

Chapter 6

The Low Molecular Weight Components

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Summary

Photosystem 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 Photosystem 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

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the Photosystem II protein components are highly conserved, from the primitive cyanobacterium *Gloeobacter violaceus* PCC 7421 to the vascular plant *Arabidopsis thaliana*. Advances in purification of Photosystem II samples and protein identification have confirmed the presence of several novel proteins and provide new tools for the analysis of Photosystem II in various mutants and under different growth conditions. Recent structural studies have also contributed tremendously to our understanding of the organization of Photosystem 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 Photosystem 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 O₂ 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 its rapid removal of 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 helices (Zouni et al., 2001). 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). 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). 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.

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 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

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

1 are missing in *G. violaceus*, may be related to the
2 development of the thylakoid membrane system, since
3 it is the only organism that lacks thylakoids. Another
4 anomalous organism is *P. marinus* MED 4, which
5 lacks *psbU* and *psbV* since *P. marinus* MIT 9313, a
6 close relative, retains both of them. *P. marinus* MED
7 4 is known to be a high-light adapted ecotype with
8 multiple *hli*p and photolyase genes (Hess et al., 2001).
9 Such adaptation may be coupled with the absence of
10 two extrinsic proteins in PS II.

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

28 Studies on the biochemical composition of PS II
29 are providing correlations between the structure and
30 genetic data from various organisms. Comprehensive
31 analysis of a highly active, His-tag purified PS II
32 from *Synechocystis* 6803 using the latest protein
33 identification technologies revealed a number of
34 PS II subunits that had never been seen in such a
35 purified protein complex (Kashino et al., 2002a). A
36 similar study in *T. vulcanus* (Kashino et al., 2002b)
37 revealed that the protein make up of PS II in the two
38 organisms is very similar, with only a few subtle
39 variations, such as a different apparent molecular
40 weight of the cytochrome *b*₅₅₉ large subunit. Irrgang
41 and coworkers (Irrgang et al., 2001) examined PS II
42 core components from both *T. elongatus* and spinach
43 to show that there are expected differences in some
44 of the subunit components, but the behavior of the
45 complexes in forming monomers and dimers is es-
46 sentially the same.

47 In this chapter, we will discuss the low molecular
48 weight protein subunits of PS II with respect to the
49 new discoveries that contribute to a better overall
50 understanding of the function of the entire protein
51 complex. Most of the proteins discussed are less
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53 than 10 kDa, but a few are between 10 and 13 kDa. 53
54 This group includes the heme-binding heterodimer, 54
55 cytochrome *b*₅₅₉, whose function has been a subject of 55
56 intense debate for almost thirty years. The majority of 56
57 the proteins covered in this review consists of single 57
58 helix, membrane-spanning proteins that are usually 58
59 organized peripherally to the large integral proteins. 59
60 Many of these proteins are not essential for PS II 60
61 activity, but they appear to be important in regulation 61
62 or biogenesis. PS II also has small soluble, extrinsic 62
63 proteins, associated with either the stromal/cytoplasmic 63
64 or luminal surface of PS II. The small extrinsic 64
65 proteins discussed here do not have a known role in 65
66 oxygen evolution activity as do the larger extrinsic 66
67 proteins described in Chapter 5 of this book. Most 67
68 of the small PS II subunits found in cyanobacteria 68
69 are also present in plants and encoded by genes in 69
70 the chloroplast genome. 70

71 II. Membrane Spanning Subunits 72

73 A. *PsbE/F* — Cytochrome *b*₅₅₉ 74

75 This enigmatic member of the PS II complex is a 75
76 heme-linked heterodimer of the single membrane 76
77 spanning proteins, PsbE and PsbF. Cytochrome 77
78 (Cyt) *b*₅₅₉ was first biochemically characterized by 78
79 Garewal and Wasserman (Garewal and Wasserman, 79
80 1974b), who developed an efficient Triton-based 80
81 purification method. Using this purification method 81
82 (Garewal and Wasserman, 1974a), Cyt *b*₅₅₉ was iso- 82
83 lated and sequenced (Herrmann et al., 1984; Widger 83
84 et al., 1985). The protein contains one α subunit, PsbE, 84
85 and one β subunit, PsbF, each of which contributes 85
86 one histidine residue to coordinate a single heme to 86
87 give its unique two-chain structure. PsbE is 9 kDa 87
88 and PsbF is 4 kDa and the proteins are 83 and 39 88
89 amino acids, respectively. 89

90 In most PS II-containing bacteria, the proteins are 90
91 encoded by the *psbEFLJ* operon. The gene, *psbF*, 91
92 was actually found because its ribosome-binding region 92
93 overlaps the stop codon for *psbE* (Herrmann et al., 93
94 1984). Genetic analysis of Cyt *b*₅₅₉ in *Synechocystis* 94
95 6803 (Pakrasi et al., 1988, 1990, 1991) and 95
96 *Chlamydomonas reinhardtii* (Morais et al., 1998, 96
97 2001) indicated that it is required for PS II activity but 97
98 may not be involved in primary electron transport. A 98
99 recent study of tobacco chloroplast mutants indicated 99
100 that PsbE and PsbF are each required for the stable 100
101 insertion of the other, and without these proteins, the 101
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Table 1. List of Photosystem II genes in representative genomes

Photosystem II gene	<i>Synechocystis</i> sp. PCC 6803	<i>Anabaena</i> sp. PCC 7120	<i>N. punctiforme</i> ATCC 29133	<i>Trichodesmium</i> <i>erythraeum</i>	<i>T. elongatus</i> BP-1	<i>P. marinus</i> MED4	<i>P. marinus</i> MIT9313	<i>G. violaceus</i> PCC 7421	<i>Synechococcus</i> sp. WH8102	<i>Arabidopsis</i> <i>thaliana</i>
<i>psbA</i> : D1 reaction center protein	<i>slr1311</i> <i>slr1867</i> <i>slr1181</i>	<i>al3372</i> <i>al4592</i> <i>alr4866</i> <i>alr3742</i>	4 genes	yes	<i>tlr1843</i> <i>tlr1844</i> <i>tlr1477</i>	<i>PMM0223</i>	<i>PMT0419</i> <i>PMT1532</i>	<i>gl13144</i> <i>gl10779</i> <i>glr1706</i> <i>glr2322</i> <i>glr2656</i>	<i>SYNW0983</i> <i>SYNW1470</i> <i>SYNW1919</i> <i>SYNW2151</i>	chloroplast
<i>psbB</i> : CP47 chlorophyll-binding protein	<i>slr0906</i>	<i>alr0138</i>	yes	yes	<i>tlr1530</i>	<i>PMM00315</i>	<i>PMT1665</i>	<i>glr2999</i>	<i>SYNW1982</i>	chloroplast
<i>psbC</i> : CP43 chlorophyll-binding protein	<i>slr0851</i>	<i>alr4291</i>	yes	yes	<i>tlr1631</i>	<i>PMM11158</i>	<i>PMT1180</i>	<i>glr2324</i>	<i>SYNW0676</i>	chloroplast
<i>psbD</i> : D2 reaction center protein	<i>slr0849</i> <i>slr0927</i>	<i>alr4290</i> <i>alr4548</i>	yes	yes	<i>tlr1630</i> <i>tlr0455</i>	<i>PMM11157</i>	<i>PMT1179</i>	<i>glr2323</i>	<i>SYNW0677</i> <i>SYNW2232</i>	chloroplast
<i>psbE</i> : cytochrome b559 large subunit	<i>ssr3451</i>	<i>asr3845</i>	yes	yes	<i>tsr1541</i>	<i>PMM0297</i>	<i>PMT1896</i>	<i>gsr0856</i>	<i>SYNW0204</i>	chloroplast
<i>psbF</i> : cytochrome b559 small subunit	<i>smr0006</i>	<i>asr3846</i>	yes	yes	<i>tsr1542</i>	<i>PMM0298</i>	<i>PMT1897</i>	<i>gsr0857</i>	<i>SYNW0203</i>	chloroplast
<i>psbH</i> : small membrane-spanning protein	<i>ssi2598</i>	<i>asr0846</i>	yes	yes	<i>tsr1386</i>	<i>PMM0251</i>	<i>PMT1837</i>	<i>gsr3002</i>	<i>SYNW0269</i>	chloroplast
<i>psbI</i> : small membrane-spanning protein	<i>smi0001</i>	<i>asr1277</i>	yes	yes	<i>tsr1074</i>	<i>PMM0253</i>	<i>PMT1840</i>	<i>gsr3634</i>	<i>SYNW0266</i>	chloroplast
<i>psbJ</i> : small membrane-spanning protein	<i>smr0008</i>	<i>asr3848</i>	yes	yes	<i>tsr1544</i>	<i>PMM0300</i>	<i>PMT1899</i>	<i>gsr0859</i>	<i>SYNW0201</i>	chloroplast
<i>psbK</i> : small membrane-spanning protein	<i>smi0005</i>	<i>asr0885</i>	yes	yes	<i>tsr10176</i>	<i>PMM0272</i>	<i>PMT1863</i>	<i>gsr2807</i>	<i>SYNW0243</i>	chloroplast
<i>psbL</i> : small membrane-spanning protein	<i>smr0007</i>	<i>asr3847</i>	yes	yes	<i>tsr1543</i>	<i>PMM0299</i>	<i>PMT1898</i>	<i>gsr0858</i>	<i>SYNW0202</i>	chloroplast
<i>psbM</i> : small membrane-spanning protein	<i>smi0003</i>	<i>asr0883</i>	yes	yes	<i>tsr2052</i>	<i>PMM0317</i>	<i>PMT1663</i>	<i>gsr2997</i>	<i>SYNW1980</i>	chloroplast
<i>psbO</i> : manganese-stabilizing protein	<i>slf0427</i>	<i>alr3854</i>	yes	yes	<i>tlr0444</i>	<i>PMM0228</i>	<i>PMT1800</i>	<i>glr3691</i>	<i>SYNW0303</i>	Al1906680 Al1978450 Al11977090 Al2928605 Al2930790 Al2939470 Al3555330 Al3956650 Al4915510 Al5911450
<i>psbP</i> : 23 kDa extrinsic protein	<i>slr1418</i>	<i>alr3076</i>	yes	yes	<i>tlr2075</i>	<i>PMM1098</i>	<i>PMT1078</i>	<i>glr1440</i>	<i>SYNW0927</i>	Al4921280 Al4905180 Al3901440
<i>psbQ</i> : 16 kDa extrinsic protein	<i>slr1638</i>	<i>alr1355</i>	yes	yes	<i>tlr2057</i>	no	no	no	<i>SYNW2505</i>	Al1979040 Al1944575 Al1944575
<i>psbR</i> : 10 kDa extrinsic protein	no	no	no	no	no	no	no	no	no	chloroplast
<i>psbS</i> : light harvesting complex regulator	no	no	no	no	no	no	no	no	no	chloroplast
<i>psbTc</i> : small membrane-spanning protein	<i>smr0001</i>	<i>asr0137</i>	yes	yes	<i>tsr1531</i>	<i>PMM0314</i>	<i>PMT1666</i>	<i>gsr3000</i>	<i>SYNW1983</i>	Al3921050 Al1951400
<i>psbTn</i> : extrinsic protein	no	no	no	no	no	no	no	no	no	no
<i>psbU</i> : 12 kDa extrinsic protein	<i>slr1194</i>	<i>alr1216</i>	yes	yes	<i>tlr2409</i>	no	<i>PMT0178</i>	<i>glr2873</i>	<i>SYNW2192</i>	no
<i>psbV</i> : cytochrome c550	<i>slf0258</i>	<i>alr0259</i>	yes	2 genes	<i>tlr1285</i> <i>tlr1284</i>	no	<i>PMT1427</i>	<i>glr2337</i> <i>glr2338</i>	<i>SYNW1537</i>	no
<i>psbW</i> : small membrane-spanning protein	no	no	no	no	no	no	no	no	no	Al2930570
<i>psbX</i> : small membrane-spanning protein	<i>smi0002</i>	<i>asr0941</i>	yes	yes	<i>tsr2013</i>	<i>PMM0062</i>	<i>PMT1590</i>	<i>gsr1874</i>	<i>SYNW0334</i>	Al2906520
<i>psbY</i> : small membrane-spanning protein	<i>smi0007</i>	<i>asr7025</i>	yes	yes	<i>tsr0836</i>	<i>PMM1117</i>	<i>PMT1048</i>	no	<i>SYNW0898</i>	Al1967740
<i>psbZ</i> : small membrane-spanning protein	<i>slr1281</i>	<i>asr3992</i>	yes	yes	<i>tsr1967</i>	<i>PMM1644</i>	<i>PMT0080</i>	no	<i>SYNW0081</i>	chloroplast
<i>psb27</i> : 11 kDa extrinsic protein	<i>slr1645</i>	<i>alr1258</i>	yes	yes	<i>tlr2464</i>	<i>PMM0507</i>	<i>PMT1260</i>	no	<i>SYNW1772</i>	Al1903600 Al1905385
<i>psb28</i> : 13kD extrinsic protein	<i>slr1398</i>	<i>alr0801</i> <i>alr1082</i>	yes	yes	<i>tlr0493</i>	<i>PMM0926</i>	<i>PMT0604</i>	<i>gsr0928</i>	<i>SYNW1065</i>	Al4928660

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D1 subunit is not stable (Swiatek et al., 2003). Further supporting the connection between D1 stability and Cyt b_{559} is evidence from Lupinkova and colleagues (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 cytochrome b_{559} hemes in a his-tagged PS II preparation, while Kashino and colleagues (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

Table 1, continued. '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 (Institute, 2002; 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 TIGR *Arabidopsis* database (Research, 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.

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 oxygen evolution activity and caused the HP cytochrome to convert to LP. Mizusawa and colleagues 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 (Mizusawa et al., 1997).

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). 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 (Ferreira et al., 2004; Kamiya and Shen, 2003; Zouni et al., 2001), 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 slow build up of reducing equivalents on the acceptor

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1 side during illumination. In this model, the acceptor
2 side of PS II is oxidized by LP Cyt b_{559} and the HP
3 form reduces the donor side. A major difference
4 between this model and the Chl Z quenching model
5 is that it is best applied under limited photo-stress as
6 opposed to constant illumination.

7 Both models incorporate Cyt b_{559} into PS II activity
8 as part of an alternate electron pathway. It appears
9 evident that Cyt b_{559} is not required for water oxida-
10 tion, even though it is important in the function
11 of PS II under physiological conditions. A recent
12 study by Morais and colleagues (Morais et al., 2001)
13 used mutations in the heme-binding site of Cyt b_{559}
14 in *C. reinhardtii* to show that redox activity of the
15 cytochrome is not necessary for O₂ evolution from
16 PS II. The authors mention in the discussion that there
17 could be a very low level of heme binding in vivo
18 which would allow for the small number of assembled
19 PS II centers in their mutant (Morais et al., 2001).
20 It is unclear if there is normal PS II activity in the
21 complete absence of a Cyt b_{559} heme. Much progress
22 has been made towards speculating the role of Cyt
23 b_{559} in PS II, which allows for grand debates. Further
24 studies are required to determine how the presence
25 of Cyt b_{559} and its redox contributions influence the
26 activity of PS II (Chapter 12).

27 *B. PsbH*

30 The PsbH subunit has a molecular weight in the range
31 of 6–10kDa. It was identified in PS II preparations
32 from higher plants (spinach and wheat), the green
33 alga *C. reinhardtii*, and the cyanobacteria *T. vulcanus*
34 by N-terminal sequencing (Hird et al., 1986; Michel
35 and Bennett, 1987; Dedner et al., 1988; Koike et
36 al., 1989). The *psbH* gene was identified based on
37 sequence homology in *Synechocystis* 6803 (Abdel-
38 Mawgood and Dilley, 1990) and more recently PsbH
39 was identified as a protein in *Synechocystis* 6803
40 thylakoids by MALDI/MS analysis (Abdel-Mawgood
41 and Dilley, 1990; Szabo et al., 2001; Kashino et al.,
42 2002a). Using a mutant with a His-tag at the N-ter-
43 minus of the PsbH protein, Buchel and colleagues
44 (Buchel et al., 2001) investigated the location of the
45 PsbH protein in *C. reinhardtii* PS II by gold label-
46 ing the protein using a Ni-NTA linker. Their results
47 indicate a position close to the two transmembrane
48 helices of Cyt b_{559} . Although they were unable to
49 identify cross-linked products between PsbH and Cyt
50 b_{559} , PsbH did cross-link to PsbX, which has been
51 shown to be close to Cyt b_{559} . More recently, PsbH

53 was assigned to a helix adjacent to PsbX in the PS II
54 structure (Ferreira et al, 2004).

55 The *psbH* gene is co-transcribed with the Cyt b_6f
56 complex genes *petC* and *petA* in higher plants, but is
57 monocistronic in *Synechocystis* 6803 (Mayes et al.,
58 1993). In *C. reinhardtii*, the *psbB*, *psbT* and *psbH*
59 genes are located in the same gene cluster; however,
60 deletion mutagenesis showed that *psbB/T* and *psbH*
61 are transcribed separately since disruption at either
62 locus had no effect on the other (Summer et al., 1997).
63 A similar *psbB/T/H* cluster is also found in some cya-
64 nobacteria such as *G. violaceus*, suggesting that the
65 cluster may reflect an ancient gene arrangement.

66 In spinach and *C. reinhardtii*, PsbH undergoes light
67 dependent phosphorylation at a threonine residue
68 (position 2 or 3 in the mature peptide depending
69 on the species) on the stromal side of the thylakoid
70 membrane, which is usually followed by a region of
71 basic amino acids (Dedner et al., 1988; Michel and
72 Bennett, 1987). Recent mass spectroscopic analyses
73 of higher plant thylakoid proteins have identified
74 two phosphorylation sites on PsbH. In *Arabidopsis*,
75 PsbH is phosphorylated at Thr2 and Thr4 (Vener
76 et al., 2001). Gomez and colleagues (Gomez et al.,
77 2002) also found direct evidence for a second phos-
78 phorylation site on PsbH from spinach and pea. In
79 their MS analysis, two 80Da adducts on PsbH were
80 identified, corresponding to a doubly phosphorylated
81 PsbH peptide. Additionally, they observed two 32Da
82 adducts associated with the doubly phosphorylated
83 PsbH peptide, which they hypothesize is the result
84 of oxidative modification.

85 Sundby and colleagues (Sundby et al., 1989)
86 reported that sodium bicarbonate has a negative ef-
87 fect on the phosphorylation of PsbH in spinach and
88 that depletion of endogenous bicarbonate stimulated
89 phosphorylation of PsbH. To account for the observed
90 competition between bicarbonate and phosphoryla-
91 tion, they suggest that basic amino acids may provide
92 a binding site for the bicarbonate anion.

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

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1 Deletion of *psbH* in *Synechocystis* 6803 results
2 in a mutant capable of photoautotrophic growth
3 at a reduced rate. O₂ evolution, fluorescence, and
4 thermoluminescence measurements indicate that
5 electron flow from Q_A to Q_B is impaired (Mayes et al.,
6 1993). It was concluded that PsbH is not required for
7 cyanobacterial PS II assembly and function in vivo,
8 but the polypeptide probably optimizes electron flow
9 by interacting with the Q_B site on the D1 protein. In
10 further analysis of the *psbH* deletion mutant, Ko-
11 menda and colleagues (Komenda et al., 2002) also
12 found weakened attachment of CP47 to the D1-D2
13 heterodimers as well as weakened binding of bicar-
14 bonate to the acceptor side of PS II.

15 Quite a different phenotype was observed in the
16 *C. reinhardtii* mutant lacking PsbH (O'Connor et
17 al., 1998; Summer et al., 1997). Although PS II core
18 proteins are translated and inserted into the thylakoid
19 membrane as in wild type cells, PS II centers do not
20 accumulate. The PS II deficiency is also observed in
21 dark grown cells suggesting the effect is not due to
22 photoinhibition. Therefore, PsbH plays an important
23 role in PS II assembly and stability in *C. reinhardtii*.
24 Interestingly, O'Connor and colleagues (O'Connor
25 et al., 1998) also found that the site-directed mutant
26 Thr3Ala of PsbH in *C. reinhardtii*, which eliminates
27 the identified phosphorylation site, has a phenotype
28 identical to wild type. A slight, but consistent decrease
29 in O₂ evolution activity was observed in Thr3Ala
30 compared to wild type. This may result from a slight
31 structural change in PsbH upon phosphorylation that
32 affects activity. Also, it is likely that another phos-
33 phosphorylation site exists in the *C. reinhardtii* protein
34 since two phosphorylation sites were identified in
35 higher plant PsbH.

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

C. PsbI

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

70 A *C. reinhardtii psbI* deletion mutant is capable
71 of photoautotrophic growth under low light condi-
72 tions, but not high light conditions. The amount of
73 assembled PS II centers and O₂ evolution activity is
74 10–20% that of wild type as determined by variable
75 fluorescence yield and steady state O₂ evolution
76 activities. Unlike other PS II-deficient mutants, the
77 high light sensitivity of the *psbI* deletion mutant
78 persisted when the mutant was grown on medium
79 containing acetate (Kunstner et al., 1995). Thus in
80 *C. reinhardtii*, PsbI is required for both assembly and
81 function of PS II.

82 A different phenotype was observed for the
83 *Synechocystis* 6803 *psbI* deletion mutant. The cya-
84 nobacterial mutant had a loss of only 25–30% PS II
85 activity compared to wild type. Other PS II proteins
86 accumulated to wild type levels with no significant
87 destabilization of PS II centers, and the *Synechocystis*
88 6803 mutant is only slightly more light sensitive than
89 wild type (Ikeuchi et al., 1995b). The conclusion from
90 the *Synechocystis* 6803 mutant indicates that PsbI is
91 not absolutely required for PS II assembly and func-
92 tion in cyanobacteria, but optimizes PS II activity.

93 PS II centers have not been isolated for further bio-
94 chemical analysis from either of the mutants described
95 above. Because of their more stable PS II centers, the
96 thermophilic cyanobacterium *T. elongatus* represents
97 a better system for isolating mutant PS II centers for
98 more detailed biochemical analyses. Katoh and Ikeu-
99 chi (Katoh and Ikeuchi, 2001a) created such a *psbI*
100 deletion mutant and found the isolated PS II centers
101 to be exclusively monomeric. The mutant also had
102 decreased light harvesting efficiency. These results
103 demonstrate that PsbI plays a major structural role

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1 in PS II dimer assembly and the functional assembly
2 of the antenna complex, but is not required for PS II
3 function in vitro.

4 *D. PsbJ*

5 The *PsbJ* subunit is encoded by the *psbJ* gene, a
6 member of the *psbEFLJ* operon that is conserved in
7 most oxygenic photosynthetic organisms. The *psbJ*
8 gene was determined to be the second open reading
9 frame closely downstream of the genes encoding the
10 subunits of Cyt b_{559} , and was given its name because
11 of a presumed association with PS II (Cushman et al.,
12 1988). The protein encoded by *psbJ* in *Synechocystis*
13 6803 was determined to be localized in the thylakoid
14 membrane and have a role in PS II activity (Lind et
15 al., 1993). *PsbJ* is predicted to be 4.1 kDa and is 40
16 amino acids long with 9 amino acid-long N/C-termi-
17 nal extensions flanking a single membrane spanning
18 helix. Even though *PsbJ* was predicted to be part of
19 PS II for many years, it was first seen in an isolated
20 PS II preparation in a recent study by Kashino and
21 coworkers (Kashino et al., 2002a). More recently, it
22 has been identified to be a component of the crystal-
23 lized PS II complex (Ferreira et al., 2004).

24 Mutations in *psbJ* have been made in both *Synecho-*
25 *cystis* 6803 (Lind et al., 1993) and tobacco (Hager et
26 al., 2002; Regel et al., 2001), which showed that *PsbJ*
27 is required for photoautotrophic growth in tobacco
28 but not in *Synechocystis* 6803. A major difference in
29 PS II activity between cyanobacterial and tobacco
30 $\Delta psbJ$ mutants is that the electron flow from Q_A^- to
31 Q_B is slowed by only 40% in the *Synechocystis* 6803
32 mutant and by almost two orders of magnitude in the
33 tobacco mutant (Regel et al., 2001). Single flash O_2
34 evolution experiments in both *Synechocystis* 6803 and
35 tobacco indicated that *PsbJ* is required for the stability
36 of the S_n -states of the Mn cluster in the O_2 evolution
37 apparatus (Regel et al., 2001). In the *Synechocystis*
38 6803 *psbJ* mutant, the decay of the S_3 state via back
39 electron flow from the acceptor side is twenty times
40 faster than in wild type cells (Regel et al., 2001). In
41 tobacco, young $\Delta psbJ$ leaves have slightly impaired
42 PS II driven photochemistry while mature leaves are
43 severely damaged (Hager et al., 2002), suggesting
44 that light-induced photodamage is accumulated in the
45 absence of *PsbJ*. The damage accumulated in mature
46 $\Delta psbJ$ tobacco leaves includes the loss of OE (oxygen
47 enhancer) proteins, but this appears to be a general
48 result of the damage and not directly resulting from
49 the absence of *PsbJ* since those proteins are properly
50 assembled in younger leaves. Like many of the other
51 small subunits of PS II, it is unclear what the exact
52 role of *PsbJ* is in the complex.

53 assembled in younger leaves. Like many of the other
54 small subunits of PS II, it is unclear what the exact
55 role of *PsbJ* is in the complex.

56 *E. PsbK*

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

81 Because *PsbK* remains bound to PS II after
82 removal of LHCII, but is not present in the PS II
83 reaction center core, it was concluded that *PsbK*
84 was not essential for PS II activity (Murata et al.,
85 1988). However, *PsbK* inactivation in *C. reinhardtii*
86 chloroplasts results in mutants that are unable to ac-
87 cumulate normal amounts of PS II and cannot grow
88 photoautotrophically. Growth rates returned to wild
89 type levels on acetate-containing medium. Pulse la-
90 beling experiments confirmed a PS II destabilization
91 in the mutant rather than a reduction in polypeptide
92 synthesis. This phenotype suggests that the *PsbK*
93 protein is necessary for PS II stabilization in vivo
94 (Takahashi et al., 1994).

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

5 A detailed biochemical analysis of the PS II centers
6 in these mutants was not possible, but recently
7 a deletion mutant in the thermophilic bacterium
8 *T. elongatus*, afforded such a study (Kato and
9 Ikeuchi, 2001a). The mutant phenotype was similar
10 to that observed for the *Synechocystis* 6803 mutant.
11 Cells were capable of photoautotrophic growth at
12 slightly, but reproducibly slower rates under various
13 temperature conditions. The O₂ evolution activity of
14 the PsbK-depleted PS II particles was comparable to
15 wild type at low concentrations of DCBQ. At higher
16 DCBQ concentrations, PsbK-depleted PS II particles
17 had a lower O₂ evolution activity (Kato and Ikeuchi,
18 2001a). A similar acceptor dependency was also
19 observed for PsbX in the same organism (Kato and
20 Ikeuchi, 2001b).

21 *F. PsbL*

22 Another member of the *psbEFLJ* operon is the gene
23 encoding the small PS II subunit, PsbL. It is predicted
24 to be 38 amino acids long with a single membrane
25 spanning helix and a 17-amino acid soluble N-ter-
26 minal extension. The PsbL helix is located near the
27 dimer interface with PsbT and PsbM in cyanobacteria
28 (Ferreira et al, 2004). A *Synechocystis* 6803 $\Delta psbL$
29 mutant lacks a functional PS II complex (Anbudurai
30 and Pakrasi, 1993; Kitamura et al., 1994; Ozawa et
31 al., 1997) while the tobacco mutant retains residual
32 PS II activity (Swiatek et al., 2003). Tobacco $\Delta psbL$
33 cannot grow photoautotrophically and is hypersensi-
34 tive to light (Swiatek et al., 2003). Additionally, the
35 PsbJ subunit is lost in the tobacco $\Delta psbL$ mutant, but
36 PsbJ does not seem to be required for the presence
37 of PsbL (Swiatek et al., 2003).

38 In vitro reconstitution of hydrophobic components
39 in a partially disintegrated PS II complex has only
40 been successful for PsbL protein (Nagatsuka et al.,
41 1991). When PQ-depleted PS II partial complex
42 consisting of CP47/D1/D2/PsbI/PsbW was recon-
43 stituted with PQ and PsbL, functional Q_A activity
44 was restored. Such reconstitution is specifically de-
45 pendent on the addition of PsbL (Kitamura et al.,
46 1994). Further analysis using recombinant PsbL
47 showed that the C-terminal part of PsbL is crucial
48 for recovering the Q_A electron transfer activity in the
49 reconstituted complexes (Ozawa et al., 1997). These

studies suggest that PsbL is necessary for electron
transfer from Q_A.

52 *G. PsbM*

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

73 *H. PsbN*

74 Based on a partial N-terminal sequence of a 4.7 kDa
75 polypeptide in isolated PS II preparation from *T. vul-*
76 *canus*, Ikeuchi and coworkers (Ikeuchi et al., 1989b)
77 named it the PsbN protein. The *psbN* gene is present
78 in cyanobacteria as well as the chloroplast genomes of
79 a number of plants and algae. Zouni and colleagues
80 (Zouni et al., 2001) have stated that the crystallized
81 PS II complex from *T. elongatus* has the PsbN protein,
82 although the primary data supporting this statement
83 has not been published yet. Subsequently, a helix
84 near PsbK was tentatively named PsbN in the most
85 recent PS II crystal structure (Ferreira et al, 2004).
86 In contrast, Kashino and coworkers (Kashino et al.,
87 2002b) have shown that the original assignment of the
88 PsbN protein in *T. vulcanus* was incorrect, and this
89 protein is actually PsbT_c (see below). Therefore, the
90 presence of PsbN in PS II remains ambiguous.

91 *I. PsbS*

92 The integral PS II subunit, PsbS, is a 22 kDa protein
93 found in plants and not in cyanobacteria. PsbS was
94 originally found as a coprecipitant in an antibody pull
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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 O₂ 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 27.

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 O₂ 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 (a) PsbT_c is closely associated with D1, and (b) PsbT_c may act as a scaffold for the replacement of photo-inactivated D1.

K. *PsbT_N*

The second PsbT protein is a ~5 kDa 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 luminal 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 luminal 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 and colleagues (Lorkovic

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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 spinach 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 and coworkers (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 thaliana* antisense mutant of PsbW with a >96% decrease in *psbW* transcript was generated by Shi and colleagues (Shi et al., 2000). The mutant contained no dimeric PS II supercomplex, but retained normal electron transfer properties. O₂ 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 CO₂ conditions. The mutant had a lower O₂ 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 (Kato 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 into 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 and coworkers (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 O₂ 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 O₂ evolution, indicating that it is not essential for photosynthesis and is not an important manganese ligand in the oxygen-evolving center (Meenam 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 *ycf9* 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 luminal 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 $\Delta psbZ$ 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 $\Delta psbZ$ mutants with different interpretations of the function of *PsbZ*. The mutant studied by Baena-Gonzales and coworkers (Baena-Gonzalez 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 O₂ evolution was unchanged in the $\Delta psbZ$ 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 $\Delta psbZ$ 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 and coworkers (Swiatek et al., 2001) indicates that as a connection between PS II and LHCII, *PsbZ* helps mediate non-photochemical 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

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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 *Synecho-*

cystis 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 review, 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.

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