often present in multiple isoforms (Obokata et al. 1993). These proteins are synthesized as precursors and imported into chloroplasts. The precursors of PsaF and PsaN have bipartite transit sequences that target them to thylakoid lumen (Mant et al. 1994; Nielsen et al. 1994). The genes encoding most PS I subunits of barley (Okkels et al. 1992; Kjærulff et al. 1993; Knoetzel and Simpson 1993), spinach (Flieger et al. 1993), *Chlamydomonas reinhardtii*, *Synechocystis* sp. PCC 6803 (Chitnis et al. 1993; Xu et al. 1994e, 1995), *Synechococcus elongatus* (Mühlenhoff et al. 1993) and *Synechococcus* sp. PCC 7002 have been cloned and characterized (see the review by Bryant 1992 for pre-1992 sources). The deduced primary sequences of PS I proteins have provided clues about their structure and function. In addition, biochemical resolution-reconstitution experiments and subunit-deficient cyanobacterial mutants have identified roles of PS I subunits.

PsaA-PsaB

These homologous, highly conserved, and hydrophobic subunits form the catalytic core of PS I (Fish et al. 1985a,b; Fish and Bogorad 1986). Examination of the amino acid sequences of PsaA-PsaB deduced from their genes revealed several structural features. First, PsaA and PsaB contain 11 hydrophobic domains each. These regions are 19–25 residues length and may cross membranes (Fish et al. 1985a; Kirsch et al. 1986). At least eight of these regions form transmembrane helices, while one may be positioned parallel to the membrane plane (Krauß et al. 1993; Fromme et al. 1994). Second, most charged residues are present in the extramembrane loops and may be involved in interaction with smaller subunits and diffusible electron carriers. Third, a high count of histidine residues is found in the transmembranal helices (Fish et al. 1985a,b). These histidine residues were proposed to be involved in coordinating the Chl molecules associated with PS I (Fish et al. 1985a). Fourth, the conserved cysteine residues in PsaA and PsaB (two in each protein) provide the ligands to the bound iron-sulfur cluster F_X. Fifth, PsaA and PsaB contain leucine zipper motifs (Kossel et al. 1990; Webber and Malkin 1990). Significance of these features is being tested through structural analysis and site-directed mutagenesis.

PsaC

The terminal electron transfer centers (F_A and F_B) of PS I are coordinated by the 8.8-kDa protein PsaC (see review by Golbeck 1992). It is a acidic hydrophilic protein with 81 amino acids. PsaC is a extraordinarily conserved protein; a comparison of the deduced amino acid sequences of PsaC from plants, algae and cyanobacteria shows that, with the exception of some conservative replacements near N-terminus and in the center of the protein, most residues are completely conserved during evolution (Bryant 1992). Therefore the function and interprotein interactions of this protein place significant constraints over its structure. The most notable feature of PsaC includes nine conserved cysteines, eight of which participate in ligating the Fe-S centers. Although some models for the conformation of PsaC have been proposed on the basis of its similarity to the bacterial ferredoxins (Dunn and Gray 1988; Golbeck 1993b), the structure of PsaC is yet to be determined.

PsaD

It is a conserved peripheral protein with 139–144 amino acids in cyanobacteria and an additional $\sim$ 23 residues in higher plants (Golbeck 1992). The extra amino acids form an extension at the N-terminus of the plant PsaD. Topological studies have shown that the N-terminal extension of eukaryotic PsaD is accessible to proteases (Lagoutte and Vallon 1992; Zilber and Malkin 1992). Although conformation of PsaD is unknown, biochemical analysis has shown that the C-terminal domain of PsaD is surface-exposed (Xu et al. 1994b). Secondary structure predictions based on Chou-Fasman or Garnier algorithms indicates significant α helical structure in PsaD. In general, PsaD is a basic protein and thus may be able to interact with PsaC and ferredoxin through electrostatic interactions. PsaD is implicated in many functions. It is required for stable organization of PsaC, PsaL and PsaE into PS I (Li et al. 1991b; Chitnis and Nelson 1992a; Xu et al. 1994a). Characterization of the PsaD-less mutants of *Synechocystis* sp. PCC 6803 has demonstrated the essential role of PsaD in interaction with ferredoxin and flavodoxin (Xu et al. 1994c).

PsaE

It is a conserved, peripheral subunit on the stromal (cytoplasmic) side of PS I. Cyanobacterial PsaE is 70–

75 amino acids long, while plant and algal PsaE has an N-terminal extension (see review by Bryant 1992). Similar to plant PsaD, the extension in spinach PsaE is accessible to exogeneous proteases (Lagoutte and Vallon 1992). In contrast, the C-terminal Glu63 and Glu67 and Lys74 in PsaE of *Synechocystis* sp. PCC 6803 are exposed on the surface of PS I (Xu et al. 1994b; see also Rousseau et al. 1993). The solution conformation of *Synechococcus* sp. PCC 7002 PsaE, proposed from NMR analysis, reveals that the protein has an antiparallel five-stranded β-sheet structure (Falzone et al. 1994a,b). In this structure, the N- and C-termini are close to each other. Therefore the N-terminal extension may make the C-terminal domain of plant PsaE inaccessible to proteases, thus providing an explanation for the apparant contradiction between the topological studies on the plant and cyanobacterial PsaE. The C-terminal surface exposed domain of PsaE may be involved in its interaction with ferredoxin, that has been demonstrated from cyanobacterial mutants (Rousseau et al. 1993; Xu et al. 1994c) and biochemical depletions (Sonoike et al. 1993; Strotmann and Weber 1993). PsaE may also be required for cyclic electron transport around PS I (Yu et al. 1993b). PsaE in the PS I complex can be chemically cross-linked to ferredoxin:$NADP^+$ oxidoreductase (Anderson et al. 1992), indicating an additional role for PsaE in interaction with ferredoxin:$NADP^+$ oxidoreductase.

PsaF

Until recently, PsaF was considered a peripheral subunit on the lumenal side of PS I (for example, see Golbeck 1992). However, several reports demonstrate that it is an integral membrane protein. Chaotropic treatments have been routinely used to distinguish between the peripheral and integral membrane proteins. PsaF resists removal from cyanobacterial PS I by 3 M NaI (Fig. 1) or by 8 M urea (Li et al. 1991a). Similarly, PsaF from plant PS I is resistant to removal by n-butanol (Oh-oka et al. 1988) or by alkaline salt treatment (Tjus and Andersson 1991). In contrast, treatment of PS I complexes with high concentration of detergents, such as Triton X-100, removes PsaF from the core complex (Bengis and Nelson 1977). Extraction of a membrane-protein by Triton X-114 or organic solvents depends on the overall hydrophobicity of the protein. Spinach PsaF partitions with Triton-X 114, indicating its hydrophobic nature (Zilber et al. 1990; Tjus and Andersson 1991). The amino acid sequences of PsaF deduced from the gene sequences reveal two moderately hydrophobic regions in this protein (Chitnis et al. 1991). A cross-linked product that is recognized by both anti-PsaE and anti-PsaF antibodies can be obtained by treatment of PS I with N-ethyl-3-3 [dimethylaminopropyl] carbodiimide or with glutaraldehyde, showing that regions of PsaE and PsaF are in close proximity (D.A. Bryant, personal communication; V.P. Chitnis and P.R. Chitnis, unpublished results). PsaF is synthesized as a precursor protein with a transit sequence typical of proteins targeted to thylakoid lumen, suggesting the N-terminal bulk of the protein is exposed on the lumenal side and the C-terminal hydrophobic domain serves as a stop-transfer sequence. This would indicate that the C-terminal hydrophilic domain of PsaF is exposed on the *stromal (cytoplasmic) side* of the membranes and may interact with PsaE.

Function of PsaF remains controversial. Based on biochemical depletion and chemical cross-linking experiments, this subunit has been proposed to function as the plastocyanin or cytochrome c_6 docking site on the oxidizing side of PS I (Bengis and Nelson 1977; Nechushtai and Nelson 1981; Wynn and Malkin 1988; Hippler et al. 1989; Wynn et al. 1989a,b). In contrast, the PsaF-less mutant strains of *Synechocystis* sp. PCC 6803 do not show any defect in their photoautotrophic growth, cytochrome c_6-dependent $NADP^+$ photoreduction activity of PS I or $P700^+$ rereduction kinetics (Chitnis et al. 1991; Xu et al. 1994c,e). Biochemical depletion of PsaF also does not affect activity of cyanobacterial PS I (Hatanaka et al. 1993).

PsaG

This is a moderately conserved subunit that contains $\sim$ 100 amino acids (Golbeck 1992). It has only been detected in eukaryotic PS I. Despite the initial discrepancies in the prediction of its transmembrane helices (Steppuhn et al. 1988; Franzen et al. 1989; Okkels et al. 1992), it is now believed that PsaG contains two putative transmembrane helices placed towards the termini and a highly hydrophilic large extramembranal loop. From the positive inside rule (Gavel et al. 1991), the N-terminus of PsaG is predicted to be in the lumen (Okkels et al. 1992). PsaG shares some sequence homology with PsaK. PsaG may play a role in interaction with LHCI.

PsaH

It is another PS I subunit that has only been detected in algae and plants (Ikeuchi 1992). It is ~ 95 amino acids in length and has conserved primary structure (Golbeck 1992). The mature PsaH protein contains an N-terminal acidic domain, a central hydrophobic domain and a C-terminal basic region. PsaH is an extrinsic subunit on the stromal side of PS I (Tjus and Andersson 1991; Zilber and Malkin 1992). Like PsaG, PsaH may play a role specific for PS I from plant and algae.

PsaI

The deduced amino acid sequences of PsaI from plants and cyanobacteria shows high degree of conservation (Xu et al. 1995). PsaI of the *Synechocystis* sp. PCC 6803 does not contain obvious consensus sequences for binding of prosthetic groups. Unlike *psaI* of another cyanobacterium *Anabaena variabilis* ATCC 29413 (Sonoike et al. 1992), the *psaI* gene from *Synechocystis* codes for the mature polypeptide without a presequence. The PsaI subunits contain a central hydrophobic domain flanked by hydrophilic N- and C-termini (Scheller et al. 1989b). The hydrophobic region potentially could make a kinked transmembrane helix. PsaI in intact spinach thylakoids is not accessible to external proteases (Zilber and Malkin 1992). Furthermore, PsaI is resistant to removal by chaotropic agents (Scheller et al. 1989b; Xu et al. 1994a,d). Therefore, PsaI is an integral membrane protein containing one transmembrane helix. The N-terminus of PsaI may be exposed on the lumenal side of thylakoids. PsaI of *Anabaena variabilis* has a presequence that is predicted to assist thylakoid translocation. The C-terminal hydrophilic domain of PsaI in barley contains several positively charged residues (Scheller et al. 1989b). If the positive inside rule for predicting topology of membrane proteins (Von Heijne and Gavel 1988) applies to PsaI in barley, the C-terminal domain of this protein may face the stromal (cytoplasmic) side of the photosynthetic membranes. While PsaI in cyanobacterial and higher plants shares some common features in hydropathy profile, the C-terminus of cyanobacterial and liverwort PsaI contains several negatively charged residues. The significance of these differences in overall charges in the C-termini of PsaI from cyanobacterial and higher plants is not understood. PsaI may be involved in correct organization of PsaL (Xu et al. 1995).

PsaJ

PsaJ is a 4.4-kDa hydrophobic protein, which has been identified in PS I preparations from cyanobacteria (Ikeuchi et al. 1991, 1993; Xu et al. 1994e) and higher plants (Ikeuchi et al. 1990). Its deduced amino acid sequence reveals a highly conserved sequence of 22 amino acids which may span the membrane. The hydrophilic N-terminal domain of PsaJ is only 4–5 amino acids long while the C-terminal domain has overall negative charge and > 15 amino acids (Golbeck 1992). PsaJ is not required for the electron-transfer function of PS I (Xu et al. 1994e). It, however, may interact with PsaF and PsaE (Xu et al. 1994d). The exact nature of these interactions or their importance in vivo is not known. Orientation of PsaJ with respect to thylakoids is not clear. It is likely that the relatively large C-domain of PsaJ may be on the stromal (cytoplasmic) side and interacts with PsaE.

PsaK

It is an integral membrane-protein that is tightly associated with the PsaA-PsaB complex. It can be removed from this complex by treatment with thiol reagents, without affecting PS I charge separation (Wynn and Malkin 1990). Deduced primary sequences of PsaK from higher plants and cyanobacteria do not show high degree of homology (Golbeck 1992), however, they do contain two hydrophobic domains predicted to be membrane-spanning alpha-helices. From positive inside rule (Gavel et al. 1991), the N-terminus of PsaK may be in the lumen of thylakoids. The comparison of the deduced primary sequences of PsaK and PsaG shows significant similarity, suggesting that an ancestral gene has been duplicated in a chloroplast progenitor to evolve into *psaG* and *psaK*, but remained single in cyanobacteria (Kjærulff et al. 1993). The role of PsaK in the function and structure of PS I remains to be studied.

PsaL

A comparison of the deduced primary structures of PsaL shows a greater diversity than seen in other subunits (Golbeck 1992). However, the protein contains several conserved regions of homology, most notably in the hydrophobic regions. Hydropathy analysis of PsaL suggests the presence of a hydrophilic N-terminal domain followed by two potential transmembrane domains (Chitnis et al. 1993). A relatively

large N-terminal domain of PsaL in stacked spinach thylakoids resists proteolysis and is predicted to be located on the stromal side (Zilber and Malkin 1992). This subunit is required for PS I trimer formation and is a component of the connecting domain that links three monomers to form a trimer (Chitnis and Chitnis 1993).

PsaM

It is a small polypeptide (~ 3 kDa, ~ 29 amino acids) and has recently been detected in cyanobacterial PS I (Ikeuchi et al. 1993; Xu et al. 1994c). The amino acid sequences have a rather short (~ 17 amino acids) hydrophobic domain in the protein, flanked by hydrophilic termini. Figure 1 clearly shows that PsaM cannot be removed by chaotropes, indicating that PsaM is an integral membrane-protein. Distribution of charged residues in PsaM suggests that N-terminus of PsaM may be exposed in the lumen of thylakoids (Ikeuchi et al. 1993). Although PsaM has not been shown to be present in plant PS I preparations, an open reading frame coding for a PsaM-like peptide is present in the liverwort chloroplast genome (Ohyama et al. 1986).

PsaN

This 9 kDa extrinsic polypeptide of plant PS I is located on the lumenal side of thylakoids (He and Malkin 1992). It does not bind any cofactors and is not required for $NADP^+$ photoreduction. Recently a barley cDNA clone encoding the PsaN subunit of PS I was isolated (Knoetzel and Simpson 1993). The mature PsaN is 85 amino acids in length, with no putative membrane-spanning region. Its transit peptide routes this protein to thylakoid lumen by a *sec*-independent pathway (Mant et al. 1994). In contrast, PsaF is routed by a *sec*-dependent pathway. Considering the presence of a bacterial-type presequence for a cyanobacterial PsaF, it is likely that PsaN was added to PS I after the divergence of chloroplasts and cyanobacteria. This subunit has not yet been detected in the PS I preparations from cyanobacteria. Function of PsaN is not known.

Molecular genetic studies to reveal structure-function relations in PS I

In recent years, biochemical and genetic methods have been elegantly combined and extensively used to identify functions of PS I polypeptides and to decipher structure-function relations. The cyanobacterium *Synechocystis* sp. PCC 6803 has been the most popular organism for molecular genetic dissection of the photosynthetic apparatus. A glucose-tolerant strain of *Synechocystis* can be grown under photoheterotrophic and light-activated heterotrophic conditions, enabling one to obtain mutants defective in the photosynthetic electron transport (Anderson and McIntosh 1991). This cyanobacterium has been used to generate the earliest subunit-deficient PS I mutants (Chitnis et al. 1989a,b). Later other cyanobacterial species and the green alga *Chlamydomonas reinhardtii* have also been used by several groups to create PS I mutants. Here we review the use of molecular genetics to study structure-function relationships in the electron transfer subunits of PS I and to identify functions of the accessory subunits of PS I.

Mutagenesis of the PS I subunits that bind electron-transfer centers

Biochemical analyses have demonstrated that the PsaA, PsaB, and PsaC subunits of PS I bind electron-transfer centers. Several subunit-deficient mutants lacking one or more of these subunits have been generated. Inactivation of *psaA* or *psaB* of *Synechocystis* sp. PCC 6803 results in the absence of PS I in the thylakoids of mutants, showing that these subunits cannot form homodimers and the presence of PsaA-PsaB heterodimer is essential for the assembly of the complex (Smart et al. 1992, 1994; Smart and McIntosh 1993). Although these mutant strains required light-activated photoheterotrophic conditions for growth, other groups have generated PsaA- or PsaB-less cyanobacterial mutants in *Synechocystis* sp. PCC 6803 (Shen et al. 1993), *Anabaena variabilis* (Toelge et al. 1991; Nyhus et al. 1993), or *Synechococcus* sp. PCC 7002 (D.A. Bryant, personal communication) that can be grown under low light intensity or in dark. A mutant strain of *Chlamydomonas reinhardtii* with an inactive *psaB* due to a frame-shift mutation is also available (Bingham et al. 1991). All these mutants have similar phenotypes with respect to PS I assembly and function.

With the availability of subunit-deficient mutants, vigorous site-directed mutagenesis of PsaA-PsaB is now focused on identifying the residues that bind different cofactors. An obvious choice for such approach was mutations in the cysteine residues that have been proposed as F_x ligands. When Cys565 in PsaB of *Syne-*

chocystis sp. PCC 6803 was mutated to serine, histidine or aspartic acid, the mutations greatly reduced accumulation of PS I (Smart et al. 1993). The serine mutant contains sufficient PS I to perform detailed EPR analysis. The C565S mutant PS I contains a [3Fe-4S] and [4Fe-4S] clusters at F_X that are capable of electron transfer to F_A/F_B (Warren et al. 1993). Therefore, Cys565 coordinates F_X, which in turn is essential for stable assembly of PS I core. Mutation of another cysteine residue in *Chlamydomonas reinhardtii* has also indicated a similar role of F_X in PS I assembly (Webber et al. 1993). Mutagenesis of the leucine zipper motif in PsaB does not provide support for a major structural role of the leucine zipper in reaction center dimerization (Smart et al. 1993).

Mutant strains with inactive *psaC* have been generated in *Anabaena variabilis* (Mannan et al. 1991), *Synechocystis* sp. PCC 6803 (L. McIntosh, personal communication), and *Chlamydomonas reinhardtii* (Takahashi et al. 1991). Although all these mutants lack EPR signals that can be attributed to F_A/F_B, the lack of PsaC has different effects on the assembly of the complex in different organisms. PS I core is not stably assembled in the PsaC-less mutant of *Chalmydomonas reinhardtii*, but is assembled and functional in charge separation in the cyanobacterial mutants. PsaD and PsaE cannot be detected in the membranes of PsaC-less cyanobacterial mutants (Mannan et al. 1994).

An in vitro reconstitution approach has been successfully used to determine role of the conserved cysteines in PsaC (Zhao et al. 1990, 1992; Li et al. 1991b). PsaD, PsaE and PsaC of *Synechococcus* sp. PCC 7002 have been produced in *Escherichia coli* for in vitro reconstitution with a biochemical prepared PS I core ($P700\text{-}F_X$). These experiments showed that the substitution of Cys14 by an aspartate led to the formation of a [3Fe-4S] center at F_B, while the similar substitution at Cys51 led to a [3Fe-4S] center at F_A. These results indicate that the F_A cluster is liganded by Cys 21, 48, 51, and 54 while Cys 11, 14, 17 and 58 are ligated to the cluster F_B (Zhao et al. 1992). Phenotype of the reconstituted PsaC mutants shows that reduction of F_A and F_B are independent of each other, thus favoring two parallel pathways of electron transfer through PsaC. Further site-directed mutations showed that the ninth cysteine (Cys34) does not participate in ligating either F_A or F_B (Yu et al. 1993a). This approach allows functional characterization of the proteins before and after assembly into PS I (Yu et al. 1993a).

Targeted mutagenesis to reveal functions of the accessory subunits

The accessory subunits of PS I do not bind any redox-active cofactors. The conservation of the predicted primary sequences of these PS I polypeptides from cyanobacteria and higher plants suggests a high selection pressure during evolution. The cyanobacterial and chloroplast PS I have similar structure and mechanism of electron transfer. This suggests important roles for these polypeptides. Biochemical depletion and targeted mutagenesis can be used to study roles of the accessory subunits in PS I function and organization. Subunit-specific cyanobacterial mutants with deletion or interruption of the *psaD* (Chitnis et al. 1989b), *psaE* (Chitnis et al. 1989a; Rousseau et al. 1993; Zhao et al. 1993), *psaF* (Chitnis et al. 1991; Xu et al. 1994e), *psaI* (Xu et al. 1995), *psaJ* (Xu et al. 1994d), *psaK* (D.A. Bryant, personal communication), *psaL* (Chitnis and Chitnis 1993; Chitnis et al. 1993), and *psaM* (D.A. Bryant, personal communication) genes have been generated and characterized. Several combinatorial mutants that lack more than one accessory subunit are also available (Chitnis and Chitnis 1993; Cohen et al. 1993; Xu et al. 1994c). The study of these mutants have revealed the following functions of the accessory subunits.

(a) Interaction with soluble electron acceptors

The accessory subunits provide topological surfaces for accurate docking and orientation of the soluble electron carriers on the reducing sides of PS I, thereby allowing a mechanism to modulate PS I activity. Plastocyanin and cytochrome c_6 interact with the oxidizing side of PS I while ferredoxin and flavodoxin transiently associate with the reducing side of PS I.

Reducing site of PS I is required for the reduction of ferredoxin. Flavodoxin replaces ferredoxin in cyanobacteria growing in iron-depleted medium (Morand et al. 1994). Ferredoxin or flavodoxin mediated $NADP^+$ photoreduction, and a direct examination of ferredoxin or flavodoxin reduction have been used to characterize the function of subunit-deficient PS I complexes from *Synechocystis* sp. PCC 6803 (Rousseau et al. 1993; Xu et al. 1994c). PsaD-less cyanobacterial membranes are unable to reduce $NADP^+$ via ferredoxin (Xu et al. 1994c). Also, the absence of PsaD in these membranes drastically reduces flavodoxin reduction rates (< 10% of wild type). In contrast, the lack of PsaE has more severe effect on ferredoxin reduction

than on flavodoxin reduction. It seems that interaction of flavodoxin with PS I has less stringent structural requirements (Xu et al. 1994c). Also, extramembrane regions of PsaL, PsaJ or PsaF may not form an essential part of the reducing site of PS I because the PS I complexes of mutants lacking these subunits have normal ferredoxin- or flavodoxin-mediated $NADP^+$ reduction activity (Xu et al. 1994c). Therefore, PsaD, PsaE and PsaC are the essential components of the reducing side of PS I. Protease accessibility and NHS-biotinylation experiments have shown that a C-terminal domain of PsaD is exposed on the surface of PS I (Xu et al. 1994b). Deletions in the C-terminus of PsaD do not abolish the ability of the mutant proteins to assemble into PS I in vitro (Chitnis and Nelson 1992a). Therefore, the surface-exposed domain is not essential for the assembly of PsaD into PS I complex and may interact with ferredoxin. These results are indeed consistent with biochemical characterization of the reducing site by topographical and chemical cross-linking investigations (Zilber and Malkin 1988; Lelong et al. 1994; Xu et al. 1994b).

Besides the linear electron transfer that results in $NADP^+$ photoreduction, PS I also participates in cyclic electron flow. Although the identity of electron acceptor in the cyclic pathway is not known, characterization of PsaE-less strain of *Synechococcus* sp. PCC 7002 (Yu et al. 1993b) has shown that PsaE is essential for cyclic electron flow around PS I. Recently obtained PsaM-less mutant of *Synechococcus* sp. PCC 7002 also shows defect in cyclic electron flow (D.A. Bryant, personal communication).

(b) Protection of the electron transfer centers

P700*/P700 has the lowest calculated redox potential (−1290 mV) for any biological redox center. Other electron transfer centers are also extremely electronegative (−1010 to −530 mV) (see reviews by Golbeck and Bryant 1991; Setif 1992). Protection of these centers is an essential condition for PS I to function in an oxygenic photosynthetic environment. Some accessory subunits of PS I may provide physical isolation of the electron transfer centers, protect them from wasteful oxidation and allow only regulated oxidation to occur. Turnover of PS I is increased in the PsaE-less *Synechocystis* mutant strain (Chitnis and Nelson 1992b). The PsaD-PsaE-less mutant strain of *Synechocystis* sp. PCC 6803 dies under higher light intensity or temperature due to photooxidative damage (Q. Xu and P.R. Chitnis, unpublished results). PsaD and PsaE may have protective roles on the reducing side of PS I.

(c) Structural and regulatory role in the organization of PS I

Some accessory subunits interact with other subunits and orient them properly. Other accessory subunits, like PsaL are essential components of the trimeric quaternary structure of PS I, that has been demonstrated in the photosynthetic membranes of cyanobacteria (Kruip et al. 1994). Several PsaL-less mutant strains have been obtained in *Synechocystis* sp. PCC 6803; trimeric PS I cannot be obtained from the PsaL-less strains (Chitnis and Chitnis 1993; Chitnis et al. 1993). Therefore, PsaL is essential for the formation of stable PS I trimers. Furthermore, PsaL in monomers, but not in the trimers is susceptible to thermolysin digestion, suggesting the positioning of PsaL in the trimer-forming domain of PS I. Recently, addition of Ca^{+2} was found to stimulate formation of PS I trimers. Calcium binds to the PsaL subunit of PS I, thus suggesting a regulatory role of PsaL in trimer formation (V.P. Chitnis and P.R. Chitnis, unpublished results).

PS I (Table 1), PS II (see review by Pakrasi and Vermaas 1992) and Cyt b_6/f (Pierre and Popot 1993) complexes contain many hydrophobic polypeptides of ≤ 4 kDa. These proteins have one putative transmembrane helix flanked by short hydrophilic, charged domains. PsaJ, PsaI and PsaM polypeptides of PS I belong to this group (Table 1). Recent characterization of PsaI- and PsaJ-less mutants of *Synechocystis* sp. PCC 6803 indicates that these polypeptides are 'nuts and bolts' in the structure of PS I. They assist in the correct organization of other subunits by anchoring peripheral proteins and stabilizing transmembrane helices in lipid bilayer. Analysis of PS I organization in a PsaJ-less mutant of *Synechocystis* sp. PCC 6803 has revealed that PsaJ may interact with both PsaE and PsaF (Xu et al. 1994d). It is possible, for example, that a PsaJ-PsaF interaction may involve stabilization of the transmembrane helix of PsaF. The putative transmembrane region of PsaF is shorter and less hydrophobic than typical transmembrane helices (Chitnis et al. 1991). The highly hydrophobic transmembrane helix of PsaJ may stabilize the PsaF helix. Targeted inactivation of *psaI* in *Synechocystis* sp. PCC 6803 has revealed a role of PsaI in the normal organization of the PsaL subunit of PS I (Xu et al. 1995). PsaL is removed from the PS I core after detergent extraction of the PsaI-less membranes. A structural interaction between PsaL and PsaI

may stabilize association of PsaL with the PS I core. PsaL in the wild-type and PsaI-less membranes equally resists chaotropic extraction, but exhibits an increased susceptibility to proteolysis in the mutant strain. Similar role of PsaM in organization of other subunits, perhaps PsaK, has not been identified.

Thus the accessory polypeptides of PS I interact with soluble electron acceptors, protect the redox centers, and assist in PS I assembly and organization of the complex. In addition the hydrophobic accessory subunits may interact with other membrane proteins, such as pigment-carrier proteins. In cyanobacteria, they may also participate in energy transfer from phycobilisomes to PS I, either by interacting with phycobilisomes or by binding Chl a molecules that are crucial in energy transfer. Changes in state transitions in some cyanobacterial mutants indicate such role for PsaK, PsaF, and PsaJ (D.A. Bryant, personal communication). Integral membrane-proteins in plants and algae, especially PsaG and PsaH, may interact with LHCI. These functions of accessory proteins of PS I are yet to be demonstrated.

Structure and organization of PS I

PS I structure has been studied by negative staining electron microscopy and X-ray crystallography. Organization of PS I subunits has also been inferred from their hydropathy profiles determined from deduced amino acid sequences, nature of their transit peptides and from biochemical experiments involving protease accessibility, antibody epitope mapping, chemical cross-linking and reconstitution. Although a clear picture of the organization of PS I subunits may be forthcoming from the finer resolution of PS I crystal-structure, topological explorations of the PS I complexes aid and complement interpretation of X-ray crystallographic data. Based on the available information, Fig. 2 presents a model for the organization for PS I polypeptides in cyanobacteria.

Electron microscopy and X-ray crystallographic studies

Electron microscopy has provided valuable information on the global shape and size of different PS I complexes (Boekema et al. 1994) and X-ray crystallography has permitted a somewhat more detailed knowledge of secondary and tertiary structural elements of the reaction center core subunits (Krauß et al. 1993; Fromme et al. 1994).

The negative staining electron microscopy, mainly performed on the PS I complexes isolated from cyanobacteria, predicted the complex to be either an ellipsoid of 18 × 8 nm (Williams et al. 1983), or a disk shape with a diameter of about 19 nm and a thickness of 6 nm (Boekema et al. 1987). The trimeric form of the cyanobacterial PS I was measured to be a triangular disk of 18 × 6 × 6 nm and the monomeric PS I has been described to be a pear-shape of 15 × 9 × 6 nm (Ford et al. 1988; Ford and Holzenburg 1988). The reaction centers have a ridge of 2.5–3.0 nm height projecting from one side of the membrane while their other side is rather flat and exhibits a shallow, central indentation (Ford et al. 1990; Bottcher et al. 1992; Kruip et al. 1993). This ridge has been proposed to be composed of the PsaC, PsaD and PsaE subunits. The other side, presumably the lumenal side, is flat, but has a 3 nm deep indentation in the center, perhaps caused by a partial separation between PsaA and PsaB where plastocyanin can dock during electron transfer (Bottcher et al. 1992; Kruip et al. 1993). Several subunit-deficient cyanobacterial mutants are now being used in electron microscopy studies to determine approximate locations of PS I subunits in the overall architecture of PS I. For example, electron microscopy of the wild type and PsaF-PsaJ-less PS I trimers from *Synechocystis* sp. PCC 6803 suggest that the lack of PsaF and PsaJ cause changes in a contiguous area on the opposite side from the trimer-forming domain (E. Boekema, J. Kruip, P.R. Chitnis and M. Rögner, unpublished results).

These electron microscopy studies have yielded valuable information concerning the overall shape and size of PS I complexes. These studies do not (and cannot) provide detailed structure of the complex. The exact packing of eleven or more proteins in a compact PS I structure will only be understood from atomic resolution X-ray diffraction analysis of PS I crystals. PS I of *Synechococcus elongatus* (Witt et al. 1987), *Mastigocladus laminosus* (Almog et al. 1991), *Synechococcus* sp. PCC 7002 (Tsiotis et al. 1993) and *Synechocystis* sp. PCC 6803 (Reilly and Nelson 1988) have been crystallized. The three dimensional structure of trimeric PS I from *Synechococcus elongatus* has been proposed at 6 Å resolution (Krauß et al. 1993). According to this model, a monomer of PS I consists of a 'catalytic domain' and a smaller 'connecting domain' that links monomers to form a trimer. The absence of trimers in the PsaL-less mutants of *Synechocystis* sp. PCC 6803 indicates that the smaller domain with two

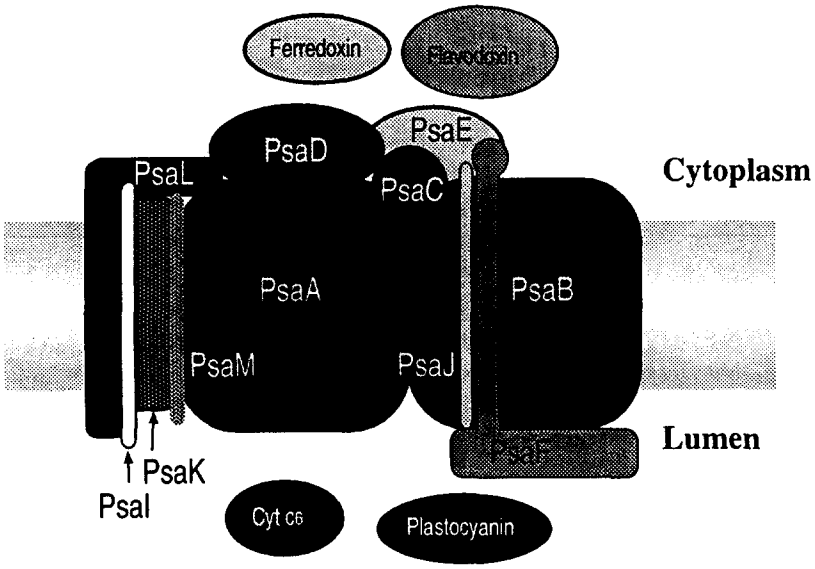

Fig. 2. A model for the organization of PS I polypeptides in cyanobacteria. The rationale behind the specific spatial arrangement shown in the model is discussed in the text and in Table 2. Exact location of PsaM with respect to other subunits is not known. Also, PsaA and PsaB in this model are arbitrarily assigned.

transmembrane helices (*j* and *k* in (Krauß et al. 1993)) may belong to PsaL (Chitnis and Chitnis 1993). The crystal-analysis of PS I has indicated locations of the [4Fe-4S] clusters F_X, F_A and F_B, 28 α-helices and 45 Chl *a* molecules. PsaA and PsaB were proposed to contain eight transmembrane helices each (Krauß et al. 1993). The two core subunits were found to relate to each other by a two-fold rotation symmetry axis that passes through the Fe-S cluster F_x. Although the 6 Å structure has provided some tentative assignments regarding organization of PS I complex, a more accurate assignment of the different protein subunits within the complex could not be accomplished due to the low resolution of this analysis.

Topology of the PS I complex

Biochemical studies have shown that two large subunits (PsaA and PsaB) and some low molecular weight subunits (PsaF, PsaL, PsaK, PsaG, PsaI, PsaJ, and PsaM) are integral membrane-proteins with highly hydrophobic transmembrane domains (Ortiz et al. 1985; Zilber et al. 1990; Tjus and Andersson 1991; Xu et al. 1994c,e). PsaD, PsaC, PsaE, and PsaH are peripheral proteins on the stromal (cytoplasmic) side of photosynthetic membranes (Li et al. 1991a; Tjus and Andersson 1991; Lagoutte and Vallon 1992). PsaN is a peripheral protein on the lumenal side of membranes (He and Malkin 1992).

What regions of PS I proteins are exposed on the surface? Proteases, and surface-labeling techniques are used to address this question. Early studies demonstrated that the PS I core (PsaA-PsaB) is an integral membrane-proteins complex (Ortiz et al. 1985; Malkin 1986). However, considerable parts of the PsaA-PsaB extend outside the membrane into stroma and lumen (Sadewasser and Sherman 1981; Ortiz et al. 1985). Recent investigations using proteases indicated that the N-terminal domains of PsaA and PsaB in plant and cyanobacterial PS I are more accessible to proteases (Zilber and Malkin 1992; Xu et al. 1994e), suggesting that the C-terminal domains are involved in the formation of PsaA-PsaB heterodimer and in interaction with other subunits. Chemical modification of arginine residues in PsaA-PsaB core and trypsin-treatment of the core indicate that arginyl residues in PsaA-PsaB interact with PsaC (Rodday et al. 1993). Another study using antibodies to synthetic peptides has shown that residues 413–421 of PsaA are exposed on the stromal surface of the membrane (Vallon and Bogorad 1993). Immunogold labeling also indicates that residues 371–379 and 497–505 are exposed on the lumenal surface of the thylakoids (Vallon and Bogorad 1993). Therefore the F_x center and the N-termini of the core proteins is located on the stromal (cytoplasmic) side of the

thylakoids. Accessibility of primary amines to NHS-biotin is yet another criterion used to probe the protein surfaces that are exposed to aqueous phase. Both PsaA and PsaB have surface-exposed lysine residues (Xu et al. 1994b). Further studies are necessary to accurately identify the surface topology of the PS I core.

On the reducing side of PS I, some domains of the PsaD, and PsaE subunits of PS I are exposed to proteases (Zilber and Malkin 1992; Chitnis and Chitnis 1993; Xu et al. 1994c). Only limited cleavage sites are accessible for proteolytic digestion of spinach PsaD (Zilber and Malkin 1992). The N-terminal domain of PsaD, that is absent in the cyanobacterial PS I, is exposed to the stroma whereas the bulk of the protein is protected. Further partial mapping of exposed epitopes indicated that another region between Met 74 and Met 140, that includes a cluster of basic amino acids, is also accessible to proteases (Lagoutte and Vallon, 1992). Proteolytic treatment of spinach PsaE revealed that the N-terminal domain of this protein is also exposed to the stroma (Lagoutte and Vallon, 1992; Zilber and Malkin, 1992). In a detailed topological study of the reducing side of cyanobacterial PS I, the surface-exposed domains were identified using NHS-biotinylation and proteases (Xu et al. 1994b). A lysine-containing region of PsaC and the C-terminal regions of PsaE and PsaD are exposed on the surface of cyanobacterial PS I. In plant PS I, PsaH is also exposed on stromal side, but its surface topology is not known (Zilber and Malkin, 1992).

The topology of smaller hydrophobic proteins of PS I (PsaF, PsaL, PsaK, PsaG, PsaI, PsaJ, and PsaM) is not clearly understood (Zilber and Malkin 1992). In intact plant thylakoids, PsaF is not accessible to proteases. PsaL contains a protease-accessible cleavage site, probably in the loop between two carboxyl-proximal proposed transmembranal spans, indicating that this loop is stromal-exposed. A central part of PsaK is also exposed on the stromal side of the membranes (Zilber and Malkin, 1992). Surface-exposed domains in other PS I subunits are not known.

How are PS I proteins organized with respect to each other? Chemical cross-linking, reconstitution experiments, and phenotypes of subunit-deficient mutants have revealed several subunit interactions in PS I (Table 2). This data can be used to propose a model for the organization of PS I proteins (Fig. 2). PsaD, PsaE and PsaC are in contact with each other and a considerable part of PsaC is probably buried under PsaD and PsaE (Oh-Oka et al. 1989; Xu et al. 1994a). These studies are supported by the analysis of subunit-specific cyanobacterial mutants. PsaC is more accessible to chaotropic agents in PsaD-less mutant while PsaD and PsaE do not assemble in vivo into PS I of PsaC-less cyanobacterial mutant (Mannan et al. 1994). Cross-linking experiments with glutaraldehyde have identified additional nearest-neighbor pairs in cyanobacterial PS I: PsaD-PsaE, PsaE-PsaF, and PsaL-PsaD (Xu et al. 1994a). Examination of PS I in PsaD-less and PsaL-less strains of *Synechocystis* sp. PCC 6803 have further confirmed the importance of PsaD-PsaL interaction in their assembly into PS I complex (Xu et al. 1994a). In similar studies, structural interactions between PsaJ and PsaF and between PsaI and PsaL have been shown (Xu et al. 1994d, 1995). PsaL and PsaI are required for the formation of PS I trimers (Chitnis and Chitnis 1993; Xu et al. 1995) and may constitute the connecting domain of PS I, that was identified in X-ray crystallographic analysis (Krauß et al. 1993). The connecting domain may be linked to the catalytic domain through PsaD-PsaL interactions. PsaF and PsaJ may be localized close to each other to the opposite side from the connecting domain. The only transmembrane helices that can be attributed to the PsaK and PsaM hydrophobic subunits are between the connecting domain and the catalytic domain. This placement is consistent with the accessibility of PsaK to proteases in monomeric PS I, but not in trimeric PS I (Chitnis and Chitnis 1993).

Interaction of PS I with soluble electron carriers

Interactions on the reducing side of PS I

The interprotein electron transfer on the reducing side of PS I is a complex process. At least three proteins (PsaC, PsaD, and PsaE) in PS I must interact with ferredoxin for electron transfer to occur (Rousseau et al. 1993; Xu et al. 1994c). The interaction between ferredoxin and PS I involves electron transfer in a series of iron-sulfur centers. Recently, kinetics of reduction of soluble cyanobacterial ferredoxin by cyanobacterial PS I were investigated by flash absorption spectroscopy (Setif and Bottin 1994). This study revealed the existence of three different first order components with $t_{1/2}$ of $\sim$ 500 ns, 20 μs and 100 μs. The 500 ns phase corresponds to electron transfer from F_A/F_B to ferredoxin. These analyses also indicated the presence of at least two types of PS I-ferredoxin complexes, all competent in electron transfer.

Table 2. Subunit Interactions in PS I complex

Subunit	Interacting subunit	Evidence[a]	Reference
PsaA	PsaB	Resolution	(Fish and Bogorad 1986)
		X-ray	(Krauß et al. 1993)
PsaA-PsaB	PsaC	Resolution	(Golbeck et al. 1988)
PsaA-PsaB	PsaD, PsaE	Resolution	(Li et al. 1991b)
	Other subunits	EM	(Kruip et al. 1993)
		X-ray	(Krauß et al. 1993)
PsaC	PsaD	Reconstitution	(Li et al. 1991b)
		Cross-linking	(Oh-Oka et al. 1989; Xu et al. 1994a)
		Genetic	(Mannan et al. 1994)
PsaC	PsaE	Cross-linking	(Oh-Oka et al. 1989; Xu et al. 1994a)
		Genetic	(Mannan et al. 1994)
PsaD	PsaE	Reconstitution	(Chitnis and Nelson 1992a)
		Cross-linking	(Oh-Oka et al. 1989; Xu et al. 1994a)
		Topological	(Xu et al. 1994c)
PsaD	PsaL	Cross-linking	(Xu et al. 1994a)
		Topological	(Xu et al. 1994a,e)
PsaE	PsaF	Cross-linking	(D.A. Bryant, personal communication; V.P. Chitnis, P.R. Chitnis, unpublished results)
		Topological	(Xu et al. 1994d,e)
PsaE	PsaJ	Topological	(Xu et al. 1994d)
PsaF	PsaJ	Topological	(Xu et al. 1994d)
PsaI	PsaL	Topological	(Xu et al. 1995)

[a] Evidence: The *resolution*-reconstitution experiments show requirement of one protein for reconstitution of the other subunit. *EM* (electron microscopic) and *X-ray* diffraction analysis show interaction between tow proteins. Chemical *cross-linking* of two proteins by homo- or hetero-bifunctional reagents demonstrate nearest-neighbor relations between two proteins while the *topological* studies involving treatment with chaotropes and proteases indicate subunit interactions. The absence or decreased accumulation of specific proteins in the subunit-specific *genetic* mutants also indicate assembly requirements and subunit interactions.

Which proteins of PS I interact with ferredoxin? Ferredoxin accepts electrons from F_A-F_B of PsaC, implying that these proteins should contact each other. The physical association between PsaC and ferredoxin has not yet been demonstrated. The major obstacle in the association of PsaC and ferredoxin is the unfavorable electrostatic interactions; both PsaC and ferredoxin have strong electronegative surfaces at the physiological pH. Therefore docking proteins are required to facilitate the interaction by providing clusters of oppositely charged amino acids. PsaD and PsaE are required for the interaction and reduction of ferredoxin (Rousseau et al. 1993; Xu et al. 1994c).

What types of interactions are involved between PS I and ferredoxin? Current evidence suggests that the interactions are electrostatic in nature. Spectroscopic study of ferredoxin reduction by PS I suggests that complex formation precedes electron transfer and the rate constants for complex formation depend on ionic concentration, especially magnesium ions, suggesting electrostatic interactions between ferredoxin and PS I (Hervas et al. 1992; Setif and Bottin 1994). The zero-length cross-linker N-ethyl-3-(dimethylaminopropyl)-carbodiimide, which reacts carboxyl and amino groups of proteins, can be used to cross-link ferredoxin to PsaD (Zanetti and Merati 1987; Zilber and Malkin 1988; Wynn et al. 1989a). The lysyl residue at position

106 of PsaD from *Synechocystis* sp. PCC 6803 can be cross-linked to the glutamyl residue at the position 93 in ferredoxin from the cyanobacterium (Lelong et al. 1994). The phenotype of PsaD-less cyanobacterial mutants is consistent with the functional requirement of PsaD for ferredoxin-docking (Xu et al. 1994c). The nature of interactions between PsaE and ferredoxin are not known.

Interactions on the oxidizing side of PS I

The interaction of plastocyanin with PS I is not clearly understood. Spectroscopic measurements revealed that two types of interactions between plastocyanin and plant PS I. A fast phase with $t_{1/2}$ of 12–14 μs and a second slower phase with life-time $t_{1/2}$ of 200 μs (Haehnel et al. 1980; Bottin and Mathis 1985). The fast rate can be attributed to the association of plastocyanin in the vicinity of P700, whereas the slower phase may represent a distant plastocyanin population (Haehnel et al. 1980; Bottin and Mathis 1985). PsaF is essential for the fast electron transfer (Hippler et al. 1989). In cyanobacteria and algae, plastocyanin and cytochrome c_6 can donate electrons to PS I (Diaz et al. 1994; Hervas et al. 1994). Plastocyanin and Cyt c_6 from *Anabaena* sp. PCC 7119 have similar association constants for the complex formation with spinach PS I (Medina et al. 1993). Thermodynamic analysis has shown that PS I-mediated oxidation of plastocyanin as well as Cyt c_6 from an alga takes place with a two-step kinetics (Diaz et al. 1994). In contrast, laser flash kinetic analysis of cyanobacterial Cyt c_6 and plastocyanin oxidation by PS I suggests an apparent one-step bimolecular collisional mechanism (Hervas et al. 1994). At physiological pH, Cyt c_6 is more efficient than plastocyanin in donating electrons to the cyanobacterial PS I (Hervas et al. 1994).

Which regions in plastocyanin interact with PS I? Site-directed mutagenesis has shown that a hydrophobic region containing Leu12 on the surface of the spinach plastocyanin is crucial for the association and for electron transfer to PS I (Nordling et al. 1991). In a separate study, when Gly10 or Ala90 in a conserved flat hydrophobic surface around the copper ligand His87 of spinach plastocyanin were replaced by the bulky hydrophobic Leu, the formation of the PC-PS I complex was abolished. Therefore, these two residues are part of the structures required for the docking of plastocyanin on PS I and for the efficient electron transfer from plastocyanin to $P700^+$ (Haehnel et al. 1994). What constitutes the oxidizing site on the lumenal surface of PS I? PsaF has been proposed to function as a docking site for plastocyanin or Cyt c_6 (see earlier discussion on PsaF). However, PsaF is not essential for the electron transfer (Xu et al. 1994e). Based on a low resolution surface fitting model, the helices of PsaA and PsaB that are parallel to the lumenal membrane-surface have been proposed to form the plastocyanin-docking site (Fromme et al. 1994). These helices contain a conserved structural motif that is shared by NdhH subunit of NADH:plastoquinone oxidoreductase and the CD helix in L and M subunits of the reaction center of purple bacteria (Kuhn et al. 1994). All these enzymes interact with soluble electron carriers and may have similar mechanism for docking. Functional significance of the putative docking sites on PsaA-PsaB needs to be tested by site-directed mutagenesis.

The current model for the plastocyanin-PS I interaction and electron transfer in plants contains a four-step mechanism (Haehnel et al. 1994). First, long-range electrostatic interactions between the negative patch of plastocyanin and positive residues on PsaF are formed. In a second stage, the flat hydrophobic patch of plastocyanin docks tightly on a hydrophobic site, probably comprised of the two membrane-attached helices, of PsaA-PsaB. These two association steps allow the fast electron transfer with a $t_{1/2}$ of 12–14 μs. The interaction ends with a facilitated release of the oxidized plastocyanin.

Acknowledgements

We thank John Golbeck, Don Bryant, Lee McIntosh, and Matthias Rogner for helpful discussions and for communicating unpublished data. Our unpublished work was supported by grants from the National Science Foundation (MCB9202751 and MCB9405325 to PRC), the United States Department of Agriculture (92–37306–7661 to PRC) and the United States – Israel Binational Agricultural Research & Development Fund (IS-2191–92RC to RN and PRC). This paper represents contribution 95-306-J from the Kansas Agricultural Experimental Station.

References

Almog O, Shoham G, Michaeli D and Nechushtai R (1991) Monomeric and trimeric forms of the Photosystem I reaction center of *Mastigocladus laminosus*: Crystallization and preliminary characterization. Proc Natl Acad Sci USA 88: 5312–5316

Anderson B, Scheller H and Moller B (1992) The PS I-E subunit of Photosystem I binds ferredoxin:$NADP^+$ oxidoreductase. FEBS Lett 311: 169–173

Anderson JM and Melis A (1983) Localization of different photosystems in separate regions of chloroplast membranes. Proc Natl Acad Sci USA 80: 745–749

Anderson SL and McIntosh L (1991) Light-activated heterotrophic growth of the cyanobacterium *Synechocystis* sp. strain PCC 6803: A blue-light-requiring process. J Bacteriol 173: 2761–2767

Bengis C and Nelson N (1977) Subunit structure of chloroplast Photosystem I reaction center. J Biol Chem 252: 4564–4569

Bingham SE, Xu R and Webber AN (1991) Transformation of chloroplasts with the psaB gene encoding a polypeptide of the Photosystem I reaction center. FEBS Lett 292: 137–140

Boekema EJ, Dekker JP, Van Heel MG, Rogner M, Saenger W, Witt I and Witt HT (1987) Evidence for a trimeric organization of the Photosystem I complex from the thermophilic cyanobacterium *Synechococcus* sp. FEBS Lett 217: 283–286

Boekema EJ, Boonstra AF, Dekker JP and Rogner M (1994) Electron microscopic structural analysis of Photosystem I, Photosystem II, and the cytochrome b6/f complex from green plants and cyanobacteria. J Bioenerg Biomemb 26: 17–29

Bottcher B, Graber P and Boekema EJ (1992) The structure of Photosystem-I from the thermophillic cyanobacterium *Synechococcus* sp. determined by electron-microscopy of 2-dimensional crystals. Biochim Biophys Acta 1100: 125–136

Bottin H and Mathis P (1985) Interaction of plastocyanin with the Photosystem I reaction center: A kinetic study by flash spectroscopy. Biochemistry 24: 6453–6460

Bruce BD and Malkin R (1988a) Structural aspects of Photosystem I from *Dunaliella salina*. Plant Physiol 88: 1201–1206

Bruce BD and Malkin R (1988b) Subunit stoichiometry of the chloroplast Photosystem I complex. J Biol Chem 263: 7302–7308

Bryant D (1992) Molecular biology of Photosystem I. In: Barber J (ed) The Photosystems: Structure, Function and Molecular Biology, pp 501–549. Elsevier, Amsterdam

Chitnis PR and Nelson N (1992a) Assembly of two subunits of the cyanobacterial Photosystem I on the n-side of thylakoid membranes. Plant Physiol 99: 239–246

Chitnis PR and Nelson N (1992b) Biogenesis of Photosystem I: The subunit PsaE is important for the stability of PS I complex. In: Argyroudi-Akoyunoglou J (ed) Chloroplast Biogenesis, pp 285–290. Plenum Press, New York

Chitnis PR, Reilly PA, Miedel MC and Nelson N (1989a) Structure and targeted mutagenesis of the gene encoding 8-kDa subunit of Photosystem I of the cyanobacterium *Synechocystis* sp. PCC 6803. J Biol Chem 264: 18374–18380

Chitnis PR, Reilly PA and Nelson N (1989b) Insertional inactivation of the gene encoding subunit II of Photosystem I of the cyanobacterium *Synechocystis* sp. PCC 6803. J Biol Chem 264: 18381–18385

Chitnis PR, Purvis D and Nelson N (1991) Molecular cloning and targeted mutagenesis of the gene *psaF* encoding subunit III of Photosystem I from the cyanobacterium *Synechocystis* sp. PCC 6803. J Biol Chem 266: 20146–20151

Chitnis VP and Chitnis PR (1993) PsaL subunit is required for the formation of Photosystem I trimers in the cyanobacterium *Synechocystis* sp. PCC 6803. FEBS Lett 336: 330–334

Chitnis VP, Xu Q, Yu L, Golbeck JH, Nakamoto H, Xie D-L and Chitnis PR (1993) Targeted inactivation of the gene psaL encoding a subunit of Photosystem I of the cyanobacterium *Synechocystis* sp. PCC 6803. J Biol Chem 268: 11678–11684

Cohen Y, Chitnis VP, Nechushtai R and Chitnis PR (1993) Stable assembly of PsaE into cyanobacterial photosynthetic membranes is dependent on the presence of other accessory subunits of Photosystem I. Plant Mol Biol 23: 895–900

Diaz A, Hervas M, Navarro JA, De la Rosa MA and Tollin G (1994) A thermodynamic study by laser flash photolysis of plastocyanin and cytochrome c_6 oxidation by Photosystem I from the green alga *Monoraphidium braunii*. Eur J Biochem 222: 1001–1007

Dunn PPJ and Gray JC (1988) Localization and nucleotide sequence of the gene for the 8 kDa subunit of Photosystem I in pea and wheat chloroplast DNA. Plant Mol Biol 11: 311–319

Falzone CJ, Kao Y-H, Zhao J, Bryant DA and Lecomte TTJ (1994a) The three-dimensional solution structure of PsaE from the cyanobacterium *Synechococcus* sp. strain PCC 7002: A Photosystem I protein that shows structural homology with SH3 domain. Biochemistry 33: 6052–6062

Falzone CJ, Kao Y-H, Zhao J, MacLaughlin KL, Bryant DA and Lecomte TTJ (1994b) ^1H and ^{15}N NMR assignments of PsaE, a Photosystem I subunit from the cyanobacterium *Synechococcus* sp. PCC 7002. Biochemistry 33: 6043–6051

Fish LE, Kuck U and Bogorad L (1985a) Analysis of the two partially homologous P700 chlorophyll a proteins of maize Photosystem I: Predictions based on the primary sequences and features shared by other chlorophyll proteins. In: Steinback KE, Bonitz S, Arntzen CJ and Bogorad L (eds) Molecular Biology of the Photosynthetic Apparatus, pp 111–120. Cold Spring Harbor Laboratory, Cold Spring, New York

Fish LE, Kuck U and Bogorad L (1985b) Two partialy homologous adjacent light-inducible maize chloroplast genes encoding polypeptides of the P700 chlorophlly *a* protein complex of Photosystem I. J Biol Chem 260: 1413–1421

Fish LE and Bogorad L (1986) Identification and analysis of the maize P700 chlorophyll *a* apoproteins PS I-A1 and PS I-A2 by high pressure liquid chromatography analysis and partial sequence determination. J Biol Chem 261: 8134–8139

Flieger K, Oelmueller R and Herrmann RG (1993) Isolation and characterization of cDNA clones encoding a 18.8kDa polypeptide, the product of the gene *psaL*, associated with Photosystem I reaction center from spinach. Plant Mol Biol 22: 703–709

Ford RC and Holzenburg A (1988) Investigation of the structure of trimeric and monomeric Photosystem I reaction centre complexes. EMBO J 7: 2287–2293

Ford RC, Pauptit R and Holzenburg A (1988) Structural studies on improved crystals of the Photosystem I reaction center from *Phormidium laminosum*. FEBS Lett 238: 385–389

Ford RC, Hefti A and Engel A (1990) Ordered arrays of the Photosystem I reaction centre after reconstitution: Projections and surface reliefs of the complex at 2 nm resolution. EMBO J 9: 3067–3075

Franzen L-G, Frank G, Zuber H and Rochaix J-D (1989) Isolation and characterization of cDNA clones encoding Photosystem I subunits with molecular masses 11.0, 10.0 and 8.4 kDa from *Chlamydomonas reinhardtii*. Mol Gen Genet 219: 137–144

Fromme P, Schubert W-D and Krauss N (1994) Structure of Photosystem I: Suggestions on the docking sites for plastocyanin, ferredoxin and the coordination of P700. Biochim Biophys Acta 1187: 99–105

Gavel Y, Steppuhn J, Herrmann R and Von Heijne G (1991) The 'positive-inside rule' applies to thylakoid membrane proteins. FEBS Lett 282: 41–46

Golbeck JH (1992) Structure and function of Photosystem I. Annu Rev Plant Physiol Plant Mol Biol 43: 293–324

Golbeck JH (1993a) Shared thematic elements in photochemical reaction centers. Proc Natl Acad Sci USA 90: 1642–1646

Golbeck JH (1993b) The structure of Photosystem I. Curr Opinions Struct Biol 3: 508–514

Golbeck JH and Bryant DA (1991) Photosystem I. Curr Top Bioenerg 16: 83–177

Golbeck JH, Mehari T, Parrett K and Ikegami I (1988) Reconstitution of the Photosystem I complex from the P700 and FX-containing reaction center core protein and the F_A/F_B polypeptide. FEBS Lett 240: 9–14

Gross E (1993) Plastocyanin: Structure and function. Photosynth Res 37: 103–116

Haehnel W, Propper A and Krause H (1980) Evidence for complexed plastocyanin as the immediate electron donor of P-700. Biochim Biophys Acta 593: 384–399

Haehnel W, Jansen T, Gause K, Klosgen RB, Stahl B, Michel D, Huvermann B, Karas M and Herrmann R (1994) Electron transfer from plastocyanin to Photosystem I. EMBO J 13: 1028–1038

Hatanaka H, Sonoike K, Hirano M and Katoh S (1993) Small subunits of Photosystem I reaction center complexes from *Synechococcus elongatus*. II. Is the *psaF* gene productrequired for oxidation of cytochrome c-553? Biochim Biophys Acta 1141: 45–51

He WZ and Malkin R (1992) Specific release of a 9-kDa extrinsic polypeptide of Photosystem I from spinach chloroplasts by salt washing. FEBS Lett 308: 298–300

Hervas M, Navarro J and Tollin G (1992) A laser-flash spectroscopy study of the kinetics of electron transfer from spinach Photosystem I to spinach and algal ferredoxins. Photochem Photobiol 56: 319–324

Hervas M, Ortega JM, Navarro JA, De la Rosa MA and Bottin H (1994) Laser flash kinetic analysis of *Synechocystis* sp. PCC 6803 cytochrome c_6 and plastocyanin oxidation by Photosystem I. Biochim Biophys Acta 1184: 235–241

Hippler M, Ratajczak R and Haehnel W (1989) Identification of the plastocyanin binding subunit of Photosystem I. FEBS Lett 250: 280–284

Ikeuchi M (1992) Subunit proteins of Photosystem I. Plant Cell Physiol 33: 669–676

Ikeuchi M, Hirano A, Hiyama T and Inoue Y (1990) Polypeptide composition of higher plant Photosystem I complex: Identification of *psaI*, *psaJ* and *psaK* gene products. FEBS Lett 263: 274–278

Ikeuchi M, Nyhus KJ, Inou Y and Pakrasi HB (1991) Identities of four low-molecular-mass subunits of the Photosystem I complex from *Anabaena variabilis* ATCC 29413: Evidence for the presence of the *psaI* gene product in a cyanobacterial complex. FEBS Lett 287: 5–9

Ikeuchi M, Sonoike K, Koike H, Pakrasi H and Inoue Y (1993) A novel 3.5 kDa protein component of cyanobacterial Photosystem I complexes. Plant Cell Physiol 33: 1057–1063

Kirsch W, Seyer P and Herrmann RG (1986) Nucleotide sequence of the clustered genes for two P700 chlorophyll *a* apoproteins of the Photosystem I reaction center and the ribosomal protein S14 of the spinach plastid chromosome. Curr Genet 10: 843–855

Kjærulff S, Andersen B, Nielsen VS, Møller BL and Okkels JS (1993) The PS I-K subunit of Photosystem I from barley (*Hordeum vulgare* L.): Evidence for a gene duplication of an ancestral PS I-G/K gene. J Biol Chem 268: 18912–18916

Knoetzel J and Simpson DJ (1993) The primary structure of a cDNA for PsaN, encoding an extrinsic lumenal polypeptide of barley Photosystem I. Plant Mol Biol 22: 337–345

Kossel H, Dory I, Istvan G and Maier R (1990) A leucine-zipper motif in Photosystem I. Plant Mol Biol 15: 497–99

Krauß N, Hinrichs W, Witt I, Fromme P, Pritzkow W, Dauter Z, Betzel C, Wilson KS, Witt HT and Saenger W (1993) Three-dimensional structure of system I of photosynthesis at 6 Å resolution. Nature 361: 326–331

Kruip J, Boekema EJ, Bald D, Boonstra AF and Rogner M (1993) Isolation and Structural Characterization of monomeric and trimeric Photosystem I complexes ($P700\text{-}F_A/F_B$ and $P700\text{-}F_X$) from the cyanobacterium *Synechocystis* sp. PCC 6803. J Biol Chem 268: 23353–23360

Kruip J, Bald D, Boekema E and Rogner M (1994) Evidence for the existence of trimeric and monomeric Photosystem I complexes in thylakoid membranes from cyanobacteria. Photosynth Res 40: 279–286

Kuhn M, Fromme P and Krabben L (1994) A membrane attached α-helix: a conserved strucutral motif in bacterial reaction centers, Photosystem I, and chloroplast NADH-plastoquinone oxidoreductase. Trends Biochem Sci 19: 401–402

Lagoutte B and Mathis P (1989) The Photosystem I reaction center: structure and photochemistry. Photochem Photobiol 49: 833–44

Lagoutte B and Vallon O (1992) Purification and membrane topology of PS I-D and PS I-E, two subunits of the Photosystem I reaction center. Eur J Biochem 205: 1175–1185

Lelong C, Setif P, Lagoutte B and Bottin H (1994) Identification of amino acids involved in the functional interaction between Photosystem I and ferredoxin from *Synechocystis* sp. PCC 6803 by chemical cross-linking. J Biol Chem 269: 10034–10039

Li N, Warren P, Golbeck JH, Frank G, Zuber H and Bryant DA (1991a) Polypeptide composition of the Photosystem I complex and the Photosystem I core protein from *Synechococcus* sp. PCC 6301. Biochim Biophys Acta 1059: 215–25

Li N, Zhao J, Warren PV, Warden JT, Bryant DA and Golbeck JH (1991b) PsaD is required for the stable binding of PsaC to the Photosystem I core protein of *Synechococcus* sp. PCC 6301. Biochemistry 30: 7863–7872

Lockau W and Nitschke W (1993) Photosystem I and its bacterial counterparts. Physiol Plant 88: 372–381

Malkin R (1986) A consideration of the organization of chloroplast Photosystem I. Photosynth Res 10: 197–200

Mannan RM, Whitmarsh J, Nyman P and Pakrasi HB (1991) Directed mutagenesis of an iron sulfur protein of the Photosystem I complex in the filamentous cyanobacterium *Anabaena variabilis* ATCC 29413. Proc Natl Acad Sci USA 88: 10168–72

Mannan RM, Pakrasi HB and Sonoike K (1994) The PsaC protein is necessary for the stable association of the PsaD, PsaE, and PsaL porteins in the Photosystem I complex: Analysis of a cyanobacterial mutant strain. Arch Biochem Biophys 315: 68–73

Mant A, Nielsen VS, Knott TG, Moller BL and Robinson C (1994) Multiple mechanisms for targeting of Photosystem I subunits F, H, K, L, and N into and across the thylakoid lumen. J Biol Chem 269: 27303–27309

Medina M, Diaz A, Hervas M, Navarro JA, Gomez-Morano C, De la Rosa MA and Tollin G (1993) A comparative laser flash absorption spectroscopy study of *Anabaena* PCC 7119 plastocyanin and cytochrome c_6 photooxidation by Photosystem I particles. Eur J Biochem 213: 1133–1138

Morand LZ, Cheng RH, Krogmann DW and Ho KK (1995) Soluble electron transfer catalysts of cyanobacteia, In: Bryant DA (ed) The Molecular Biology of Cyanobacteria, pp 381–407. Kluwer Academic Publishers, Dordrecht (in press)

Mühlenhoff U, Haehnel W, Witt HT and Herrman RG (1993) Genes encoding eleven subunits of Photosystem I from the thermophilic cyanobacterium *Synechococcus* sp. Gene 127: 71–78

Nechushtai R and Nelson N (1981) Purification properties and biogenesis of *Chlamydomonas reinhardtii* Photosystem I reaction center. J Biol Chem 256: 11624–11628

Nielsen VS, Mant A, Knoetzel J, Moller BL and Robinson C (1994) Import of barley Photosystem I subunit N into the thylakoid lumen

is mediated by a bipartite presequence lacking an intermediate processing site. J Biol Chem 269: 3762–3766

Nordling M, Sigfridsson K, Young S, Lundberg LG and Hansson O (1991) Flash-photolysis studies of the electron transfer from genetically modified spinach plastocyanin to Photosystem I. FEBS Lett 291: 327–330

Nyhus KJ, Thiel T and Pakrasi HB (1993) Targeted interruption of the *psaA* and *psaB* genes encoding the reaction-centre proteins of Photosystem I in the filamentous cyanobacterium *Anabaena variabilis* ATCC 29413. Mol Microbiol 9: 979–988

Obokata J, Mikami K, Hayashida N, Nakamura M and Sugiura M (1993) Molecular heterogeneity of Photosystem I: psaD, psaE, psaF, psaH, and psaL are all present in isoforms in *Nicotiana* spp. Plant Physiol 102: 1259–1267

Oh-oka H, Takahashi Y, Kuriyama K, Saeki K and Matsubara H (1988) The protein responsible for center A/B in spinach Photosystem I: Isolation with iron sulfur cluster(s) and complete sequence analysis. J Biochem 103: 962–968

Oh-Oka H, Takahashi Y and Matsubara H (1989) Topological considerations of the 9-kDa polypeptide which contains centers A and B, associated with the 14- and 19-kDa polypeptides in the Photosystem I complex of spinach. Plant Cell Physiol 30: 869–875

Ohyama K, Fukuzawa H, Kohchi T, Shirai H, Tohru S, Sano S, Umesono K, Shiki Y, Takeuchi M, Chang Z, Aota SI, Inokuchi H and Ozeki H (1986) Chloroplast gene organization deduced from complete sequence of liverwort *Marchantia polymorpha* chloroplast DNA. Nature 322: 572–574

Okkels JS, Nielsen S, Scheller HV and Møller BL (1992) A cDNA clone from barley encoding the precursor from the Photosystem I polypeptide PS I-G: Sequence similarity to PS I-K. Plant Mol Biol 18: 989–994

Ortiz W, Lam E, Chollar S, Munt D and Malkin R (1985) Topography of the protein complexs of the chloroplast thylakiod membrane. Studies of Photosystem I using a chemical probe and proteolytic digestion. Plant Physiol 77: 389–397

Pakrasi HB and Vermaas WFJ (1992) Protein engineering of Photosystem II. In: Barber J (ed) The Photosystems: Strucutre, Function and Molecular Biology, pp 231–257. Elsevier, Amsterdam

Pierre Y and Popot J-L (1993) Identification of two 4-kDa miniproteins in the cytochrome b6f complex from *Chlamydomonas reinhardtii*. CR Acad Sci Paris 316: 1404–1409

Redinbo MR, Yeates TO and Merchant S (1994) Plastocyanin: Structural and functional analysis. J Bioenerg Biomemb 26: 49–66

Reilly P and Nelson N (1988) Photosystem I complex. Photosynth Res 19: 73–84

Rodday SM, Jun S-S and Biggins J (1993) Interaction of the F_AF_B-containing subunit with the Photosystem I core heterodimer. Photosynth Res 36: 1–9

Rousseau F, Setif P and Lagoutte B (1993) Evidence for the involvement of PS I-E subunit in the reduction of ferredoxin by Photosystem I. EMBO J 12: 1755–1765

Sadewasser DA and Sherman LA (1981) Internal and external membrane proteins of the cyanobacterium, *Synechococcus cedrorum*. Biochim Biophys Acta 640: 326–340

Scheller HV, Svedson I and Moller BL (1989a) Subunit compostion of Photosystem I and identification of center X as [4Fe-4S] iron-sulfur cluster. J Biol Chem 264: 6929–6934

Scheller HV, Okkels JS, Høj PB, Svendsen I, Roepstorff P and Møller BL (1989b) The primary structure of a 4.0-kDa Photosystem I polypeptide encoded by the chloroplast *psaI* gene. J Biol Chem 264: 18402–18406

Setif PQY (1992) Energy transfer and trapping in Photosystem I. In: Barber J (ed) Current Topics in Photosynthesis, pp 471–499. Elsevier, Amsterdam

Setif PQY and Bottin H (1994) Laser flash absorption spectroscopy study of ferredoxin reduction by Photosystem I in *Synechocystis* sp. PCC 6803: Evidence for submicrosecond and microsecond kinetics. Biochemistry 33: 8495–8504

Shen G, Boussiba S and Vermaas WFJ (1993) *Synechocystis* sp. PCC 6803 strains lacking Photosystem I and phycobilisome function. Plant Cell 5: 1853–1863

Sherman DM, Troyan TA and Sherman LA (1994) Localization of membrane proteins in the cyanobacterium *Synechococcus* sp. PCC7942: Radial asymmetry in the photosynthetic complexes. Plant Physiol 106: 251–262

Smart LB and McIntosh L (1993) Genetic inactivation of the *psaB* gene in *Synechocystis* sp. PCC 6803 disrupts assembly of Photosystem I. Plant Mol Biol 21: 177–180

Smart LB, Warren PV, Golbeck JH and McIntosh L (1993) Mutational analysis of the structure and biogenesis of the Photosystem I reaction center in the cyanobacterium *Synechocystis* sp. PCC 6803. Proc Natl Acad Sci USA 90: 1132–1136

Smart LB, Anderson SL and McIntosh L (1992) Targeted genetic inactivation of the Photosystem I reaction center in the cyanobacterium *Synechocystis* sp. PCC 6803. EMBO J 10: 3289–3296

Smart LB, Bowlby NR, Anderson SL, Sithole I and McIntosh L (1994) Genetic manipulation of the cyanobacterium *Synechocystis* sp. PCC 6803: Development of strains lacking Photosystem I for the analysis of mutations in Photosystem II. Plant Physiol 104: 349–354

Sonoike K, Ikeuchi M and Pakrasi HB (1992) Presence of an N-terminal presequence in the PsaI protein of the Photosystem I complex in the filamentous Cyanobacterium *Anabaena variabilis* ATCC 29413. Plant Mol Biol 20: 987–990

Sonoike K, Hatanaka H and Katoh S (1993) Small subunits of Photosystem I reaction center complexes from Synechococcus elongatus. II. The *psaE* gene product has a role to promote interaction between the terminal electron acceptor and ferredoxin. Biochim Biophys Acta 1141: 52–57

Steppuhn J, Hermans J, Nechushtai R, Ljungberg U, Thummler F, Lottspeich F and Herrmann RG (1988) Nucleotide sequence of cDNA clones encoding the entire precursor polypeptides for subunits IV and V of Photosystem I reaction center from spinach. FEBS Lett 237: 218–224

Strotmann H and Weber N (1993) On the function of PsaE in chloroplast Photosystem I. Biochim Biophys Acta 1143: 204–210

Takahashi Y, Goldschmidt-Clermont M, Soen S-Y, Franzen LG and Rochaix J-D (1991) Directed chloroplast transformation in *Chlamydomonas reinhardtii*: Insertional inactivation of the *psaC* gene encoding the iron sulfur protein destabilizes Photosystem I. EMBO J 10: 2033–2040

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

Thornber JP, Cogdell R, Chitnis PR, Morishige DT, Peter GF, Gomez S, Anandan S, Preiss S, Welty B, Lee A, Takeuchi T and Kerfeld C (1994) The light harvesting systems of bacteria and higher plants. In: Barber J (ed) Photosynthesis, pp 58–116. JAI Press, New York

Tjus SE and Andersson B (1991) Extrinsic polypeptides of spinach Photosystem I. Photosynth Res 27: 209–219

Toelge M, Ziegler K, Maldener I and Lockau W (1991) Directed mutagenesis of the gene *psaB* of Photosystem I of the cyanobac-

terium *Anabaena vaiabilis* ATCC 29413. Biochim Biophys Acta 1060: 233–236

Tsiotis G, Nitschke W, Haase W and Michel H (1993) Purification and crystallization of Photosystem I complex from a phycobilisome-less mutant of the cyanobacterium *Synechococcus* PCC 7002. Photosynth Res 35: 285–297

Vallon O and Bogorad L (1993) Topological study of PS I-A and PS I-B, the large subunits of the Photosystem-I reaction center. Eur J Biochem 214: 907–915

Van Grondelle R, Dekker JP, Gillbro T and Sundstrom V (1994) Energy transfer and trapping in photosynthesis. Biochim Biophys Acta 1187: 1–65

Von Heijne G and Gavel Y (1988) Topogenic signals in integral membrane proteins. Eur J Biochem 174: 671–674

Warren PV, Smart LB, McIntosh L and Golbeck JH (1993) Site-directed conversion of cysteine-565 to serine in PsaB of Photosystem I results in the assembly of [3Fe-4S] and [4Fe-4S] clusters in F_X. A mixed ligand [4Fe-4S] cluster is capable of electron transport to F_A and F_B. Biochemistry 90: 1132–1136

Webber A and Malkin R (1990) Photosystem I reaction-centre proteins contain leucine zipper motifs. A proposed role in dimer formation. FEBS Lett 264: 1–4

Webber A, Gibbs P, Ward J and Bingham S (1993) Site-directed mutagenesis of the Photosystem I reaction center in chloroplasts: The proline-cysteine motif. J Biol Chem 268: 12990–12995

Williams RC, Glazer AN and Lundell DJ (1983) Cyanobacterial Photosystem I: Morphology and aggregation behavior. Proc Natl Acad Sci USA 80: 5923–5926

Witt I, Witt HT, Gerken S, Saenger W, Dekker JP and Rogner M (1987) Crystallization of reaction center I of photosynthesis, low-concentration crystallization of photoactive protein complex from the cyanobacterium *Synechoccus* sp. FEBS Lett 221: 260–264

Wynn RM and Malkin R (1988) Interaction of plastocyanin with Photosystem I: A chemical cross-linking study of the polypeptide that binds to plastocyanin. Biochemistry 27: 5863–5869

Wynn RM and Malkin R (1990) The Photosystem I 5.5 kDa subunit (the *psaK* gene product). An intrinsic subunit of the PS I reaction center complex. FEBS Lett 262: 45–48

Wynn RM, Omaha J and Malkin R (1989a) Structural and functional properties of the cyanobacterial Photosystem I complex. Biochemistry 28: 5554–5560

Wynn RM, Luong C and Malkin R (1989b) Maize Photosystem I. Identification of the subunit which binds plastocyanin. Plant Physiol 91: 445–449

Xu Q, Armbrust TS, Guikema JA and Chitnis PR (1994a) Organization of Photosystem I polypeptides: A structural interactions between PsaD and PsaL subunits. Plant Physiol 106: 1057–1063

Xu Q, Guikema JA and Chitnis PR (1994b) Identification of surface-exposed domains on the reducing side of Photosystem I. Plant Physiol 106: 617–624

Xu Q, Jung YS, Chitnis VP, Guikema JA, Golbeck JH and Chitnis PR (1994c) Mutational analysis of Photosystem I polypeptides in *Synechocystis* sp. PCC 6803. Subunit requirements for the reduction of $NADP^+$ mediated by ferredoxin and flavodoxin. J Biol Chem 269: 21512–21518

Xu Q, Odom WR, Guikema JA, Chitnis VP and Chitnis PR (1994d) Targeted deletion of *psaJ* from the cyanobacterium *Synechocystis* sp. PCC 6803 indicates structural interactions between the PsaJ and PsaF subunits of Photosystem I. Plant Mol Biol 26: 291–302

Xu Q, Yu L, Chitnis VP and Chitnis PR (1994e) Function and organization of Photosystem I in a cyanobacterial mutant strain that lacks PsaF and PsaJ subunits. J Biol Chem 269: 3205–3211

Xu Q, Hoppe D, Chitnis VP, Odom WR, Guikema JA and Chitnis PR (1995) Mutational analysis of Photosystem I polypeptides in *Synechocystis* sp. PCC 6803. Targeted inactivation of *psaI* reveals the role of PsaI in structural organization of PsaL. J Biol Chem (In press)

Yu L, Zhao J, Lu W, Bryant DA and Golbeck JH (1993a) Characterization of the [3Fe-4S] and [4Fe-4S] clusters in unbound PsaC mutants C14D and C51D. The midpoint potentials of the single [4Fe-4S] clusters are identical to F_A and F_B in bound PsaC of Photosystem I. Biochemistry 32: 8251–8258

Yu L, Zhao J, Mühlenhoff U, Bryant DA and Golbeck JH (1993b) PsaE is required for in vivo cyclic electron flow around Photosystem I in the cyanobacterium *Synechococcus* sp. PCC 7002. Plant Physiol 103: 171–180

Zanetti G and Merati G (1987) Interaction between Photosystem I and ferredoxin: Identification by chemical cross-linking of the polypeptide which binds ferredoxin. Eur J Biochem 169: 143–146

Zhao J, Warren PV, Li N, Bryant DA and Golbeck JH (1990) Reconstitution of electron transport in Photosystem I with PsaC and PsaD proteins expressed in *Escherichia coli*. FEBS Lett 276: 175–180

Zhao J, Li N, Warren PV, Golbeck JH and Bryant DA (1992) Site-directed conversion of a cysteine to aspartate leads to the assembly of a [3Fe-4S] cluster in PsaC of Photosystem I. The photoreduction of F_A is independent of F_B. Biochemistry 31: 5093–5099

Zhao J, Snyder WB, Mühlenhoff U, Rhiel E, Warren PV, Golbeck JH and Bryant DA (1993) Cloning and characterization of the psaE gene of the cyanobacterium *Synechococcus* sp. PCC 7002: characterization of a psaE mutant and overproduction of the protein in *Escherichia coli*. Mol Micobiol 9: 183–194

Zilber AL and Malkin R (1988) Ferredoxin cross-links to a 22 kD subunit of Photosystem I. Plant Physiol 88: 810–814

Zilber AL and Malkin R (1992) Organization and topology of Photosystem I subunits. Plant Physiol 99: 901–911

Zilber AL, Wynn R, Webber A and Malkin R (1990) Organization of PS I subunits in thylakoid membranes. In: Baltscheffsky M (ed) Current Research in Photosynthesis, pp 575–578. Kluwer Academic Publishers, Dordrecht