Chapter 6

The Low Molecular Weight Proteins of Photosystem II

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Summary

Photosystem II (PS II) has a complex arrangement of membrane spanning and soluble subunits responsible for its unique role in oxygenic photosynthesis. Advances in several areas of biology have contributed significantly to our understanding of individual subunits and the PS II complex as a whole. In recent years, the genome sequences of several plants and many cyanobacteria have been completed allowing for comparisons of their genes. This information, along with previous biochemical evidence, has strengthened the conclusion that the

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T. Wydrzynski and K. Satoh (eds): Photosystem II: The Light-Driven Water:Plastoquinone Oxidoreductase, pp. 121–137.

PS II protein components are highly conserved, from the primitive cyanobacterium *Gloeobacter violaceus* PCC 7421 to the vascular plant *Arabidopsis thaliana*. Advances in purification of PS II samples and protein identification have confirmed the presence of several novel proteins and provide new tools for the analysis of PS II in various mutants and under different growth conditions. Recent structural studies have also contributed tremendously to our understanding of the organization of PS II subunits and cofactors. The combination of genomic, proteomic, and structural studies will continue to be a source of new insights into the assembly, regulation and function of PS II. This chapter highlights the contributions of such studies to our understanding of the numerous low molecular weight PS II components.

I. Introduction

Photosystem II (PS II) is a large protein complex consisting of at least 20 subunits that contribute to optimal charge separation and O_2 evolution. PS II is made up of many subunits that are highly conserved in all the organisms from which it has been isolated. The complexity of PS II is conserved between the primitive single-membrane system of *Gloeobacter violaceus* PCC 7421 and the intricately structured thylakoid membranes of chloroplasts. Despite its bulk, PS II is still assembled frequently in photosynthetic organisms because it has a very high turnover rate. PS II is the target of extensive photodamage requiring rapid removal of its damaged subunits and reassembly of new ones. Understanding PS II as a whole requires examining the complex on many different levels from ultrastructure to the function of individual components in a number of photosynthetic organisms.

Recently, great advances have been made towards determining the structural components of PS II. Innovative technologies in protein purification and crystallization, recent developments in genome sequence annotation, and the implementation of high throughput proteome analysis have shed new light on the big picture of PS II, both for its form and its functions. Zouni and coworkers achieved 3.8 Å resolution crystal structure of PS II from *Thermosynechococcus elongatus* BP-1, which provides an arrangement for many of the membrane-spanning helixes (Zouni et al., 2001; Chapter 19, Witt). PS II from another cyanobacterium, *Thermosynechococcus vulcanus*, was crystallized to 3.7 Å resolution, with a similar subunit arrangement as was seen in the Zouni study (Kamiya and Shen, 2003; Chaptere 20, Shen and Kamiya). A second structure was determined for PS II from *T. elongatus* at 3.5 Å resolution with different assignments for some of the small membrane spanning subunits (Ferreira et al, 2004; Chapter 21, Barber and Iwata). Continued efforts in purification and crystallization of PS II will shed further light on the variability in the arrangement of subunits between organisms and also under different growth conditions (Biesiadka et al., 2004.

Progress in the field of genomics has allowed broad comparisons of the PS II subunits found in cyanobacteria and plants based on predicted amino acid sequence similarities. Table 1 shows the list of PS II genes, which were obtained from the complete sets of open reading frames of *Synechocystis* sp. PCC 6803 (Kaneko et al., 1996), *Anabaena* sp. PCC 7120 (Kaneko et al., 2001), *T. elongatus* (Nakamura et al., 2002), *Prochlorococcus marinus* MED4 (Rocap et al., 2003), *Prochlorococcus marinus* MIT9391 (Rocap et al., 2003), *G. violaceus* (Nakamura et al., 2003b) and *Synechococcus* sp. WH8102 (Palenik et al., 2003), or extracted from nucleotide sequences of the complete or incomplete genome data of *Nostoc punctiforme* ATCC 29133, *Trichodesmium erythraeum*, and *Arabidopsis thaliana*. Inclusion of *Arabidopsis* in this comparison mostly among cyanobacteria allows comparison of several additional PS II subunits, because plants have acquired genes that appear not to be present in cyanobacteria. On the other hand, some cyanobacteria have PS II genes that are not present in other cyanobacteria. For example, *G. violaceus* lacks *psbY, psbZ*, *psb27* and the second copy of *psbD*, in spite of its relatively large genome size (4.6 Mb). Phylogenetic analysis of 16S rRNA indicates that *G. violaceus* is one of the most anciently

Abbreviations: Cyt – cytochrome; DCBQ – 2,6-dichloro-*p*-benzoquinone; His-tag – histidine tag; HP and LP – high and low redox potential, respectively; LHCII – light harvesting complex associated with PS II; MALDI/MS – matrix assisted laser desorption ionization/mass spectrometry; ORF – open reading frame; P680 – primary electron donor in PS II; PQ – plastoquinone; PS I and PS II – Photosystem I and II, respectively; Q_A – the primary quinone acceptor of PS II on the D2 subunit; Q_B – the secondary quinone acceptor of PS II on the D1 subunit; Sec – secretion; TGIR – The Institute for Genomic Reseach

branched species among cyanobacteria. If so, the gene composition of *G. violaceus* may suggest that the ancestral PS II consisted of a simpler subunit composition. Alternatively, components, which are missing in *G. violaceus*, may be related to the development of the thylakoid membrane system, since it is the only organism that lacks thylakoids. Another anomalous organism is *P. marinus* MED 4, which lacks *psbU* and *psbV* since *P. marinus* MIT 9313, a close relative, retains both of them. *P. marinus* MED 4 is known to be a high-light adapted ecotype with multiple *hlip* and photolyase genes (Hess et al., 2001). Such adaptation may be coupled with the absence of two extrinsic proteins in PS II.

The inclusion of *Arabidopsis* in Table 1 reveals the changes that have taken place in PS II genes during the evolution from cyanobacteria to chloroplasts. Many of the PS II components are encoded by single copy genes in the chloroplast. There are also PS II genes in the plant nuclear genome that encode subunits not found in cyanobacteria (PsbW, PsbR, PsbS, and $PsbT_N$), and presumably not necessary for a free-living photosynthetic organism. Furthermore, there are plant PS II proteins (PsbP and PsbQ) whose functional role has been examined thoroughly, whereas the function of homologs in cyanobacteria remains an open question. Table 1 is an example of the type of information that can be gathered from genomic analysis of PS II-containing organisms. Such information is useful for establishing the different components used in PS II activity in various model organisms.

Studies on the biochemical composition of PS II are providing correlations between the structure and genetic data from various organisms. Comprehensive analysis of a highly active, histidine (His)-tag purified PS II from *Synechocystis* 6803 using the latest protein identification technologies revealed a number of PS II subunits that had never been seen in such a purified protein complex (Kashino et al., 2002a). A similar study in *T. vulcanus* (Kashino et al., 2002b) revealed that the protein make up of PS II in the two organisms is very similar, with only a few subtle variations, such as a different apparent molecular weight of the cytochrome (Cyt) b_{559} large subunit. Irrgang et al. (2001) examined PS II core components from both *T. elongatus* and spinach to show that there are expected differences in some of the subunit components, but the behavior of the complexes in forming monomers and dimers is essentially the same.

In this chapter, we will discuss the low molecular weight protein subunits of PS II with respect to the new discoveries that contribute to a better overall understanding of the function of the entire protein complex. Most of the proteins discussed are less than 10 kDa, but a few are between 10 and 13 kDa. This group includes the heme-binding heterodimer, Cyt b_{559}, whose function has been a subject of intense debate for almost thirty years (Chapter 15, Faller and Rutherford). The majority of the proteins covered in this chapter consists of single helix, membrane-spanning proteins that are usually organized peripherally to the large integral proteins. Many of these proteins are not essential for PS II activity, but they appear to be important in regulation or biogenesis. PS II also has small soluble, extrinsic proteins, associated with either the stromal/cytoplasmic or lumenal surface of PS II. The small extrinsic proteins discussed here do not have a known role in oxygen evolution activity as do the larger extrinsic proteins described in Chapter 5 (Bricker and Burnap). Most of the small PS II subunits found in cyanobacteria are also present in plants and encoded by genes in the chloroplast genome.

II. Membrane Spanning Subunits

A. PsbE/F — Cytochrome b_{559}

This enigmatic member of the PS II complex is a heme-linked heterodimer of the single membrane spanning proteins, PsbE and PsbF. Cyt b_{559} was first biochemically characterized by Garewal and Wasserman (1974b), who developed an efficient Triton-based purification method. Using this purification method (Garewal and Wasserman, 1974a), Cyt b_{559} was isolated and sequenced (Herrmann et al., 1984; Widger et al., 1985). The protein contains one α subunit, PsbE, and one β subunit, PsbF, each of which contributes one histidine residue to coordinate a single heme to give its unique two-chain structure. PsbE is 9 kDa and PsbF is 4 kDa and the proteins are 83 and 39 amino acids, respectively.

In most PS II-containing bacteria, the proteins are encoded by the *psbEFLJ* operon. The gene, *psbF*, was actually found because its ribosome-binding region overlaps the stop codon for *psbE* (Herrmann et al., 1984). Genetic analysis of Cyt b_{559} in *Synechocystis* 6803 (Pakrasi et al., 1988, 1990, 1991) and *Chlamydomonas reinhardtii* (Morais et al., 1998, 2001) indicated that it is required for PS II activity but may not be involved in primary electron transport. A recent study of tobacco chloroplast mutants indicated

Table 1. List of Photosystem II genes in representative genomes

Photosystem II gene	*Synechocystis* sp. PCC 6803	*Anabaena* sp. PCC 7120	*N. punctiforme* ATCC 29133	*Trichodesmium erythraeum*	*T. elongatus* BP-1	*P.marinus* MED4	*P.marinus* MIT9313	*G.violaceus* PCC 7421	*Synechococcus* sp. WH8102	*Arabidopsis thaliana*
psbA: D1 reaction center protein	*slr1311* *sll1867* *slr1181*	*all3572* *alr3727* *alr4592* *alr4866* *alr3742*	4 genes	yes	*tlr1843* *tlr1844* *tlr1477*	*PMM0223*	*PMT0419* *PMT1532*	*gll3144* *glr0779* *glr1706* *glr2322* *glr2656*	*SYNW0983* *SYNW1470* *SYNW1919* *SYNW2151*	chloroplast
psbB: CP47 chlorophyll-binding protein	*slr0906*	*all0138*	yes	yes	*tlr1530*	*PMM0315*	*PMT1665*	*glr2999*	*SYNW1982*	chloroplast
psbC: CP43 chlorophyll-binding protein	*sll0851*	*alr4291*	yes	yes	*tlr1631*	*PMM1158*	*PMT1180*	*glr2324*	*SYNW0676*	chloroplast
psbD: D2 reaction center protein	*sll0849* *slr0927*	*alr4290* *alr4548*	yes	yes	*tlr1630* *tlr0455*	*PMM1157*	*PMT1179*	*glr2323*	*SYNW0677* *SYNW2232*	chloroplast
psbE: cytochrome b559 large subunit	*ssr3451*	*asr3845*	yes	yes	*tsr1541*	*PMM0297*	*PMT1896*	*gsr0856*	*SYNW0204*	chloroplast
psbF: cytochrome b559 small subunit	*smr0006*	*asr3846*	yes	yes	*tsr1542*	*PMM0298*	*PMT1897*	*gsr0857*	*SYNW0203*	chloroplast
psbH: small membrane-spanning protein	*ssl2598*	*asl0846*	yes	yes	*tsl1386*	*PMM0251*	*PMT1837*	*gsr3002*	*SYNW0269*	chloroplast
psbI: small membrane-spanning protein	*sml0001*	*asr1277*	yes	yes	*tsr1074*	*PMM0253*	*PMT1840*	*gsl3634*	*SYNW0266*	chloroplast
psbJ: small membrane-spanning protein	*smr0008*	*asr3848*	yes	yes	*tsr1544*	*PMM0300*	*PMT1899*	*gsr0859*	*SYNW0201*	chloroplast
psbK: small membrane-spanning protein	*sml0005*	*asl0885*	yes	yes	*tsl0176*	*PMM0272*	*PMT1863*	*gsr2807*	*SYNW0243*	chloroplast
psbL: small membrane-spanning protein	*smr0007*	*asr3847*	yes	yes	*tsr1543*	*PMM0299*	*PMT1898*	*gsr0858*	*SYNW0202*	chloroplast
psbM: small membrane-spanning protein	*sml0003*	*asl0883*	yes	yes	*tsl2052*	*PMM0317*	*PMT1663*	*gsl2997*	*SYNW1980*	chloroplast
psbO: manganese-stabilizing protein	*sll0427*	*all3854*	yes	yes	*tll0444*	*PMM0228*	*PMT1800*	*glr3691*	*SYNW0303*	At5g66570 At3g50820
psbP: 23 kDa extrinsic protein	*sll1418*	*all3076*	yes	yes	*tlr2075*	*PMM1098*	*PMT1078*	*gll1440*	*SYNW0927*	At1g06680 At1g76450 At1g77090 At2g28605 At2g30790 At2g39470 At3g55330 At3g56650 At4g15510 At5g11450
psbQ: 16 kDa extrinsic protein	*sll1638*	*all1355*	yes	yes	*tll2057*	no	no	no	*SYNW2505*	At4g21280 At4g05180 At3g01440
psbR: 10 kDa extrinsic protein	no	no	no	no	no	no	no	no	no	At1g79040
psbS: light harvesting complex regulator	no	no	no	no	no	no	no	no	no	At1g44575
psbTc: small membrane-spanning protein	*smr0001*	*asl0137*	yes	yes	*tsr1531*	*PMM0314*	*PMT1666*	*gsr3000*	*SYNW1983*	chloroplast
psbTn: extrinsic protein	no	no	no	no	no	no	no	no	no	At3g21050 At1g51400
psbU: 12 kDa extrinsic protein	*sll1194*	*alr1216*	yes	yes	*tll2409*	no	*PMT0178*	*gll2873*	*SYNW2192*	no
psbV: cytochrome c550	*sll0258*	*all0259*	yes	2 genes	*tll1285* *tll1284*	no	*PMT1427*	*gll2337* *gll2338*	*SYNW1537*	no
psbW: small membrane-spanning protein	no	no	no	no	no	no	no	no	no	At2g30570
psbX: small membrane-spanning protein	*sml0002*	*asr0941*	yes	yes	*tsr2013*	*PMM0062*	*PMT1590*	*gsr1874*	*SYNW0334*	At2g06520
psbY: small membrane-spanning protein	*sml0007*	*asr1025*	yes	yes	*tsl0836*	*PMM1117*	*PMT1048*	no	*SYNW0898*	At1g67740
psbZ: small membrane-spanning protein	*sll1281*	*asr3992*	yes	yes	*tsr1967*	*PMM1644*	*PMT0080*	no	*SYNW0081*	chloroplast
psb27: 11 kDa extrinsic protein	*slr1645*	*all1258*	yes	yes	*tll2464*	*PMM0507*	*PMT1260*	no	*SYNW1772*	At1g03600 At1g05385
psb28: 13kD extrinsic protein	*sll1398*	*all0801* *all1082*	yes	yes	*tlr0493*	*PMM0926*	*PMT0604*	gsl0928	*SYNW1065*	At4g28660

that PsbE and PsbF are each required for the stable insertion of the other, and without these proteins, the D1 subunit is not stable (Swiatek et al., 2003). Further supporting the connection between D1 stability and Cyt b_{559} is evidence from Lupinkova et al. (2002) indicating that *Synechocystis* 6803 D1 His252 is required for adduct formation with the N-terminal serine of Cyt b_{559} under damaging conditions.

Historically, one of the dilemmas in understanding the structure of Cyt b_{559} was the orientation of the subunits relative to each other and the thylakoid membrane. It was postulated that the Cyt has an $\alpha_2\beta_2$ topology with the β subunit C-terminus in the stroma and the associated heme closer to the lumen (McNamara et al., 1997). With the publication of the PS II crystal structure from *T. elongatus*, it seems that the debate is finally resolved in favor of a parallel heterodimer with the N-termini of both subunits in the stroma (Zouni et al., 2001).

Another controversy that is still unresolved regards the number of cytochrome heterodimers per PS II center. One of the major difficulties involves purifying the PS II center without disturbing the cytochromes bound to the system. In two recent studies using purified PS II with a poly-histidine tagged CP47 there are different ratios of Cyt b_{559} to PS II centers. Lakshmi and colleagues (Lakshmi et al., 2002) found 1.1 Cyt b_{559} hemes in a his-tagged PS II preparation, while Kashino et al. (2002a) found 1.9 hemes per PS II. The current crystal structure indicates that there is one Cyt b_{559} per PS II (Ferreira et al, 2004), so whether one is removed more easily or the extinction coefficient of a single heme is variable remains an open question.

An attribute of Cyt b_{559} that makes it unique as well as difficult to understand is its changing midpoint potential. The two predominant forms are the high potential (HP) and low potential (LP) with their redox midpoint at 370–435 mV and 0–80 mV, respectively. An interesting correlation between Cyt b_{559} and the O_2 evolution activity of PS II can be drawn from the contribution of electrons from the manganese cluster in forcing a shift from LP to HP cytochrome (Mizusawa et al., 1997). In this study, PS II complexes were depleted of Mn, which abolished O_2 evolution activity and caused the HP cytochrome to convert to LP. Mizusawa et al. (1997) showed that during photoactivation conditions, electron donation from the Mn cluster was sufficient to convert LP cytochrome to HP. Furthermore, this study linked the interconversion between cytochrome redox forms to Q_A^- oxidation.

An early hypothesis for the role of Cyt b_{559} in PS II was that it cycled extra electrons around the reaction center to release excess energy. A study in spinach chloroplasts revealed that oxidation of Cyt b_{559} in the presence of water oxidation inhibitors could be considered physiologically relevant under conditions when the water-splitting machinery cannot keep up with the oxidation capacity of the chlorophyll (Heber et al., 1979). It has been proposed that Cyt b_{559} is photooxidized by P680 through Chl Z (a fluorescence quencher) to protect the water oxidation complex from damage when excess energy is absorbed (Thompson and Brudvig, 1988). The cytochrome can be re-reduced by Q_B under some conditions, supporting the idea for its involvement in an electron path alternative to the water oxidation path (Buser et al., 1992). Even though the b_{559} heme is relatively far away from the Q_B site for direct electron transfer (Zouni et al., 2001; Kamiya and Shen, 2003; Ferreira et al, 2004; Chapters 19–21), recent evidence by Lupinkova and colleagues (Lupinkova et al., 2002) suggests that the link between the N-terminal serine of Cyt b_{559} and His252 on D1 could allow for an electron tunnel from Q_B to the Cyt b_{559} heme.

Another model for the function of Cyt b_{559} allowing for photoprotection and encompassing the changing midpoint potential has been called a molecular switch mechanism. Evidence supporting a single electron redox protection mechanism in PS II (Nedbal et al., 1992; Barber and De Las Rivas, 1993; Poulson et al., 1995) suggests that under increasing light intensity the LP form of Cyt b_{559} accumulates as an alternate path for electrons from P680 through pheophytin. As evidenced in (Poulson et al., 1995) the slow photochemistry of Cyt b_{559} in vivo is justified by the

Table 1, Legend. 'Yes' means the presence of a specified gene, which is confirmed by BLAST search but is not yet annotated as an ORF. Nucleotide and deduced sequence data were downloaded from the web (Nakamura et al., 2002). Complete names for the organisms included in the table are: *Synechocystis* sp. PCC 6803; *Anabaena* sp. PCC 7120; *Nostoc punctiforme* ATCC 29133; *Trichodesmium erythraeum*; *Thermosynechococcus elongatus* BP-1; *Prochlorococcus marinus* MED4; *Prochlorococcus marinus* MIT9313; *Gloeobacter violaceus* PCC 7421; *Synechococcus* sp. WH8102; and *Arabidopsis thaliana*. *Arabidopsis* genes were identified by searching the *Arabidopsis* genome with *Synechocystis* 6803 PS II genes, where applicable. The nomenclature for the nuclear genes refers to locus numbers as published in the The Institute for Genomic Research *Arabidopsis* database (2002). Nomenclature for cyanobacterial genes is as the ORFs are annotated in Cyanobase (Nakamura et al., 2003a). The table was updated Dec. 12, 2003, so annotations are current as of that date.

slow build up of reducing equivalents on the acceptor side during illumination. In this model, the acceptor side of PS II is oxidized by LP Cyt b_{559} and the HP form reduces the donor side. A major difference between this model and the Chl Z quenching model is that it is best applied under limited photo-stress as opposed to constant illumination.

Both models incorporate Cyt b_{559} into PS II activity as part of an alternate electron pathway. It appears evident that Cyt b_{559} is not required for water oxidation, even though it is important in the function of PS II under physiological conditions. A recent study by Morais et al. (2001) used mutations in the heme-binding site of Cyt b_{559} in *C. reinhardtii* to show that redox activity of the cytochrome is not necessary for O_2 evolution from PS II. The authors mention in the discussion that there could be a very low level of heme binding in vivo which would allow for the small number of assembled PS II centers in their mutant (Morais et al., 2001). It is unclear if there is normal PS II activity in the complete absence of a Cyt b_{559} heme. Much progress has been made towards speculating the role of Cyt b_{559} in PS II, which allows for grand debates. Further studies are required to determine how the presence of Cyt b_{559} and its redox contributions influence the activity of PS II (for more details see Chapter 12, Ahrling et al.).

B. PsbH

The PsbH subunit has a molecular weight in the range of 6–10 kDa. It was identified in PS II preparations from higher plants (spinach and wheat), the green alga *C. reinhardtii*, and the cyanobacterium *T. vulcanus* by N-terminal sequencing (Hird et al., 1986; Michel and Bennett, 1987; Dedner et al., 1988; Koike et al., 1989). The *psbH* gene was identified based on sequence homology in *Synechocystis* 6803 (Abdel-Mawgood and Dilley, 1990) and more recently PsbH was identified as a protein in *Synechocystis* 6803 thylakoids by MALDI/MS analysis (Abdel-Mawgood and Dilley, 1990; Szabo et al., 2001; Kashino et al., 2002a). Using a mutant with a His-tag at the N-terminus of the PsbH protein, Buchel et al. (2001) investigated the location of the PsbH protein in *C. reinhardtii* PS II by gold labeling the protein using a Ni-NTA linker. Their results indicate a position close to the two transmembrane helices of Cyt b_{559}. Although they were unable to identify cross-linked products between PsbH and Cyt b_{559}, PsbH did cross-link to PsbX, which has been shown to be close to Cyt b_{559}. More recently, PsbH was assigned to a helix adjacent to PsbX in the PS II structure (Ferreira et al, 2004).

The *psbH* gene is co-transcribed with the Cyt b_6f complex genes *petC* and *petA* in higher plants, but is monocistronic in *Synechocystis* 6803 (Mayes et al., 1993). In *C. reinhardtii*, the *psbB, psbT* and *psbH* genes are located in the same gene cluster; however, deletion mutagenesis showed that *psbB/T* and *psbH* are transcribed separately since disruption at either locus had no effect on the other (Summer et al., 1997). A similar *psbB/T/H* cluster is also found in some cyanobacteria such as *G. violaceus*, suggesting that the cluster may reflect an ancient gene arrangement.

In spinach and *C. reinhardtii,* PsbH undergoes light dependent phosphorylation at a threonine residue (position 2 or 3 in the mature peptide depending on the species) on the stromal side of the thylakoid membrane, which is usually followed by a region of basic amino acids (Dedner et al., 1988; Michel and Bennett, 1987). Recent mass spectroscopic analyses of higher plant thylakoid proteins have identified two phosphorylation sites on PsbH. In *Arabidopsis,* PsbH is phosphorylated at Thr2 and Thr4 (Vener et al., 2001). Gomez et al. (2002) also found direct evidence for a second phosphorylation site on PsbH from spinach and pea. In their MS analysis, two 80 Da adducts on PsbH were identified, corresponding to a doubly phosphorylated PsbH peptide. Additionally, they observed two 32 Da adducts associated with the doubly phosphorylated PsbH peptide, which they hypothesize is the result of oxidative modification.

Sundby et al. (1989) reported that sodium bicarbonate has a negative effect on the phosphorylation of PsbH in spinach and that depletion of endogenous bicarbonate stimulated phosphorylation of PsbH. To account for the observed competition between bicarbonate and phosphorylation, they suggest that basic amino acids may provide a binding site for the bicarbonate anion.

Cyanobacterial PsbH does not have the conserved threonine residue that serves as the phosphorylation site in photosynthetic eukaryotes. However, PsbH from *Synechocystis* 6803 is phosphorylated in vitro. *Synechocystis* 6803 PsbH contains a threonine residue at position 5, but there has been no direct evidence for its phosphorylation. Phosphorylation was light independent, but was inhibited by oxidizing conditions. When PS II membranes from plants were subjected to high light stress, PsbH was degraded (Race and Gounaris, 1993).

Deletion of *psbH* in *Synechocystis* 6803 results

in a mutant capable of photoautotrophic growth at a reduced rate. O_2 evolution, fluorescence, and thermoluminescence measurements indicate that electron flow from Q_A to Q_B is impaired (Mayes et al., 1993). It was concluded that PsbH is not required for cyanobacterial PS II assembly and function in vivo, but the polypeptide probably optimizes electron flow by interacting with the Q_B site on the D1 protein. In further analysis of the *psbH* deletion mutant, Komenda and colleagues (Komenda et al., 2002) also found weakened attachment of CP47 to the D1-D2 heterodimers as well as weakened binding of bicarbonate to the acceptor side of PS II.

Quite a different phenotype was observed in the *C. reinhardtii* mutant lacking PsbH (O'Connor et al., 1998; Summer et al., 1997). Although PS II core proteins are translated and inserted into the thylakoid membrane as in wild type cells, PS II centers do not accumulate. The PS II deficiency is also observed in dark grown cells suggesting the effect is not due to photoinhibition. Therefore, PsbH plays an important role in PS II assembly and stability in *C. reinhardtii*. Interestingly, O'Connor et al. (1998) also found that the site-directed mutant Thr3Ala of PsbH in *C. reinhardtii*, which eliminates the identified phosphorylation site, has a phenotype identical to wild type. A slight, but consistent decrease is O_2 evolution activity was observed in Thr3Ala compared to wild type. This may result from a slight structural change in PsbH upon phosphorylation that effects activity. Also, it is likely that another phosphorylation site exists in the *C. reinhardtii* protein since two phosphorylation sites were identified in higher plant PsbH.

In summary, the PsbH protein functions differently in PS II of cyanobacteria and that of photosynthetic eukaryotes as evidenced by the different mutant phenotypes. While there is some destabilization of cyanobacterial PS II in the absence of PsbH, the protein is absolutely required for PS II assembly and stability in *C. reinhardtii*. The observed impairment of Q_A to Q_B electron flow in the *Synechocystis* 6803 *psbH* mutant may in fact be due to slight structural changes in PS II. Phosphorylation of PsbH is probably a regulatory mechanism for electron flow within PS II or PS II turnover upon photoinhibition. Either situation is likely to differ between cyanobacteria and higher plants, so differences in phosphorylation are expected.

C. PsbI

The *psbI* gene in the chloroplasts of higher plants and the genome of cyanobacteria encodes a 4.8 kDa protein, 36–39 residues in length depending on the species. It was identified as an intrinsic PS II reaction center component in spinach, and N-terminal sequence data revealed it to be encoded by a chloroplast gene (Ikeuchi and Inoue, 1988). PsbI was later found in PS II reaction center preparations from *T. vulcanus* (Ikeuchi et al., 1989a). Crosslinking studies showed that the N-terminal domain of PsbI from spinach is in close contact with the D2 protein and the α subunit of Cyt b_{559} (Tomo et al., 1993). In contrast, PS II structural analysis has placed PsbI near the dimer interface, which is closer to D1 and CP43 than D2 and Cyt b_{559} (Ferreira et al, 2004).

A *C. reinhardtii psbI* deletion mutant is capable of photoautotrophic growth under low light conditions, but not high light conditions. The amount of assembled PS II centers and O_2 evolution activity is 10–20% that of wild type as determined by variable fluorescence yield and steady state O_2 evolution activities. Unlike other PS II-deficient mutants, the high light sensitivity of the *psbI* deletion mutant persisted when the mutant was grown on medium containing acetate (Kunstner et al., 1995). Thus in *C. reinhardtii*, PsbI is required for both assembly and function of PS II.

A different phenotype was observed for the *Synechocystis* 6803 *psbI* deletion mutant. The cyanobacterial mutant had a loss of only 25–30% PS II activity compared to the wild type. Other PS II proteins accumulated to wild type levels with no significant destabilization of PS II centers, and the *Synechocystis* 6803 mutant is only slightly more light sensitive than the wild type (Ikeuchi et al., 1995b). The conclusion from the *Synechocystis* 6803 mutant indicates that PsbI is not absolutely required for PS II assembly and function in cyanobacteria, but optimizes PS II activity.

PS II centers have not been isolated for further biochemical analysis from either of the mutants described above. Because of their more stable PS II centers, the thermophilic cyanobacterium *T. elongatus* represents a better system for isolating mutant PS II centers for more detailed biochemical analyses. Katoh and Ikeuchi (2001a) created such a *psbI* deletion mutant and found the isolated PS II centers to be exclusively monomeric. The mutant also had decreased light harvesting efficiency. These results demonstrate that PsbI plays a major structural role in PS II dimer assembly and the functional assembly of the antenna complex, but is not required for PS II function in vitro.

D. PsbJ

The PsbJ subunit is encoded by the *psbJ* gene, a member of the *psbEFLJ* operon that is conserved in most oxygenic photosynthetic organisms. The *psbJ* gene was determined to be the second open reading frame closely downstream of the genes encoding the subunits of Cyt b_{559}, and was given its name because of a presumed association with PS II (Cushman et al., 1988). The protein encoded by *psbJ* in *Synechocystis* 6803 was determined to be localized in the thylakoid membrane and have a role in PS II activity (Lind et al., 1993). PsbJ is predicted to be 4.1 kDa and is 40 amino acids long with 9 amino acid-long N/C-terminal extensions flanking a single membrane spanning helix. Even though PsbJ was predicted to be part of PS II for many years, it was first seen in an isolated PS II preparation in a recent study by Kashino et al. (2002a). More recently, it has been identified to be a component of the crystallized PS II complex (Ferreira et al, 2004).

Mutations in *psbJ* have been made in both *Synechocystis* 6803 (Lind et al., 1993) and tobacco (Hager et al., 2002; Regel et al., 2001), which showed that PsbJ is required for photoautotrophic growth in tobacco but not in *Synechocystis* 6803. A major difference in PS II activity between cyanobacterial and tobacco Δ*psbJ* mutants is that the electron flow from Q_A^- to Q_B is slowed by only 40% in the *Synechocystis* 6803 mutant and by almost two orders of magnitude in the tobacco mutant (Regel et al., 2001). Single flash O_2 evolution experiments in both *Synechocystis* 6803 and tobacco indicated that PsbJ is required for the stability of the S_n-states of the Mn cluster in the O_2 evolution apparatus (Regel et al., 2001). In the *Synechocystis* 6803 *psbJ* mutant, the decay of the S_3 state via back electron flow from the acceptor side is twenty times faster than in wild type cells (Regel et al., 2001). In tobacco, young Δ*psbJ* leaves have slightly impaired PS II driven photochemistry while mature leaves are severely damaged (Hager et al., 2002), suggesting that light-induced photodamage is accumulated in the absence of PsbJ. The damage accumulated in mature Δ*psbJ* tobacco leaves includes the loss of oxygen enhancer (OE) proteins, but this appears to be a general result of the damage and not directly resulting from the absence of PsbJ since those proteins are properly assembled in younger leaves. Like many of the other small subunits of PS II, it is unclear what the exact role of PsbJ is in the complex.

E. PsbK

The *psbK* gene encodes a 3.9–4.2 kDa polypeptide predicted to contain a single transmembrane region with its N-terminal domain in the thylakoid lumen. In spinach, the translation product has 98 residues, but the mature protein is only 37 amino acids in length. PsbK was first identified as a chloroplast-encoded spinach PS II component (Murata et al., 1988). The cyanobacterial PsbK subunit was first identified in *T. vulcanus* PS II preparations (Koike et al., 1989). Later, *psbK* identification and cloning in *Synechocystis* 6803 revealed a gene coding for a 45 residue mature protein with a short 8 amino acid presequence that is cleaved upon insertion into the thylakoid membrane (Ikeuchi et al., 1991). De Vitry et al. (1991) reported detectable amounts of PsbK in *C. reinhardtii* PS II core complexes. PsbK was later found in the dimer form of CP43-depleted PS II from spinach indicating a tighter association with the PS II core complex than originally reported (Rhee et al., 1998). In contrast, it was reported that PsbK present in PS II core complexes from *C. reinhardtii* is specifically bound to CP43 (Sugimoto and Takahashi, 2001). Interestingly, the PsbK helix is closely associated with CP43 in the cyanobacterial PS II crystal structure (Ferreira et al, 2004).

Because PsbK remains bound to PS II after removal of LHCII, but is not present in the PS II reaction center core, it was concluded that PsbK was not essential for PS II activity (Murata et al., 1988). However, PsbK inactivation in *C. reinhardtii* chloroplasts results in mutants that are unable to accumulate normal amounts of PS II and cannot grow photoautotrophically. Growth rates returned to wild-type levels on acetate-containing medium. Pulse labeling experiments confirmed a PS II destabilization in the mutant rather than a reduction in polypeptide synthesis. This phenotype suggests that the PsbK protein is necessary for PS II stabilization in vivo (Takahashi et al., 1994).

psbK deletion mutants in the cyanobacterium *Synechocystis* 6803 grow photoautotrophically at a two-fold reduced rate and have slightly reduced PS II activity compared to the wild type. These results demonstrate that PsbK is not essential for PS II activity in prokaryotic cells. PsbK may have other functions in cyanobacteria because addition of glucose to the medium does not fully restore wild-type growth rates as observed in other PS II deficient mutants (Ikeuchi et al., 1991).

A detailed biochemical analysis of the PS II centers in these mutants was not possible, but recently a deletion mutant in the thermophilic bacterium *T. elongatus,* afforded such a study (Katoh and Ikeuchi, 2001a). The mutant phenotype was similar to that observed for the *Synechocystis* 6803 mutant. Cells were capable of photoautotrophic growth at slightly, but reproducibly slower rates under various temperature conditions. The O_2 evolution activity of the PsbK-depleted PS II particles was comparable to the wild type at low concentrations of DCBQ. At higher DCBQ concentrations, PsbK-depleted PS II particles had a lower O_2 evolution activity (Katoh and Ikeuchi, 2001a). A similar acceptor dependency was also observed for PsbX in the same organism (Katoh and Ikeuchi, 2001b).

F. PsbL

Another member of the *psbEFLJ* operon is the gene encoding the small PS II subunit, PsbL. It is predicted to be 38 amino acids long with a single membrane spanning helix and a 17-amino acid soluble N-terminal extension. The PsbL helix is located near the dimer interface with PsbT and PsbM in cyanobacteria (Ferreira et al, 2004). A *Synechocystis* 6803 Δ*psbL* mutant lacks a functional PS II complex (Anbudurai and Pakrasi, 1993; Kitamura et al., 1994; Ozawa et al., 1997) while the tobacco mutant retains residual PS II activity (Swiatek et al., 2003). Tobacco Δ*psbL* cannot grow photoautotrophically and is hypersensitive to light (Swiatek et al., 2003). Additionally, the PsbJ subunit is lost in the tobacco Δ*psbL* mutant, but PsbJ does not seem to be required for the presence of PsbL (Swiatek et al., 2003).

In vitro reconstitution of hydrophobic components in a partially disintegrated PS II complex has only been successful for PsbL protein (Nagatsuka et al., 1991). When PQ-depleted PS II partial complex consisting of CP47/D1/D2/PsbI/PsbW was reconstituted with PQ and PsbL, functional Q_A activity was restored. Such reconstitution is specifically dependent on the addition of PsbL (Kitamura et al., 1994). Further analysis using recombinant PsbL showed that the C-terminal part of PsbL is crucial for recovering the Q_A electron transfer activity in the reconstituted complexes (Ozawa et al., 1997). These studies suggest that PsbL is necessary for electron transfer from Q_A.

G. PsbM

The PsbM subunit was originally identified by N-terminal sequencing as a 4.7 kDa protein in PS II preparations from *T. vulcanus* isolated in the presence of Triton X-100 and urea. It was found to be homologous to ORF34 from tobacco and liverwort chloroplast DNA and was predicted to have a single transmembrane segment (Ikeuchi et al., 1989b). The PsbM protein was also identified in PS II particles from *C. reinhardtii* (de Vitry et al., 1991). Analysis of *Synechocystis* 6803 PS II by Kashino et al. (2002a) confirmed the presence of the PsbM protein for the first time in that organism. The PsbM helix was also seen in the *T. elongatus* PS II crystal structure (Ferreira et al, 2004). Recently, *psbM* disruption mutant was created in *T. elongatus* but no clear phenotype was detected (C. Aoyama, M. Iwai, M. Ikeuchi, personal communication). Although it is associated with PS II from all model organisms studied, no functions have been hypothesized for PsbM.

H. PsbN

Based on a partial N-terminal sequence of a 4.7 kDa polypeptide in isolated PS II preparation from *T. vulcanus*, Ikeuchi et al. (1989b) named it the PsbN protein. The *psbN* gene is present in cyanobacteria as well as the chloroplast genomes of a number of plants and algae. Zouni et al. (2001) have stated that the crystallized PS II complex from *T. elongatus* has the PsbN protein, although the primary data supporting this statement has not been published yet. Subsequently, a helix near PsbK was tentatively named PsbN in the most recent PS II crystal structure (Ferreira et al, 2004). In contrast, Kashino et al. (2002b) have shown that the original assignment of the PsbN protein in *T. vulcanus* was incorrect, and this protein is actually $PsbT_c$ (see below). Therefore, the presence of PsbN in PS II remains ambiguous.

I. PsbS

The integral PS II subunit, PsbS, is a 22 kDa protein found in plants and not in cyanobacteria. PsbS was originally found as a coprecipitant in an antibody pull down experiment with PsbO and PsbP from spinach thylakoids (Ljungberg et al., 1984a). It has amino acid sequence homology to chlorophyll *a*/*b* binding proteins that make up the light harvesting complex (LHC) (Wedel et al., 1992). Most LHC proteins have

three membrane spanning domains, but PsbS has four such spans. Even though PsbS is suggested to bind chlorophyll (Funk et al., 1995), it was recently shown to have a very weak affinity for pigments (Dominici et al., 2002). An *Arabidopsis* mutant deficient in PS II nonphotochemical quenching, npq4, was cloned and mapped to the *psbS* locus (Li et al., 2000). Further analysis of this mutant revealed that PsbS deficient plants had normal quantum yield of electron transport and normal O_2 evolution, but decreased non-photochemical quenching when exposed to high light (Li et al., 2000). Other studies on the transcript levels of *psbS* indicate that it may play a role in the greening process of hypocotyls and is probably regulated by the phytochrome system (Adamska et al., 1996). PsbS does not appear to have a direct role in the activity of PS II, and will be discussed further for its role in pigment association in Chapter 23 (Pogson et al.).

J. $PsbT_c$

In the early 1990s, two PS II proteins were almost simultaneously named PsbT. Among them, the first one is encoded by the *ycf8* ORF in the chloroplast genomes of plants and *C. reinhardtii*, and is hence, referred to as the $PsbT_c$. This gene is usually located downstream of the *psbB* gene for the CP47 protein. $PsbT_c$ is a ~4 kDa intrinsic membrane protein, with a single predicted membrane-span. This protein is also present in cyanobacterial PS II (Kashino et al., 2002a).

The *psbT*$_c$ (*smr0001*) gene has been inactivated in *Synechocystis* 6803 (H. Katoh and M. Ikeuchi, personal communication), but the mutant has not been functionally characterized. *psbT*$_c$ was also inactivated in *T. elongatus* causing a decrease in the dimer form of PS II, but little defect was detected in the O_2 evolving activity of the complex or in photoautotrophic growth rate (Iwai et al., 2001). Interestingly, the most recent PS II structural model shows $PsbT_c$ at the dimer interface, consistent with a proposed role in dimerization (Ferreira et al, 2004). A *psbT*$_c$ inactivation mutant strain of *C. reinhardtii* has been studied in considerable detail (Monod et al., 1994; Ohnishi and Takahashi, 2001). This mutant grows well under moderate light intensities, but dies under high light. Moreover, the rates of photo-inactivation as well as photo-degradation of the PS II complex in this mutant are similar to those in the wild-type strain. In contrast, the recovery of photo-damaged PS II is significantly slower in this mutant. Radioactive pulse-chase analysis showed that the absence of $PsbT_c$ does not affect the rate of synthesis of major PS II proteins, such as D1, D2, CP47 and CP43, suggesting that $PsbT_c$ is involved in a post-translational step during the repair of PS II. Biochemical fractionation of *C. reinhardtii* thylakoid membranes has shown that $PsbT_c$ cofractionates with the D1/D2 heterodimer complex that is depleted of CP47 and CP43. Based on these data, it has been suggested: that (i) $PsbT_c$ is closely associated with D1, and (ii) $PsbT_c$ may act as a scaffold for the replacement of photo-inactivated D1.

K. $PsbT_N$

The second PsbT protein is a ~5 k Da hydrophilic protein (Ikeuchi et al., 1989c; Kapazoglou et al., 1995). The gene for this protein is localized in the nuclear genome of plants, and has recently been named the *PsbT*$_N$ gene. The precursor form of this protein has a relatively long bipartite transit peptide that localizes the mature protein in the thylakoid lumen. Hence, $PsbT_N$ is a lumenal extrinsic protein in higher plant PS II. Homologs of this protein have not been found in cyanobacteria (Table 1). The function of the protein in PS II is currently unknown.

L. PsbW

PsbW is a nuclear-encoded 4.6–6kDa protein associated with PS II particles in photosynthetic eukaryotes. It was first identified in spinach and was predicted to be an integral membrane protein with a single transmembrane domain. Its topology in the membrane is the opposite of most PS II membrane proteins as it has a lumenal N-terminus (Irrgang et al., 1995). Recently *psbW* has been characterized in the green algae *C. reinhardtii* as well. The gene codes for a protein of 115 residues containing a bipartite transit peptide of 59 residues and a 56 residue mature protein (Iwai et al., 2001). The *psbW* gene is absent from the *Synechocystis* 6803 genome and all other prokaryotes examined. (Note: In the *Synechocystis* 6803 database, *psb28* is mistakenly annotated as *psbW*. See below.)

Studies by Lorkovic et al. (1995) on spinach PsbW revealed a gene that codes for a 54 residue mature protein with an 83 residue bipartite presequence. The mRNA levels were upregulated ten-fold by light, but the transcript and the protein were also detectable in etiolated seedlings. Further characterization in spin-

ach found that 80% of the PsbW protein localizes to the grana region of the thylakoids. PsbW is located close to the D1-D2 heterodimer based on its presence in reaction center preparations (Shi and Schroder, 1997). Using the blue-native gel electrophoresis system to separate PS II monomers and dimers from pea thylakoids, Thidholm et al. (2002) found PsbW exclusively associated with dimeric PS II. They also reported that PsbW is directly assembled into PS II dimers in a process dependent on its negatively charged N-terminus. These experimental data are contradictory to the previous evidence that PsbW is closely associated with the D1-D2 heterodimer. Further evidence is needed to explain how PsbW could be associated with the PS II dimer interface and be tightly bound to D1-D2 at the same time.

An *Arabidopsis* antisense mutant of PsbW with a >96% decrease in *psbW* transcript was generated by Shi et al. (2000). The mutant contained no dimeric PS II supercomplex, but retained normal electron transfer properties. O_2 evolution activity was decreased by 50% due to the disappearance of up to 40% of the D1 and D2 proteins. The amounts of other PS II proteins were also affected to differing degrees. In a more detailed investigation of the PsbW antisense mutant (Thidholm et al., 2001), quantum yield measurements after periods of light stress showed the antisense plants to be much more sensitive to photoinhibition than wild type plants. Thus, lack of PsbW results in less stable PS II dimers. Based on this phenotype, the conclusion is that PsbW is required for PS II dimer formation and PS II stability in higher plants.

PsbW also undergoes light induced proteolysis in a process similar to that described for the D1 protein. Although PsbW contains a number of serines and threonines, it is not phosphorylated under conditions that lead to the phosphorylation of other PS II proteins. Despite its lack of phosphorylation, it is protected from degradation under conditions when other PS II proteins are phosphorylated. These results support the conclusion that the PsbW protein becomes susceptible to proteolysis when PS II is destabilized as a result of D1 photodamage (Hagman et al., 1997).

M. PsbX

PsbX, a nuclear-encoded, 4.1 kDa membrane protein associated with PS II, was first identified in spinach (Ikeuchi et al., 1989c). The PsbX protein has been characterized in plants and cyanobacteria by biochemical and genetic analysis. In plants, it cross-links to Cyt b_{559}; however, it is not present in isolated PS II reaction centers (Shi et al., 1999). In cyanobacterial PS II structures, PsbX has been assigned to the helix that lies closest to the Cyt b_{559} helices (Ferreira et al, 2004; Kamiya and Shen, 2003). The PsbX mRNA levels were also found to be tightly light regulated such that no mRNA is detectable in the dark (Shi et al., 1999).

The deletion of *psbX* in the cyanobacterium *Synechocystis* 6803 did not affect growth rate, electron transport, or water oxidation, but the number of functionally active PS II centers was only 30% of wild type. PsbX mRNAs were observed under various light conditions suggesting that the transcripts are not as tightly light-regulated in cyanobacteria compared to higher plants (Funk, 2000). A PsbX deletion mutant in the thermophilic cyanobacterium *T. elongatus* also had a photoautotrophic phenotype, but showed a growth defect under low CO_2 conditions. The mutant had a lower O_2 evolution activity in the presence of high concentrations of artificial quinone acceptors compared to wild type, suggesting the PsbX protein is involved in quinone turnover at the Q_B site (Katoh and Ikeuchi, 2001b).

Like the PsbW subunit, the PsbX protein is inserted into the membrane by a mechanism independent of the previously characterized translocation machineries despite the presence of a bipartite transit peptide (Kim et al., 1996). Tissier and colleagues (Tissier et al., 2002) reported that the C-terminal acidic residues are essential for the maturation of PsbX. A single Glu to Val substitution at position 5 prevents proper insertion in to the membrane. Processing is partially restored by the hydrophilic residue Asn, implying that this domain is important for cleavage. Substitution of the C-terminal Glu residues with Val causes the cleavage site to be buried in the membrane, but insertion is not affected. These results suggest that there are stringent requirements on the peptide sequence of PsbX for insertion into the membrane and cleavage by the thylakoid processing protease, even though the mechanism of insertion is not defined.

N. PsbY

The small PS II subunit, PsbY, was first identified as a component of spinach and tobacco PS II preparations by Gau et al. (1995). It is a 4–5 kDa, single membrane-spanning protein with a 14–15 amino acid C-terminal soluble domain. In spinach and

Arabidopsis psbY encodes a 20–23 kDa precursor protein with a bipartite signal peptide targeting it to the chloroplast thylakoid membrane (Gau et al., 1998; Mant and Robinson, 1998). The precursor protein contains four hydrophobic regions, two of which resemble the signal peptides preceding the PsbW and PsbX mature protein sequence that is used for Sec-independent insertion into the thylakoid membrane (Thompson et al., 1999). Initial activity studies of PsbY concluded that it was a manganese requiring L-arginine metabolizing enzyme suspected to be important to the function of the manganese cluster in the O_2 evolution center of PS II (Gau et al., 1995). A *Synechocystis* 6803 mutant in *psbY* was able to grow normally under photoautotrophic conditions and had normal rates of PS II O_2 evolution, indicating that it is not essential for photosynthesis and is not an important manganese ligand in the O_2-evolving center (Meetam et al., 1999). PsbY was found to be part of a purified PS II complex from *Synechocystis* 6803 (Kashino et al., 2002a), but was not identified in the most recent PS II crystal structure (Ferreira et al, 2004). The functional role of PsbY in PS II remains to be determined.

O. PsbZ

PsbZ is a relatively new addition to the group of proteins known to be part of PS II. Encoding PsbZ is a gene that was located in many chloroplast genomes and was named *ycf 9* for hypothetical chloroplast open reading frame. The gene usually encodes a protein of about 62 amino acids with a predicted molecular mass of 6.5 kDa. It is predicted to have two membrane spanning domains but is most likely cleaved by a lumenal peptidase between the membrane spanning regions.

The brief history of this open reading frame was summed up in a recent review (Eckardt, 2001), which discussed the difficulty in determining the function of the protein because of the lack of homoplasmic mutants in chloroplasts. One of the first tobacco Δ*psbZ* mutants did not exhibit a phenotype in normal growth conditions, but the authors found that the protein was associated with the light-harvesting complex of PS II (Ruf et al., 2000). Two more papers published almost concurrently examined tobacco Δ*psbZ* mutants with different interpretations of the function of PsbZ. The mutant studied by Baena-Ganzales et al. (2001) still contained at least 5% of the wild-type gene, which could have produced a sufficient amount of the wild-type protein to influence the phenotype of the mutant. This study found that the rate of PS II O_2 evolution was unchanged in the Δ*psbZ* mutant and the rate of electron flow from PS II to PS I was enhanced, and suggested that PsbZ is involved in regulating electron transport depending on the prevailing conditions. The second Δ*psbZ* paper published almost simultaneously as the above study examined transplastomic knockouts in both tobacco and *C. reinhardtii* and concluded that PsbZ is necessary for the stability of the PS II-LHCII super complex (Swiatek et al., 2001). Because two organisms were examined and complementary experiments were performed in each, the Swiatek study conclusively supports a role for PsbZ that was suggested in the other manuscripts. The work by Swiatek et al. (2001) indicates that as a connection between PS II and LHCII, PsbZ helps mediate nonphotochemical quenching when PS II is exposed to light exceeding the water oxidation capacity of PS II, either in higher than normal light intensities or in low temperatures. In the cyanobacterial PS II structure, the two PsbZ helices are located on the perimeter of the dimer near CP43 (Ferreira et al, 2004). Thus, an analogous location in plant PS II would be consistent with PsbZ coordinating light harvesting protein association. More evidence is required to determine the mode of coordination of PsbZ between PS II and LHCII in plants. The mechanism of PsbZ activity is yet to be discovered.

III. Extrinsic Subunits

A. PsbR

PsbR is a 10 kDa extrinsic protein found only in plants and identified as a soluble protein released from spinach PS II preparations (Ljungberg et al., 1984b). Antisense potato plants were generated containing 1–3% of the normal PsbR protein levels to determine if the protein were necessary for PS II activity, but there was no decrease in either PS II accumulation or growth rate (Stockhaus et al., 1990). So far, analysis of this protein has not indicated a functional role in water oxidation.

B. Psb27

The Psb27 subunit was originally identified from the N-terminal sequence of a ~11 kDa polypeptide component of a purified PS II preparation from

Synechocystis 6803 (Ikeuchi et al., 1995a). This protein was originally named PsbZ. As discussed above, a distinctly different and smaller PS II protein is now called PsbZ (Swiatek et al., 2001). According to a recent nomenclature, the 11 kDa protein of PS II has been named Psb27 (Kashino et al., 2002a).

The Psb27 polypeptide in *Synechocystis* 6803 has one predicted membrane-spanning domain followed by a cleavage site for signal peptidase II. Thus, the Psb27 protein is expected to be a lumen-localized extrinsic protein of PS II. The Arabidopsis homologs (At1g03600 and At1g05385) of Psb27 were found in a proteomics study of the thylakoid lumen (Peltier et al., 2002). Although it is associated with PS II from many model organisms (Table 1), this protein was not identified in the cyanobacterial PS II crystal structure (Ferreira et al, 2004). Furthermore, no mutant has been described for this subunit, and no function has been hypothesized.

C. Psb28

The Psb28 subunit was originally identified from the N-terminal sequence of a ~13 kDa polypeptide component of a purified PS II preparation from *Synechocystis* 6803 (Ikeuchi et al., 1995a). This protein was originally named Psb13. A homologue of this protein is the deduced product of *ycf79*, an open reading frame in the chloroplast genome of *Porphyra*. Finally, the protein was also named PsbW. According to a recently published nomenclature, Psb13 has been renamed Psb28, and a different and smaller PS II protein is called PsbW (see above) (Kashino et al., 2002a).

The Psb28 protein is predicted to have no hydrophobic membrane-spanning domain and to be localized in the stroma/cytoplasm. If this prediction is correct, Psb28 is the only known extrinsic stromal/cytoplasmic protein in PS II. There was no unassigned density in the recently published PS II crystal structure that could be attributed to Psb28 on the stromal surface of PS II (Ferreira et al, 2004).

A knockout mutation of the *psb28* gene in *Synechocystis* 6803 results in reduced growth rate under photosynthetic conditions when calcium and chloride is not added to the growth medium (H. Katoh and M. Ikeuchi, personal communication). Future detailed studies of this interesting mutant strain may unravel the functional role of the Psb28 protein in PS II.

IV. Conclusion

Clearly, there is still much to be learned about the role of each subunit and how it contributes to the activity of the PS II complex. There are 18 small proteins discussed in this chapter, and there are possibly more polypeptides in various organisms that are important to PS II function. PS II is an extremely complex enzyme, which evolved before the intricate membrane systems found in most photosynthetic organisms were developed, suggesting that the task of light-induced water oxidation was not a small hurdle to jump. Functional studies, biochemical analyses, and structural arrangements from multiple photosynthetic organisms are necessary for understanding how all of the individual units of PS II work together in unique intricacy.

Acknowledgments

The preparation of this chapter was supported by funding from the National Science Foundation, Department of Energy and United States Department of Agriculture. LET and JLR were supported by graduate student fellowships from the National Science Foundation.

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