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Review

# The low molecular mass subunits of the photosynthetic supracomplex, photosystem II

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**Abstract**

The photosystem II (PSII) complex is located in the thylakoid membrane of higher plants, algae and cyanobacteria and drives the water oxidation process of photosynthesis, which splits water into reducing equivalents and molecular oxygen by solar energy. Electron and X-ray crystallography analyses have revealed that the PSII core complex contains between 34 and 36 transmembrane α-helices, depending on the organism. Of these helices at least 12–14 are attributed to low molecular mass proteins. However, to date, at least 18 low molecular mass (<10 kDa) subunits are putatively associated with the PSII complex. Most of them contain a single transmembrane span and their protein sequences are conserved among photosynthetic organisms. In addition, these proteins do not have any similarity to any known functional proteins in any type of organism, and only two of them bind a cofactor. These findings raise intriguing questions about why there are so many small protein subunits with single-transmembrane spans in the PSII complex, and their possible functions. This article reviews our current knowledge of this group of proteins. Deletion mutations of the low molecular mass subunits from both prokaryotic and eukaryotic model systems are compared in an attempt to understand the function of these proteins. From these comparisons it seems that the majority of them are involved in stabilization, assembly or dimerization of the PSII complex. The small proteins may facilitate fast dynamic conformational changes that the PSII complex needs to perform an optimal photosynthetic activity.


*Keywords:* Small protein; PS2; Function; Arabidopsis; *Synechocystis*

## 1. Introduction

During the last few years, two important breakthroughs in the research field of photosynthesis have been made. First, the genomes of several photosynthetic organisms have been or are in the progress of being fully sequenced. Completely sequenced genomes include those of the higher plant thale cress, referred to as Arabidopsis (*Arabidopsis thaliana*) [1] and the prokaryote cyanobacterium *Synechocystis* sp. PCC 6803 (hereafter named *Synechocystis* 6803 [2]) and *Anabaena* sp. PCC 7120 [3]. Genomic sequencing of other photosynthetic organisms is in progress, including, inter alia, the important crop rice [4], the tree *Populus* [5] and the green alga *Chlamydomonas reinhardtii* http://baha-ma.jgi-psf.org/prod/bin/chlamy/home.chlamy.cgi). The second scientific advance related to photosynthesis is the 3D structural modeling of several supra-membrane protein complexes involved in photosynthetic electron transport. The first large membrane protein complex whose structure was determined to 3-Å resolution was the reaction center from the purple bacterium *Rhodopseudomonas viridis* [6]. This was followed by the 2.5-Å structure of the photosystem I (PSI) complex from *Synechococcus elongatus* [7], and recently the first structural maps of photosystem II (PSII) were obtained, using both electron and X-ray crystallography, from spinach and cyanobacteria [8–11]. These two important breakthroughs have helped usher in the post-genomic era, in which large scale genome-wide screening, prediction and crystal structure analyses can be contemplated, and indeed have already been initiated (see, for instance Refs. [12–14]).

Regardless of the technique or method used to understand the structure and function of supraprotein complexes, one group of proteins that creates special problems is the

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low molecular mass proteins. In this review we define low molecular mass proteins as polypeptides with molecular masses of less than 10 kDa, corresponding to 90 or less amino acid residues. A search through the SWISS-PROT and TrEMBL databases revealed that about 7% of all protein entries have molecular masses of less than 10 kDa. A more specific screen of the Arabidopsis genome revealed that at least 1245 proteins belong to this class, accounting for 4–5% of the total number of proteins in the organism (B.J. Haas, personal communication). Small proteins are found in all kinds of organisms, in all cellular fractions, and they are normally highly conserved between related organisms. This group of proteins is often missed, neglected or even sometimes ignored, reflecting fundamental difficulties, as well as the scope for error or confusion when dealing with them. For instance, genes coding for small proteins are easily overlooked in genomic sequence analysis, or ignored as short raw sequence fragments of other genes or pseudogenes, since most gene prediction programs are not yet properly tuned for the identification of short proteins. Another difficulty appears when visualizing and analyzing these small proteins with conventional biochemical methods such as SDS-PAGE. Many small proteins cannot be detected clearly on ordinary polyacrylamide gels as they migrate close to the dye front. Thus, high-resolution gel systems have to be employed. Since many of the small subunits are integral membrane proteins that are difficult to solubilize, even two-dimensional electrophoresis is not sufficient to resolve them. Furthermore, low molecular mass proteins often diffuse away from gels or PVDF membranes during staining, if no pre-fixation is performed. In addition, some small proteins lack basic amino acid residues and therefore do not stain well during conventional Coomassie or silver staining procedures. Therefore, more careful work needs to be done to identify additional small proteins and to discover their localization and function.

In this review we discuss the low molecular mass proteins of one supramembrane protein complex, PSII in plants and cyanobacteria. The issues we address are the possible reasons that PSII contains so many low molecular mass subunits, and their possible functions.

## 2. The PSII core complex

The PSII complex is located in the thylakoid membranes of higher plants, algae and cyanobacteria and drives the oxidation of water during photosynthesis, in which water is split into reducing equivalents and molecular oxygen by solar energy. This reaction has had a profound influence on the biosphere, ultimately enabling the development of aerobic life forms on Earth. For specific discussions on photosynthetic oxygen evolution and the biogenesis and assembly of PSII, see recent reviews [15–17]. The PSII complex, which has a total mass of between 450 and 700 kDa (depending on the organism, and preparation procedures used [18]), is composed of about 30 protein subunits, some encoded in the nucleus and others in the chloroplast.

Using electron crystallography, structural information on the PSII supramolecular organization has been obtained from spinach [18] and *S. elongatus* [18]. In addition, structural information has been obtained from *A. thaliana* [19] and *Prochloron didemni* [20]. There seems to be a consensus that the PSII core monomer of higher plants contains 34 transmembrane α-helices and that cyanobacterial PSII contains two additional α-helices. The finding that the latter has 36 transmembrane α-helices comes from X-ray crystallographic data on the PSII complexes of *S. elongatus* [10] and *Thermosynechococcus vulcanus* [11] at 3.8- and 3.7-Å resolution, respectively. Fig. 1 shows a 3D structural model of PSII core dimer from spinach with 34 transmembrane α-helices derived by electron crystallography. Excluded from this core model are all the major (LHCII) and minor antenna (CP24, CP26 and CP29) proteins which are detached during extraction, if special care is not taken to keep them attached [19]. Also excluded from the PSII core is the PsbS protein, predicted to have four transmembrane helices, which seems to be the last subunit to be detached from the PSII core because it is absent in the LHCII–PSII supercomplexes [21]. However, there have been some recent

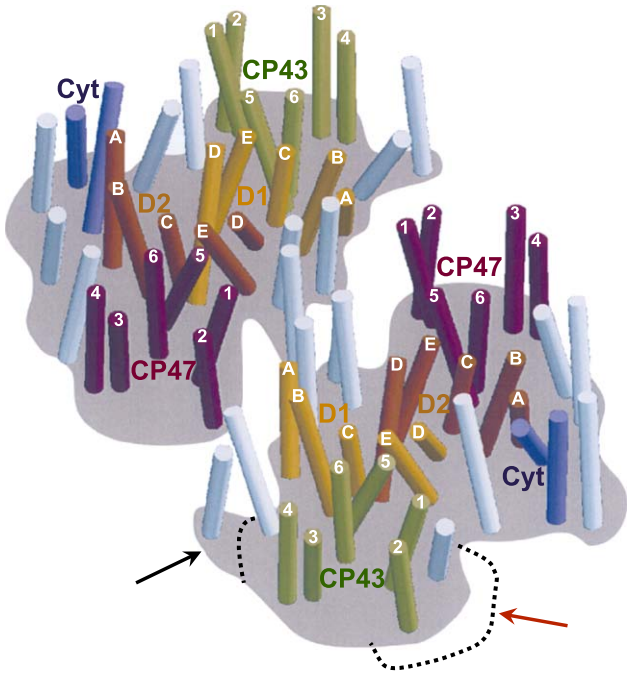


Fig. 1. 3D structural model of the PSII core dimer of spinach derived by electron crystallography (showing an oblique view). Reaction center proteins D1 and D2 are shown in yellow and orange, while inner antennae proteins CP43 and CP47 are shown in green and purple. The white and blue (Cyt $b_{559}$) coded α helices represent the small subunits of the PSII core complex. The area marked by the black arrow is missing in the cyanobacterial PSII core, and the red arrow indicates the area where three additional helices are found in cyanobacteria compared to the higher plant spinach. Reprinted by permission of Federation of the European Biochemical Societies from Ref. [132].

reports that the PsbS protein could occur in the PSII dimer [22]. For a detailed discussion on the location of the PsbS protein, see Ref. [23]. These observations indicate that in the PSII core from higher plants, the reaction center proteins D1 and D2 each provide five α-helices, and the core antennae CP43 and CP47 six α-helices each, accounting for 22 α-helices in total, and suggesting that 12 α-helices are provided by low molecular proteins. This implies that roughly 35% of the transmembrane α-helices in the PSII core are components of small transmembrane α-helix proteins. Fig. 1 illustrates the large amount of α-helices contained in a PSII dimer. The transmembrane helices in PsbE and PsbF subunits of cytochrome $b_{559}$ are shown in blue, while the helices of other low molecular mass proteins are shown in white. Also shown in Fig. 1 (lower monomer) are the two regions of the PSII core that seem to differ between higher plants and cyanobacteria. The transmembrane helix marked by a black arrow is missing in the cyanobacterial PSII core, while the red arrow marks the area where three additional transmembrane helices are found in cyanobacteria [8]. Nevertheless, there are at least 10 helices to be assigned in higher plants and at least 12 in cyanobacteria, since only the PsbE and PsbF subunits of cytochrome $b_{559}$ have been unambiguously localized in either of these kinds of PSII complexes. It is not clear at the moment whether this difference between higher plants and cyanobacteria is due to structural differences between the two PSII core complexes, or merely to some differences in stability that cause some proteins to be lost when extracting the PSII core from higher plants.

## 3. Low molecular mass proteins in PSII

The first evidence for low molecular mass subunits associated with the PSII complex was presented by Ljungberg et al. [24] who isolated a hydrophilic 5-kDa protein (corresponding to PsbTn) from PSII and showed that the PSII complex contained several other small, unidentified subunits. They were named, according to their apparent molecular masses, the 10-, 8-, 7-, 6.5-, 5.5-, 5- and 4-kDa proteins. Henrysson et al. [25] added the 6.8-, 3.7- and the 3.2-kDa proteins to the list of small subunits in PSII. To date, there are now 12 known polypeptides, with molecular masses ranging from 3.2 to 5.9 kDa in PSII. Due to their similar, small sizes, it is difficult to separate them from each other by means of one-dimensional electrophoresis, even with high-resolution gel systems. This is further confounded because their apparent masses depend on the type of SDS-PAGE used. There is still a lack of specific antisera against some of these proteins, as they are too short to have strongly antigenic regions. This confusing situation led to discussion about whether some of these small polypeptides were really specific subunits or "only" fragments of larger proteins. However, micro-sequencing, which enabled better and faster protein identification, revealed that they are new, previously unknown proteins. Several of the low molecular mass subunits of PSII were sequenced [26–29] and the first corresponding gene products, PsbK [30] and PsbI [31,32], were identified. The protein nomenclature was correlated to the gene names to avoid the confusing names related to the apparent molecular masses of the proteins.

Dramatic improvements and developments have been made in protein detection and identification techniques during the last decade. Highly sensitive fluorescence techniques combined with the latest improvements in ion mass spectrometric techniques, which allow the detection and identification of some femtomoles of proteins (roughly corresponding to 0.1 ng), now complement the traditional Coomassie brilliant blue staining. These new techniques have confirmed that PSII contains a large set of low molecular mass subunits [13,14]. Molecular biological methods have also yielded new insights into this group of PSII subunits.

In the following sections, the structural and functional aspects of 16 PSII subunits with masses below 10 kDa are summarized and described. With respect to protein sequences, we have chosen two model organisms, Arabidopsis and *Synechocystis* 6803, since the genomes of both of these organisms have been fully sequenced and are freely available on-line at the addresses http://www.kazusa.or.jp/cyano/cyano.html and http://www.tigr.org/tdb/e2k1/ath1/. It should be noted that protein and gene sequences for many of the low molecular mass subunits, from many other species, are also available. For a quick overview and sequence comparison between the two model organisms with respect to low molecular mass proteins, see Figs. 2 and 3, while the functions of all low molecular mass proteins of PSII are summarized in Table 1.

### 3.1. PsbE and PsbF

Cytochrome $b_{559}$ (Cyt $b_{559}$) is encoded by the chloroplast genes *psbE* and *psbF* and is comprised of two low molecular mass polypeptides, α and β subunits, with molecular masses of 9 and 4 kDa, respectively. Each of the polypeptides has one transmembrane helix. It is widely accepted that the holocytochrome $b_{559}$ is a heterodimer comprising one PsbE subunit, one PsbF subunit, and a heme cofactor [33]. Two histidine residues from each α and β subunit coordinate the heme [33]. X-ray crystallography has confirmed the presence of such a heterodimer in the PSII complexes [10,11]. The Cyt $b_{559}$ is closely associated with PSII in all oxygenic photosynthetic organisms. The α and β subunits of the Cyt $b_{559}$ are components of the minimal PSII reaction center complex that is still capable of primary charge separation [34]. Cyt $b_{559}$ is a b-type cytochrome, but it has a unique feature with respect to its midpoint reduction potential (Em). High-, low- and intermediate-potential forms of Cyt $b_{559}$ have been observed in intact chloroplasts, PSII membranes and isolated D1/D2/Cyt $b_{559}$ complexes [33]. The function of Cyt $b_{559}$ has been


Fig. 2. Low molecular mass subunits of PSII from *Synechocystis* 6803. Stroma: polypeptides with their N-terminus in the stromal side of the thylakoid membrane. Lumen: polypeptides with their N-terminus in the lumenal side. Accession numbers are indicated in parentheses. Transmembrane helices are underlined. Positively charged amino acid residues (H, K and R) are shown in red; negatively charged amino acid residues (D and E) in black; polar amino acid residues (S, T, N and Q) in pink; hydrophobic amino acid residues (A, V, L, I, W, Y, F and M) in green, and other amino acid residues (G, P, and C) in blue.

perplexing researchers for a long time, and numerous suggestions for its role in PSII have been made (for a review see Ref. [33] and references therein). Although Cyt $b_{559}$ is a redox-active protein, it is unlikely to be involved in the primary electron transport in PSII due to its very slow photooxidation and photoreduction kinetics. However, progress in the characterization of Cyt $b_{559}$ polypeptides, the physical properties of the heme, the electron transport reactions involving Cyt $b_{559}$ and, more recently, structural details suggest that Cyt $b_{559}$ could participate in a secondary electron transfer pathway within PSII [35]. These observations and the results of other studies on Cyt $b_{559}$ mutants indicate that Cyt $b_{559}$ is involved in protecting PSII from photoinhibition [33,37]. In addition, evidence from gene deletion studies and site-directed mutagenesis in *Synechocystis* 6803 suggests that Cyt $b_{559}$ is required for the assembly of functional PSII complexes [38–40]. In PsbE null mutants of *C. reinhardtii*, no PSII activity could be detected [36]. The absence of PSII activity was found to be due to the loss of PSII complexes, and the reduced amount of protein was probably due to posttranscriptional down-regulation of the PSII reaction center proteins. Inactivation of PsbE or PsbF gene in higher plant tobacco also abolishes PSII [41]. In summary, both PsbE and PsbF are essential components for PSII assembly, and they are probably involved in electron transport mechanisms that help to protect PSII from photodamage.

### 3.2. PsbH

The PsbH protein was originally named 10- or 9-kDa phosphoprotein in higher plant chloroplasts [42]. It is encoded by the plastome in algae and higher plants. PsbH is also present in cyanobacteria, where it exhibits 56% amino acid identity with the corresponding protein from Arabidopsis. The protein contains 63–90 amino acids, depending on the species, with molecular masses between 7.0 and 9.9 kDa. The protein from *Synechocystis* 6803 contains only 63 amino acids, and the truncation, with respect to homologous forms, is at the N-terminus of the protein. In higher plants, the phosphorylation sites of PsbH are located at the N-terminus [43]. Recently, it has been demonstrated by mass spectrometric analysis that a form of PsbH that can be doubly phosphorylated is modified at two threonine residues near the N-terminus [44]. A major consequence of the truncation of the PsbH in *Synechocystis* 6803 is that at least the N-terminal phosphorylation sites found in higher plant PsbH are not available, so it is an open question whether *Synechocystis* 6803 PsbH may be phosphorylated at an additional site. In higher plants and green algae, reversible phosphorylation is light-dependent and redox-controlled [45]. A site-directed PsbH mutant from *C. reinhardtii* lacking one of the phosphorylation sites is able to grow photoautotrophically and displays similar PSII biochemical and biophysical activities to that of wild type [46]. Hence, the role of phosphorylation of PsbH remains to be fully elucidated.

PsbH is an intrinsic membrane protein with a single transmembrane helix and its N-terminal region has been suggested to be exposed to the stromal side of the thylakoid membrane [43]. Using a His-tagged PsbH mutant and gold labeling, combined with electron micrographic and cross-linking studies, it has been revealed that the N-terminus of the PsbH protein is located close to Cyt $b_{559}$ [47]. Another near neighbor of PsbH could be the PsbX protein [47]. A replacement of *Synechocystis* 6803 PsbH (7.0 kDa) with the

Table 1
Mutants and functions of low molecular mass proteins of PSII

| Protein | Mutant from prokaryote cyanobacteria | Mutant and antisense line from eukaryote Arabidopsis/*Chlamydomonas*/tobacco | Function |
|---|---|---|---|
| PsbE/F | No photoautotrophic growth<br>Barely detectable levels of the D1 and D2 protein | No photoautotrophic growth<br>No PSII activity<br>No PSII complexes | PSII assembly at early steps<br>Photoprotection |
| PsbH | Slower photoautotrophic growth<br>Slower electron transfer from $Q_A$ to $Q_B$<br>Slower recovery from photoinhibition<br>Less stable PSII core complexes<br>Destabilized bicarbonate biding to PSII | No larger functional PSII complexes<br>No PSII activity | PSII stabilization and assembly<br>Electron transport at acceptor side of PSII<br>Photoprotection, Bicarbonate binding |
| PsbI | Photoautotrophic growth, Less oxygen production (70–80% of WT), Slightly more sensitive to light, No PSII dimer isolated | Photoautotrophic growth only at low light,<br>Much less oxygen production (only 10–20% of WT), Less PSII,<br>Sensitive to high light | PSII dimerisation/stabilization, Maintenance of PSII structure and function under high light |
| PsbJ | Slower photoautotrophic growth<br>Reduced oxygen evolution<br>Electron transfer from $Q_A$ to $Q_B$ impaired<br>Reduced amount of functional PSII | No photoautotrophic growth<br>Hypersensitive to light<br>Low efficiency in PSII electron transfer<br>Loss of PsbP | Assembly of water splitting complex<br>Involved in electron transfer within PSII |
| PsbK | Slightly reduced growth rate and electron transport | No photoautotrophic growth<br>Only 10% of PSII left<br>No PSII activity | Plastoquinone binding<br>Maintaining PSII dimeric form |
| PsbL | No photoautotrophic growth,<br>No oxygen evolution,<br>No herbicide binding | No photoautotrophic growth,<br>Pale green leaves when grow in sucrose-containing media,<br>No or residual photosynthetic activity,<br>Monomeric PSII | Donor side electron transfer, Assembly of PSII,<br>Maintaining PSII dimeric form |
| PsbM | Photoautotrophic growth<br>Normal PSII activity<br>Faster doubling time grown at very low light | No mutants reported | Unknown |
| PsbN | Deleted together with PsbH<br>Photoautotrophic growth<br>No additional changes in PSII | No mutants reported | Might not be a ubiquitous PSII subunit |
| PsbR | The protein does not exist | No change in plant morphology<br>Retardation of $QA^-$ re-oxidation<br>Lower oxygen evolution rate | pH-dependent stabilising protein for PSII<br>Docking protein for PSII extrinsic proteins |
| PsbTc | Photoautotrophic growth<br>Normal oxygen evolution<br>Decrease in PSII dimers | Photoautotrophic growth<br>Sensitive to high light | Recovery of photodamaged PSII<br>PSII dimerization/stabilization |
| PsbTn | The protein does not exist | No mutants reported | Unknown |
| PsbW | The protein does not exist | Photoautotrophic growth<br>Less PSII reaction center<br>No PSII dimer isolated<br>More vulnerable to high light and slower recovery from photoinhibition | PSII dimerization<br>Photoprotection |
| PsbX | Photoautotrophic growth<br>Normal oxygen evolution<br>Less amount of PSII<br>Growth defect at low $CO_2$ | No mutants reported | Binding or turnover of quinone molecules at $Q_B$ site |
| PsbY | Photoautotrophic growth<br>Near normal oxygen evolution, electron transport and PSII content | No mutants reported | Unknown |
| PsbZ | Photoautotrophic growth<br>Slightly reduced PSII complexes | Photoautotrophic growth<br>Dwarf phenotype under low light<br>Decrease in Chlorophyll content<br>Reduced amount of CP26<br>No PSII–LHCII supercomplexes isolated | Linker between LHCII and PSII core |

maize homolog (7.7 kDa) resulted in slight conformational modification of the $Q_B$ pocket of the D1 polypeptide, suggesting that there is a close structural interaction between the D1 protein and PsbH [48]. PsbH is already present in etiolated tissue [49], indicating that the protein may be involved in early stages of PSII assembly.

The possibility that PsbH has a role in regulating $Q_A$ to $Q_B$ electron transfer was suggested by Packham [50].

Inactivation of the *psbH* gene in *Synechocystis* 6803 [51] led to a phenotype that can grow photoautotrophically, but has a slower growth rate and slightly higher sensitivity to light than the wild type. This gene inactivation did not block the formation of PSII complexes, but the electron transfer from $Q_A$ to $Q_B$ was impaired [51] due to the slower recovery from photoinhibition in the mutant [52]. Recent studies on the PsbH mutant indicate that PSII core complexes are less stable in the deletion mutant than those of the wild type, and deletion of PsbH has inhibitory effects on bicarbonate binding to PSII [53].

Disruption of the *psbH* gene in *C. reinhardtii* caused a PSII-deficient phenotype with no detectable functional PSII complexes or PSII activity [46,54], apparently due to severely impaired formation of high molecular weight forms of PSII [54].

Biochemical data obtained from PSII complexes isolated from spinach suggest that PsbH, together with other PSII phosphoproteins, may be required for D1 protein turnover by regulating dimeric and monomeric PSII transition through their phosphorylation and dephosphorylation [55]. This has been further substantiated by the recent demonstration that PsbH is required for the rapid degradation of the photodamaged D1 protein and insertion of newly synthesized D1 into the thylakoid membrane [56]. In summary, the PsbH protein is essential for PSII activity in eukaryotes. It may play a role in regulating PSII assembly/stability and may also be involved in repair of photodamaged PSII under high light.

### 3.3. PsbI

The PsbI protein, previously named the 4.8-kDa protein, is encoded by the plastome. PsbI is a universal component of PSII and is highly conserved (e.g. there is 71% amino acid identicality between the Arabidopsis and *Synechocystis* 6803 proteins). The protein contains 36 to 38 amino acids in most species, with molecular masses ranging between 4.1 and 4.5 kDa. Sequence analysis indicates that it has a single transmembrane span. The N-terminus of this protein is formyl-Met [57] and is located on the stromal side of the thylakoid membrane [58]. It has been found to be tightly associated with the D1/D2 heterodimer [31, 32], and analysis of its cross-linked proteins indicates that the nearest neighbors of PsbI are D2 and the α subunit of Cyt $b_{559}$ [58].

The function of PsbI has been investigated in *C. reinhardtii*, *Synechocystis* 6803 and *Thermosynechococcus elongatus* (formerly *Synechcoccus*) strain BP-1 [59–61]. Inactivation of the *psbI* gene in *Synechocystis* 6803 and *T. elongatus* strain BP-1 caused similar reductions in PSII activity. The *ΔpsbI* mutants can grow photoautotrophically, but their PSII oxygen evolution activity is only 70–80% of wild-type levels [60,61]. The *Synechocystis* 6803 *ΔpsbI* mutant is also slightly more sensitive to light than the wild type [61], and in the case of *T. elongatus ΔpsbI* mutant, no dimeric PSII could be isolated [60]. It was suggested that while PsbI is not essential for PSII photochemistry in cyanobacteria, it may be involved in processes that help to optimize PSII function, such as the dimerization of PSII or stabilization of PSII dimers [60,61].

Deletion of the *psbI* gene in *C. reinhardtii* caused a more severe disruption in PSII [59] compared to that in cyanobacteria [60,61]. Although the *C. reinhardtii ΔpsbI* mutant can grow photoautotrophically under dim light, its growth rate is extremely sensitive to high light. Both the number of PSII units and the oxygen evolution activity from the mutant were found to be only 10–20% of wild-type levels. PsbI in *C. reinhardtii* may be involved in assembly and stabilization of PSII, especially in the maintenance of normal PSII structure and function under high light [59]. The possibility cannot be ruled out that PsbI is also involved in the regulation of electron transfer within PSII in green algae and higher plants.

### 3.4. PsbJ

The plastidic *psbJ* gene is located in a gene cluster together with *psbE*, *psbF* and *psbL* in most photosynthetic organisms. PsbJ is one of the most hydrophobic proteins in the thylakoid membrane, containing 39–42 amino acid residues, with a molecular mass between 4.0 and 4.4 kDa. Sequence analysis suggests that the protein has a single transmembrane helix with its N-terminus extending to the stromal side. The protein from *A. thaliana* displays 65% identity with that from *Synechocystis* 6803. The PsbJ protein has recently been demonstrated to be a bona fide component of PSII in the cyanobacterium *Synechocystis* 6803 [14,62]. However, the protein has not yet been identified in PSII preparations from higher plants, or from the thermophilic cyanobacterium *T. vulcanus* [63] despite the fact that the gene cluster *PsbEFJLJ* is present and is highly conserved among higher plants and cyanobacteria.

Mutants lacking the PsbJ protein (*ΔpsbJ*) have been generated in both *Synechocystis* 6803 and tobacco [62,64–66], and the effects of the mutations were greater in the latter. For example, *Synechocystis* 6803 *ΔpsbJ* could grow photoautotrophically, although its growth rate was slower than wild type [64]. In contrast, the tobacco *ΔpsbJ* mutant was not able to grow photoautotrophically at all [66].

In *Synechocystis* 6803, inactivation of the *psbJ* gene caused a decrease in PSII oxygen evolution [62,64]. The reason for this low activity was suggested by Lind et al. [64] to be related to the reduced amount of functional PSII. It can be concluded that the PsbJ protein may not be essential for photochemical activity of PSII, but it may be directly or indirectly involved in the efficient assembly and/or maintenance of the PSII complexes in the thylakoid membrane in *Synechocystis* 6803 [64].

When the tobacco *ΔpsbJ* mutant was grown on synthetic medium under standard light conditions, young leaves were green, while mature leaves were white. However, under low light, no photobleaching of the leaves was observed, indi-

cating that the *ΔpsbJ* mutant is hypersensitive to light [66]. The lifetime of the reduced primary acceptor $Q_A^-$ was more than 100-fold higher in the tobacco *ΔpsbJ* mutant than in the wild type, apparently because both the forward electron flow from $Q_A^-$ to $Q_B$ and the electron flow back to the oxidized Mn cluster of the donor side are impaired in this mutant [62]. These results are consistent with the finding that the extrinsic PsbP protein, which is itself required to optimize the water-splitting process in PSII, is lost in the tobacco *ΔpsbJ* mutant [66]. Thus, the incomplete assembly of the water-splitting complex reduces photosynthetic performance [66]. In addition, PsbJ has a proposed involvement in the control of PSI accumulation through PSII activity [66].

### 3.5. PsbK

The chloroplast-localized *psbK* gene is present in both higher plants and cyanobacteria, and mature PsbK protein has been found in PSII core complexes from the thermophilic cyanobacteria, *Synechococcus vulcanus* [29] and *S. elongatus* [10], *Synechocystis* 6803 [14], the green alga *C. reinhardtii* [67,68] and higher plants [28,30,69]. The PsbK precursor contains a hydrophobic presequence ranging from 5 to 24 amino acids long, and some of the longer presequences have a putative transmembrane span that may play a role in the insertion of the protein in the thylakoid membrane. It is noteworthy that the putative orientation of the PsbK in the thylakoid membrane is opposite to that of most chloroplast-encoded, low molecular mass proteins such as PsbE, PsbF, PsbH, PsbI, PsbJ, PsbL, PsbM and PsbN. In all species examined so far mature PsbK protein contains 37 amino acids and has molecular masses between 4.1 and 4.3 kDa. Accordingly, it is the most conserved small protein in PSII. The Arabidopsis PsbK shares 76% identity with the *Synechocystis* 6803 homolog. PsbK has been recently found to be tightly associated with CP43 [70].

Inactivation of the *psbK* gene in *Synechocystis* 6803 resulted in only slightly reduced growth and electron transport rates [71,72] and in a decreased number of PSII reaction centers [71]. It is likely that PsbK is not crucial for PSII activity and assembly in *Synechocystis* 6803. However, deletion of the *psbK* gene in *Chlamydomonas* destabilized the PSII complex, and the amount of PSII in the resulting mutants was less than 10% of wild-type levels [73]. The transformants were unable to grow photoautotrophically and no PSII activity was detected, suggesting that this protein is indispensable for PSII function in *C. reinhardtii* [73]. The PsbK protein in *C. reinhardtii* was also found to be specifically bound to the CP43 kDa protein [67]. The divergence in results obtained with *C. reinhardtii* and *Synechocystis* 6803 indicates that the stability requirements of their photosynthetic complexes may differ [73]. Biochemical analysis of subcore complexes of PSII from spinach has shown that dimeric CP47–reaction center complexes contain the small proteins PsbK and PsbL, together with 1–2 molecules of plastoquinone, while monomeric complexes do not [69]. This is consistent with the data from the *C. reinhardtii ΔpsbK* mutant, since dimeric PSII is purported to be more stable and more resistant to proteolysis than monomeric forms. Therefore, it has been suggested that the PsbK protein is involved in binding plastoquinone and in maintaining the dimeric organization of PSII [69].

### 3.6. PsbL

The highly conserved PsbL protein is encoded by the plastome, and the corresponding gene is part of the *psbEFLJ* gene cluster in *Synechocystis* 6803 and higher plants [74]. The protein contains 37–39 amino acid residues with molecular masses between 4.3 and 4.5 kDa depending on the species. Primary sequence analysis suggests that PsbL has one transmembrane helix. PsbL from Arabidopsis has 69% identity to that from *Synechocystis* 6803. It has also been suggested that the N-terminus of the protein is located at the stromal side of the thylakoid membrane [75].

PsbL has been detected in PSII core complexes from higher plants [28] and cyanobacteria [14,69]. Analysis of protein compositions of monomeric and dimeric forms of the CP47–RC complexes shows that the PsbL protein is only present in the dimeric form, indicating that the PsbL protein may have a role in maintaining PSII dimeric organization [69].

Inactivation of the *psbL* gene in *Synechocystis* 6803 results in a total loss of PSII-mediated oxygen evolution, and mutants with inactive forms appear to be unable to grow photoautotrophically. Interestingly, no binding of herbicides that normally bind D1/D2 proteins was detected in these mutants, despite immunological detection of D1 and D2 protein. This was suggested to be due to changes on the acceptor side of PSII [74]. This finding was further explored in a set of experiments using either isolated PsbL or overproduced wild-type and mutated proteins of PsbL, and by reconstitution of plastoquinone-9 into PSII core complexes isolated from spinach. The conclusion from these experiments was that the PsbL protein could partially facilitate the rebinding of plastoquinone to restore $Q_A$ activity in the isolated PSII core complexes [76–78]. However, the cited group later suggested that PsbL was involved in the oxidation of TyrZ by $P_{680}^+$ on the donor side of $P_{680}$. The amino acid residues essential for this putative role are located in the carboxyl terminal part of the protein [75]. This interpretation is also supported by studies of PsbL mutants from tobacco. These plants were found to be unable to grow photoautotrophically, and if sucrose was added they only grew slowly with pale green leaves: no photosynthetic activity was detected in them [80]. Recently, PsbL was also shown to be required for the stable assembly of CP43 into PSII cores [65]. In summary, PsbL is essential for the normal function of PSII. Its deletion causes fatal damage to PSII in both cyanobacteria and tobacco plants, especially for donor side

electron transfer, PSII core assembly and maintenance of the dimeric form of PSII.

### 3.7. PsbM

The PsbM protein is encoded by the *psbM* gene in the plastome. It contains 31–38 amino acid residues, with calculated molecular masses between 3.5 and 4.2 kDa. The protein has been predicted to have one transmembrane helix [81,82]. The N-terminus of the protein from *Synechocystis* 6803 has been predicted to be located at the stromal side. Sequence analysis suggests that the PsbM protein is one of the most hydrophobic proteins in the thylakoid membrane. The PsbM protein from Arabidopsis shares 54% identity to that from *Synechocystis* 6803.

The PsbM protein has also been detected in PSII complexes isolated from *C. reinhardtii* [68], *Synechocystis* 6803 [83] and *S. vulcanus* [10,82]. The presence of this protein in PSII of *Synechocystis* 6803 [14] and pea [13] was recently confirmed using proteomic techniques. A *Synechocystis* 6803 mutant was created and found to be capable of photoautotrophic growth, and to have oxygen evolution rates comparable to those of wild type. However, the doubling time of the mutant cells was faster than WT when cells were grown at very low light intensities (Nugent and Bishop personal communication). Mutation studies of the PsbM protein in higher plants or *Chlamydomonas* have not yet been reported. Therefore, the function of this protein remains unknown.

### 3.8. PsbN

The PsbN protein, encoded by the plastome, contains 43 amino acids and has a molecular mass of 4.5–5.0 kDa in most species. The PsbN protein is conserved (49% identity between *Arabidopsis* and *Synechocystis* 6803). It has been predicted to be an intrinsic membrane protein possessing a single transmembrane helix [81,82] with the N-terminus extending into the stromal side.

The effects of deleting both PsbN and PsbH on PSII activity in *Synechocystis* 6803 were the same as those of solely deleting the PsbH protein, indicating that PsbN is not essential for photoautotrophic growth and normal PSII function [51]. No other functional studies of this protein have been reported.

No PsbN protein has been found in either the PSII fraction of *Synechocystis* 6803 [83], or purified His-tagged CP47-containing PSII complexes from *Synechocystis* 6803 [14].

In the first study of this protein, it was reported that PsbN was associated with PSII core oxygen-evolving complex from the thermophilic cyanobacterium *S. vulcanus* [82]. Recently, these data were also used for the evaluation of the PSII crystal structure by Zouni et al. [10]. However, the location of PsbN was reexamined by Kashino et al. [63] and it was found that this protein was not located in PSII. The PSII associated protein that originally was regarded as a gene product of an open reading frame (ORF43), hence the name *psbN* gene [82], is actually the *psbTc* gene product [63]. The reasons for this misassignment was that only a short N-terminal sequence of the protein was obtained at that time, that only limited sequence databases were available, and that these two proteins have some homology in the N-terminal region (see Figs. 2 and 3 and Ref. [63]). Thus, the only direct experimental data supporting that the PsbN protein is a PSII protein have been disposed [63]. Moreover, there is no direct evidence indicating the presence of the PsbN protein in PSII complexes from *Synechocystis* 6803 [14], algae and higher plants [13]. Therefore, it is not currently clear whether PsbN is a bona fide PSII component or not.

### 3.9. PsbR

The *psbR* gene is found only in the nucleus of green algae and higher plants. It encodes a 12.8–14.6-kDa protein precursor containing 126–141 amino acids. The presequence of PsbR (29 to 42 amino acids long, depending on species) is considerably shorter than that of other lumenal proteins (65 to 84 residues) and lacks the large hydrophobic domain in front of the processing site [79,84]. It has been suggested that the hydrophobic C-terminal region of the mature protein may serve as a non-cleavable transfer domain, which, together with the N-terminal transit peptide, directs the protein into the chloroplast and across the thylakoid membrane [79,84]. The percentage of identity between Arabidopsis and *Chlamydomonas* PsbR is 41%. The mature PsbR protein, previously named the 10-kDa polypeptide, contains 99 amino acids with a molecular mass of 10.3 kDa in most species. Predictions indicate that it contains a transmembrane span close to its C-terminus [84], and a long N-terminal tail with $\sim$ 70 amino acids.

PsbR could not be removed from the membrane by treatment with high salt concentrations unless detergent was included [85–87], indicating that it is an intrinsic protein. This hydrophobic property has been confirmed by its partition to the hydrophobic phase during purification and its solubility in aqueous solution in the presence of detergents [87]. The PsbR protein does not bind any cofactors [87]. Based on biochemical studies [85–87] and sequence analysis [79,84], the majority of the PsbR protein is purportedly located on the lumenal side of the thylakoid membrane.

It has been proposed that PsbR provides a binding site for the extrinsic PsbP (23 kDa) protein to the thylakoid membrane [87]. Sequence analysis shows that the N-terminal region of PsbR is highly charged, providing scope to form ion bridges with the extrinsic proteins. Thus, the charged domain together with the transmembrane span might make the PsbR protein function as a docking protein. However, it was found that when PsbS and PsbR proteins were removed from PSII, the extrinsic PsbO, PsbP and PsbQ proteins were

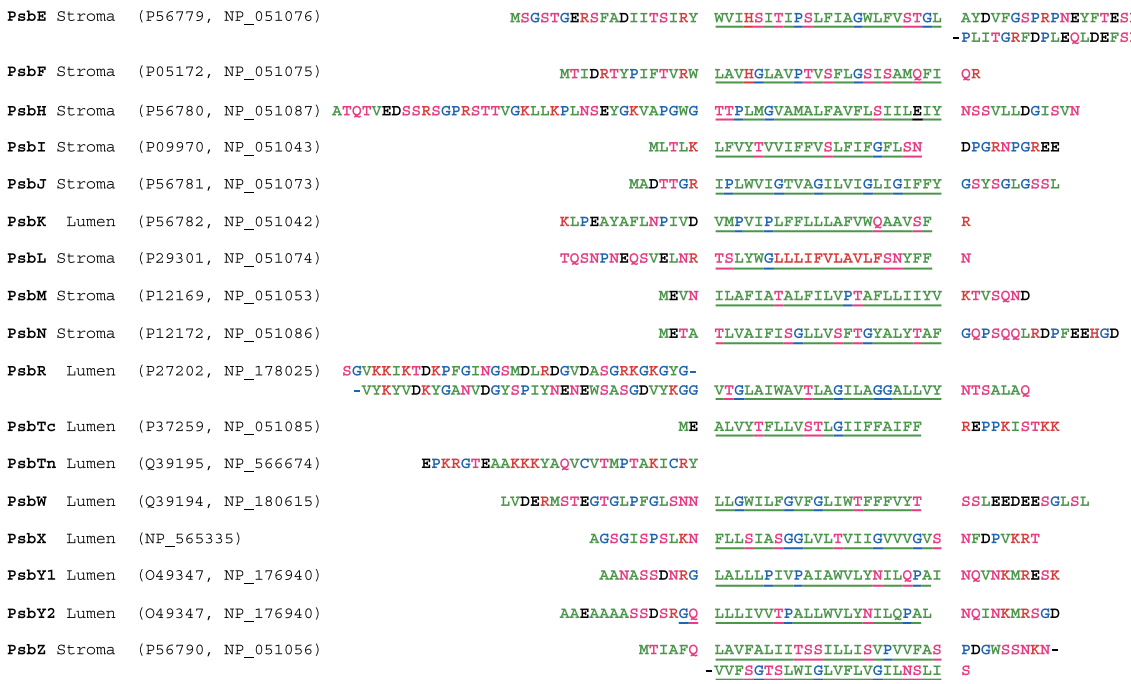


Fig. 3. Low molecular mass subunits of PSII from Arabidopsis. Stroma: polypeptides with their N-terminus in the stromal side of the thylakoid membrane. Lumen: polypeptides with their N-terminus in the lumenal side. Accession numbers are indicated in parentheses. Transmembrane helices are underlined. For color coding, see Fig. 2.

retained [85], suggesting that other factors are also involved in the docking of the PsbP protein.

*PsbR* mRNA accumulates upon light exposure and the PsbR protein was undetectable in etiolated spinach, in contrast to the expression patterns of the PsbO, PsbP and PsbQ extrinsic proteins [84]. The function of this protein has been systematically studied in transgenic potato [88]. The expression of the antisense RNA of *psbR* led to a drastic reduction in the PsbR protein level, but did not affect the content of other proteins. Furthermore, transformation with the *psbR* antisense construct did not change the plant morphology under either standard or limiting light conditions. However, deletion of PsbR caused retardation of the re-oxidation of $Q_A^-$ [88]. In addition, the steady state oxygen evolution of PsbR-deleted plants was lower and showed differences in pH dependence compared to wild type. It has been suggested that PsbR may be a pH-dependent stabilizing protein and function at both donor and acceptor sides of PSII [81,88].

### 3.10. PsbTc

The *psbTc* or *ycf8* gene is highly conserved among cyanobacteria, algae and higher plants. The lower case c in the name of this protein merely denotes that in higher plants and algae the protein is chloroplast-encoded, and is included solely because the name PsbT was used and published for another protein (PsbTn) simultaneously. The product of this gene contains 31–38 amino acids with molecular masses ranging from 3.5 to 4.4 kDa. The PsbTc protein in Arabidopsis shares ~49% identity with that in *Synechocystis* 6803. PsbTc is an intrinsic protein with a single transmembrane helix close to the N-terminus [69,89]. It has been proposed that the C-terminal tail of the protein is exposed to the stromal side of the thylakoid membrane. The predicted orientation of PsbTc, as well as PsbK, is similar to that of nuclear-encoded PsbW and PsbX, but opposite to that of other small, chloroplast-encoded PSII proteins.

PsbTc has been detected in *Synechocystis* 6803 [83], *C. reinhardtii* [89] and spinach [69]. PsbTc has been proposed to have an intimate association with the D1 and D2 proteins [69,90].

The *psbTc* gene has been disrupted in the thermophilic cyanobacterium, *T. elongatus* BP-1 [91]. The mutant can grow photoautotrophically. There are no differences in oxygen evolution rates of PSII between wild type and *ΔpsbTc*, but dimeric PSII complexes were found to be much less abundant in *ΔpsbTc* than in wild type. Therefore, PsbTc might be involved in the formation and/or stabilization of dimeric PSII complexes.

The function of this subunit has also been studied in a deletion mutant from *C. reinhardtii* [89,90]. The *ΔpsbTc* cells could grow photoautotrophically under standard conditions. However, the growth and PSII function of the mutants were impaired under high light conditions. Hence, it has been suggested that the protein is required for maintaining optimal PSII activity under adverse conditions, such as high light stress [89]. Recent studies further revealed that this chloroplast-encoded protein undergoes degradation

upon high light, but does not play a role in photoprotection. Instead, it is required for efficient recovery of photodamaged PSII [90]. It has been proposed that PsbTc in *C. reinhardtii* is involved in posttranslational event(s) during photoinhibition and repair cycles, for example during the replacement of damaged D1 protein [90].

### 3.11. PsbTn

The nuclear-encoded *psbT* gene (the corresponding protein is designated PsbTn hereafter) encodes an 11-kDa precursor protein in *Gossypium hirsutum* (Upland cotton) [92] and Arabidopsis. Neither a homologous gene nor the corresponding protein has been detected in *Synechocystis* 6803 [14]. PsbTn is a hydrophilic protein with an apparent molecular mass of 5.0 kDa according to polyacrylamide gel analyses, and was previously named the 5.0-kDa protein [24,28]. The protein has been purified and partially sequenced from PSII core complexes of spinach and wheat [24,28]. The mature protein contains only 28 amino acids with molecular masses of 3.0 kDa in *G. hirsutum* and 3.2 kDa in Arabidopsis, making it the smallest known polypeptide in the PSII complex to date. The identity between PsbTn in *G. hirsutum* and Arabidopsis is 68%.

A cDNA encoding the PsbTn precursor from *G. hirsutum* has been isolated and characterized [92]. Analysis of the deduced amino acid sequence suggested that the precursor of PsbTn bears a bipartite presequence characteristic of lumenal proteins. Import of the in vitro synthesized precursor into isolated chloroplasts confirmed this prediction. A stromal intermediate was detected, indicating that two-step processing occurs when the precursor is translocated across the chloroplast envelope and thylakoid membrane. No mutants have been reported and, thus, the function of this protein is unknown.

### 3.12. PsbW

The nuclear *psbW* gene in green algae and higher plants encodes a protein precursor that bears a transit peptide (59–83 amino acid residues in length depending on species) directing the protein into the chloroplast. The mature PsbW protein, previously known as the 6.1-kDa protein, is composed of 54 amino acid residues in spinach and Arabidopsis [93] and 56 amino acid residues in *C. reinhardtii* [94] with molecular masses of 5.9 and 6.1 kDa, respectively. The PsbW protein is predicted to have one transmembrane span [93,95], which has recently been experimentally confirmed [96]. The N-terminus of the protein was demonstrated to be located on the lumenal side and the C-terminus on the stromal side of the thylakoid membrane [93,95,97]. This could be a simple consequence of the insertion mechanism via the spontaneous pathway [98,99].

The PsbW protein has been found to be highly conserved in green algae and higher plants (there is about 50% identity between the Arabidopsis and *C. reinhardtii* homologs), but seems to be missing in cyanobacteria. However, a so-called ‘‘PsbW’’ has recently been reported from *Synechocystis* 6803, *Synechococcus* sp. PCC 7002 and several types of algae [2,100–105]. The genes encoding these proteins in algae are located in the cyanelle or chloroplast. Unlike the hydrophobic PsbW protein in higher plants, these ‘‘*Synechocystis* PsbW’’ forms are hydrophilic, and they are much larger (112–116 amino acid residues). The sequence identity between the PsbW protein in Arabidopsis and the ‘‘*Synechocystis* PsbW’’ protein is only 16% (Fig. 4A). To further confuse the situation in Arabidopsis, a soluble protein has been named PsbW-like protein (entry name: Q9M0G6), and the C-terminal domain of this protein has 44.2% identity to the ‘‘*Synechocystis* PsbW’’ protein (Fig. 4B). A molecular phylogenetic tree also clearly shows the close relationship between the Arabidopsis PsbW-like and the ‘‘*Synechocystis* PsbW’’ protein. Therefore, the ‘‘*Synechocystis* PsbW’’ protein may be the true ortholog of the PsbW-like protein in Arabidopsis. It is, however, reasonable to assume that the 13-kDa extrinsic ‘‘*Synechocystis* PsbW’’ has a totally different function from that of the eukaryotic 6-kDa membrane-spanning PsbW protein. Interestingly, a substoichiometric amount of the ‘‘*Synechocystis* PsbW’’ protein was detected in association with PSII complexes [83]. More recently, this 13-kDa protein was reported to be present in a highly active PSII preparation from *Synechocystis* 6803 with an apparent molecular mass of 10 kDa, and the authors designated the protein Psb28 [14].

The PsbW protein was found to be associated with PSII in spinach, wheat and *C. reinhardtii* [26,28,68,106]. It has been shown that the protein is tightly associated with the PSII reaction center because it could be detected in several types of PSII reaction center or subcore preparations [69,95,97]. The nearest neighbor of the PsbW protein was reported to be the large subunit of Cyt $b_{559}$ [47,95]. Hiyama et al. [107] claimed that the PsbW protein was a subunit of both PSII and PSI. However, it has been demonstrated that the PsbW protein is exclusive to PSII and was undetectable in any PSI preparations with antibodies raised against either N- or C-terminal regions of the PsbW protein [97,106] (and Scheller H.V., personal communication). Expression of the PsbW gene is light-regulated at both mRNA and protein levels in Arabidopsis [93,108] and is partly light-dependent in *C. reinhardtii* (C.L. Bishop, personal communication). The PsbW protein is present in etiolated spinach seedlings [93,108]. Its expression pattern during greening is similar to that of Cyt $b_{559}$, PsbS and the extrinsic PsbO protein, suggesting that the PsbW protein may be involved in the early steps of PSII biogenesis and assembly. The PsbW protein is organ-specific, being detectable in green organs, for example, leaves and shoots, but undetectable in roots [108].

PSII is the major target of photodamage at high light intensities. The D1 protein and, to lesser extent, the D2 and CP43 proteins are degraded under photoinhibitory condi-

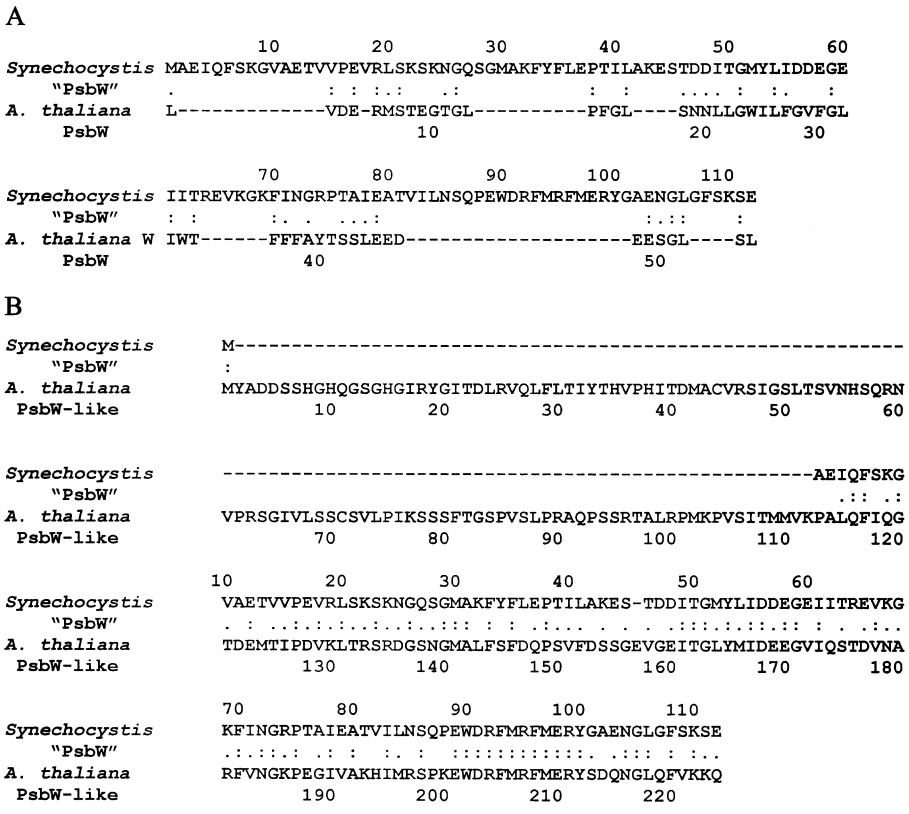


Fig. 4. Comparison of the *Synechocystis* PsbW protein with the Arabidopsis PsbW and PsbW-like proteins.

tions [109,110]. Interestingly, the nuclear-encoded PsbW protein showed almost the same rate and extent of degradation under high light stress as the D1 protein [111]. Evidence for the involvement of the PsbW protein in photoprotection has also come from studies on PsbW antisense plants. Transgenic plants lacking the PsbW protein are more vulnerable to high light stress and their recovery from photoinhibition is slower than that of the wild type [22].

Transformation of Arabidopsis plants by the *psbW* antisense construct resulted in up to 96% reductions in PsbW protein levels, but no apparent changes in phenotype were observed, except for an earlier flowering of the antisense seedlings when grown in soil [112]. Biochemical studies of transgenic Arabidopsis plants revealed that the deletion of PsbW affects the amount of most PSII core proteins, reducing the number of functional PSII complexes. In addition, no dimeric PSII complexes could be isolated in these studies, indicating that the protein is involved in stabilization of the dimer [112]. This role has recently been further substantiated by a set of experiments [113] in which newly imported PsbW was assembled quickly into dimeric PSII supercomplexes. It was also demonstrated that the N-terminal negative charges of the PsbW protein were crucial for its assembly into PSII [113].

These properties of the PsbW protein indicate that PsbW could stabilize the dimeric PSII, facilitate PSII repair after photoinhibition and guide PSII assembly.

### 3.13. PsbX

The PsbX protein is nuclear-encoded in higher plants [28,114]. A gene encoding a protein with significant similarity to the higher plant PsbX protein is present in some algae [100,103–105], where it is located in the chloroplast or cyanelle. The precursor of PsbX from Arabidopsis bears a bipartite presequence of 74 amino acid residues, which is absent in its plastid-encoded counterpart in algae, mentioned above. This suggests that the gene has been transferred from the plastid to the nucleus and acquired the required signal peptide [114]. The *psbX* gene is also found in the cyanobacteria, *S. elongatus* [115] and *Synechocystis* 6803 (*sml0002*) [2]). The PsbX of Arabidopsis and *Synechocystis* 6803 share 27% identity.

The PsbX protein contains 38–42 amino acid residues with a calculated molecular mass of 4.0–4.2 kDa, depending on the organism. Predictions according to its primary sequence indicate that PsbX has a single transmembrane span and that the N-terminus is located at the lumenal side of the membrane. The orientation of the protein has been confirmed by import studies of PsbX in Arabidopsis [98,99,114].

PsbX was named the 4.1-kDa protein when it was initially found to be associated with oxygen-evolving PSII core complexes from spinach and wheat [28]. This 4.1-kDa protein was also detected in PSII from two cyanobacteria, *S. vulcanus* [82] and *Synechocystis* 6803 [83]. Recently, the

PsbX protein has been shown to be a true component of PSII [14,116]. The higher plant PsbX is located within the PSII core, but it was undetectable in either PSIIRC or LHCII preparations [116]. The nearest neighbors of the PsbX protein in PSII are the α subunit of Cyt $b_{559}$ [47,116] and the PsbH protein [47].

Expression of the *psbX* gene is light-regulated at both the mRNA and protein levels in Arabidopsis [116]. The absence of the PsbX protein in etiolated seedlings and the response to light resemble the data obtained with chlorophyll-binding proteins, such as D1, D2 and LHCII. However, no pigments have been found to be associated with the PsbX protein, an observation that excludes the direct involvement of the protein in energy transfer. The PsbX protein is organ-specific, being detectable in green organs, such as leaves and shoots, but not in roots [116]. On the other hand, the regulation of *psbX* mRNA in *Synechocystis* 6803 under normal light conditions has been found to be independent of light, although it is expressed strongly during high light treatment [117].

Inactivation of the *psbX* gene in *Synechocystis* 6803 caused a reduction in the amount of PSII and a slight uncoupling of the antenna [117]. Nevertheless, the donor and acceptor sides of PSII functioned properly under normal and stress conditions (such as high light, and salt stress) in the *psbX* deletion mutant. Although the PsbX protein is not essential for PSII electron transport, it seems to be directly or indirectly involved in regulation of the amount of PSII [117].

The *psbX* gene has also been disrupted in the thermophilic cyanobacterium, *S. elongatus* [115]. The resulting mutant could grow photoautotrophically under standard conditions, but showed growth defects under low $CO_2$ concentrations. The PSII electron transport was much slower compared to wild type when high concentrations of the artificial electron acceptors 2,6-dichlorobenzoquinone or 2,6-dimethylbenzoquinone were used [115]. It was proposed that the binding or turnover of quinone molecules at the $Q_B$ site partially depended on the PsbX protein [115].

The mechanism of PsbX action in the regulation of the amount of PSII centers in *Synechocystis* 6803 or the binding/turnover of quinone at the $Q_B$ site in *S. elongatus* remains to be shown. Moreover, a eukaryotic PsbX knock-out mutant would be helpful for elucidating the function of this protein.

### 3.14. PsbY

The *psbY* gene in *Synechocystis* 6803 (*sml0007*) and in some algal chloroplasts (where it is known as *ycf32*) encodes a 4.0–4.2-kDa protein. However, the higher plant homolog encodes a much larger polypeptide precursor (19.5 kDa in Arabidopsis and 20.7 kDa in spinach). The precursor is synthesized in the cytosol, imported into the chloroplast, then subjected to several steps of processing and maturation to generate two thylakoid membrane proteins, PsbY-1 and PsbY-2 [118–120]. The twin proteins in Arabidopsis, with molecular masses of 4.7 and 4.9 kDa, respectively, share ~ 61% identity. It was proposed that a functional gene duplication occurred after the gene had been transferred from the chloroplast to the nucleus [118].

The amino acid identities of *Synechocystis* PsbY to the Arabidopsis PsbY-1 and PsbY-2 are 27% and 22%, respectively, indicating that the PsbY protein is not highly conserved compared to other small, plastid-encoded proteins. In Arabidopsis, both PsbY proteins have been predicted to have one transmembrane span with the N-terminus at the lumenal side of the thylakoid membrane. These predictions have been supported by in vitro protein import data [118,120]. Notably, the orientation of the *Synechocystis* PsbY in the thylakoid membrane is predicted to be opposite to its higher plant homolog (see Figs. 2 and 3).

The PsbY-1 and Y-2 proteins have been isolated from spinach PSII membrane fractions (BBY particles) [118,121]. It has been reported that the PsbY protein has two low manganese-dependent activities: catalase-like activity and an L-arginine metabolizing activity that converts L-arginine into ornithine and urea. Accordingly, the protein was previously designated L-AME [118,121]. In addition, a redox cycling assay has indicated that a redox-active group is present in the protein. Gau et al. [118] therefore proposed that a PsbY-1/PsbY-2 heterodimer or PsbY-1/PsbY-1, PsbY-2/PsbY-2 homodimer is located in the thylakoid membrane. The Trp residue located in the transmembrane region of each polypeptide should form an *o*-quinoid structure which might be involved in manganese binding.

*psbY* gene expression was demonstrated to be both light- and developmentally regulated [118]. *PsbY* mRNA was found in leaves, but not roots, stems or etiolated materials. The amount of mRNA increased during the first couple of hours of illumination, and then decreased.

A mutant strain of *Synechocystis* 6803 with inactivated *psbY* gene (*ΔpsbY*) [122] was only slightly affected in doubling time, electron transfer rate and PSII content. In addition, both forward and backward reactions through PSII were not significantly altered. The cited authors concluded that the PsbY protein does not provide an important binding site for manganese and is not essential for normal function of PSII in *Synechocystis* 6803, even under certain stress conditions, such as high light and nutritional limitations with respect to manganese, calcium and chloride ions [122].

Considering the orientation and subunit numbers, the PsbY protein in higher plants might be different from that in *Synechocystis 6803*. It is therefore possible that the PsbY protein has an important role in higher plant PSII, but not in *Synechocystis* 6803. To examine whether this proposal is correct, a knockout mutant of the *psbY* gene in higher plants is needed.

### 3.15. PsbZ

The PsbZ protein is encoded by a chloroplast gene, *psbZ*, formerly denoted *ycf9*, and sometimes also named *orf62*.

The gene is highly conserved among organisms and has been found in all photosynthetic species examined [123,124]. In most organisms the protein contains 62 amino acid residues [123,125]. The theoretical molecular mass of Arabidopsis PsbZ is 6.6 kDa. Interestingly, the *Synechocystis* PsbZ protein contains a 107-amino-acid extension to the N-terminus, giving the protein a molecular mass of 12 kDa. The degree of identity of the Arabidopsis PsbZ protein to that in *Synechocystis* is 22.4%. The Arabidopsis protein has been predicted to have two transmembrane spans, with very short N- and C-termini located on the stromal side of the thylakoid membrane [123]. The PsbZ protein has been reported to be a PSII core component in both tobacco and *Chlamydomona*s [123], and also to be present in a highly active PSII preparation from *Synechocystis* 6803 [14]. In addition, PsbZ has been co-purified with LHCII complexes from tobacco [126]. Thus, the location of the PsbZ protein seems to be at the interface of PSII and LHCII complexes [123,126,127].

Two *psbZ* mutants have been generated in *Synechocystis* 6803 (Bishop, personal communication). The mutants are capable of photoautotrophic growth and have oxygen evolution rates comparable to those of wild type. The amount of PSII in the mutants is slightly reduced. Homoplasmic *psbZ* knockout tobacco plants and *Chlamydomonas* mutant cells (*ΔpsbZ*) have been generated by a number of research groups [123,124,126]. Although all *ΔpsbZ* plants grew photoautotrophically [123,124,126], the tobacco *ΔpsbZ* developed pale leaves under standard heterotrophic growth conditions due to a reduction in chlorophyll content [123]. When tobacco *ΔpsbZ* plants were grown under dim light, their growth rates were severely retarded, generating a dwarf phenotype [123,124,126]. However, there was no significant difference in growth rates between *Chlamydomonas* wild type and *ΔpsbZ* under either standard or suboptimal condition [123].

In three independent studies the level of the antenna protein CP26 was found to be markedly reduced in *ΔpsbZ* plants [123,124,126]. The extent of the decrease in CP26 was light- and temperature-dependent [123]. Another minor antenna protein, CP29, decreased as well, but to a lesser extent [123]. The lack of the PsbZ protein as well as the reduced amounts of CP26 and CP29 caused structural changes in PSII that prevented the isolation or detection of PSII–LHCII supercomplexes in electron microscopy studies [123]. Interestingly, levels of proteins other than PSII components, such as NdhH in the NAD(P)H dehydrase complex and plastid terminal oxidase (PTOX), were also reported to be significantly reduced in *ΔpsbZ* plants [124].

The structural changes observed in PSII of *ΔpsbZ* plants were accompanied by functional modifications in PSII. Perhaps most importantly, the rates and pathways of photosynthetic electron transport in *ΔpsbZ* were reportedly altered. When chloroplasts were isolated from young plants adapted to low light conditions, rates of PSII oxygen evolution measured under low light intensities in *ΔpsbZ* were lower than in wild type [126]. As there was no significant difference in oxygen evolution between *ΔpsbZ* and wild type grown under high light [126], the reduced growth rate under low light was explained by lowered efficiency of the photosynthetic light reactions. This proposal was challenged by another study, in which thylakoid membranes were isolated from either standard-or low-light-grown tobacco [124]. It was shown that PSII oxygen evolution was similar in wild type and *ΔpsbZ* at any light intensity. These authors found that the normal electron transport from PSII to PSI was accelerated, whereas the PSII-independent cyclic electron transport pathway was impaired [124]. In the *ΔpsbZ* mutant, the steady state levels of phosphorylated PSII core proteins (D1, CP43 and PsbH) decreased, whereas the amount of phosphorylated LHCII increased, indicating that *ΔpsbZ* favored a state where LHCII detaches from PSII [123,128]. Based on fluorescence measurements, Swiatek et al. [123] propose that the PsbZ protein plays a role in non-photochemical quenching (NPQ). This suggestion has been corroborated by the finding that the xanthophyll cycle is stimulated in the *ΔpsbZ* mutant [123]. However, as the loss of PsbZ leads to reduced amounts of CP26, these effects may be secondary.

To summarize, PsbZ is likely to be a structural factor that stabilizes PSII–LHCII supercomplexes. It may also be involved in photoprotective processes under suboptimal growth conditions.

## 4. Structural summary of the low molecular subunits

In this review, data related to 16 different low molecular mass subunits that are putatively located in the PSII complex have been presented. Recently, two additional low molecular mass subunits, Psb27 [14,63] and Psb28 [14], were detected in PSII complexes from *Synechocystis* 6803 and *T. vulcanus*. In contrast, the PsbN protein has been questioned if it is a PSII protein [14]. Thus, given currently available evidence, it seems that at least 17 low molecular mass subunits may be associated with PSII. It is uncertain whether this is the final number of small subunits in the PSII complex or not, as every time an additional component has been found it was believed to be the last that remained to be discovered. Since little information is available for Psb27 and Psb28 proteins, they were not included in this summary. In addition, predictions of the secondary structure of these two proteins show that the mature proteins do not have any transmembrane helix. Therefore, excluding them from our summary would not affect the discussion of our current, overall understanding of PSII structure.

Since the first identification of low molecular mass subunits in PSII [24] their presence and significance in PSII have been questioned (see, for instance Refs. [14,122]). The data supporting a PSII location vary greatly between the different small subunits. In some cases, different PSII

preparations have been analyzed by immunoblotting or microsequencing, but often no data have been provided in resulting publications on the purity of the preparations used. New techniques, exploiting genetically engineered polyhistidine-tagged protein within the PSII complex to enhance isolation, have strongly increased the purity of the analyzed complexes. Other methods, such as HPLC separation linked to various mass spectrometric techniques, have given further support for the presence of low molecular mass subunits in PSII in both cyanobacteria [14] and higher plants [13].

### 4.1. Most small subunits are transmembrane proteins

Although the structure of none of the small proteins has been determined, all but one of the 16 low molecular mass subunits of PSII seem to be transmembrane proteins located in the thylakoid membrane (see Fig. 5). Only PsbTn has been reported to be a soluble protein that can be removed by high salt/detergent treatment from the thylakoid membrane [24]. The small size of most of these proteins allows only for one single transmembrane span, but extensions of various sizes on both sides of the membrane are possible. On the stromal side of the thylakoid membrane, only five of the proteins (PsbE, PsbF, PsbH, PsbL and PsbW) have extensions that are 13 amino acid residues long or longer. Meanwhile, on the lumen side of the membrane, six proteins (PsbE, PsbK, PsbR, PsbW and PsbY2) have putative extensions longer than 13 amino acid residues. Only the PsbE and the PsbW have significant extensions on both sides of the thylakoid membrane. This finding is of special interest when considering N- and C-terminal interactions between low molecular mass proteins and other proteins in the PSII complex. As seen in Fig. 5, several of the low molecular mass subunits may not have long enough extensions to be involved in such interactions. Indeed, biochemical analyses have shown that only the PsbE, PsbF, PsbH, PsbI and PsbW proteins are frequently found in cross-linked complexes [47,58,95,116].

### 4.2. Most small subunits are chloroplast-encoded

While the gene location (nucleus-encoded vs. plastid-encoded) for most of the low molecular mass subunits seems to be consistent among different species, the *psbX* and *psbY* genes have been identified in the plastidic genome of some algae and in the nuclear genome of higher plants such as Arabidopsis, spinach and pea. Therefore, the transfer of these genes from the chloroplast to the nucleus is presumed to have been a recent event in evolutionary terms. Despite the fact that the chloroplast genome encodes less than 100 of the almost 26, 000 proteins in Arabidopsis, nine of the small PSII proteins are encoded in the chloroplast. It has been argued that the plastid-localized genes code for proteins that are needed continuously and quickly, and may be under redox control [129,130]. This would be in good agreement with the finding that most of the chloroplast-encoded proteins (PsbE, PsbF, PsbI, PsbJ, PsbK and PsbL) have putative roles in PSII stabilization or dimerization (vide infra). This represents a critical step in the homeostasis of the PSII complex.

All nuclear-encoded, low molecular mass PSII subunits have their N-terminus on the lumenal side of the membrane. This is probably a result of the protein-targeting pathway followed by these five proteins. Most nuclear-encoded proteins of the PSII complex are targeted to the thylakoid by either the twin-arginine translocation (TAT) pathway or

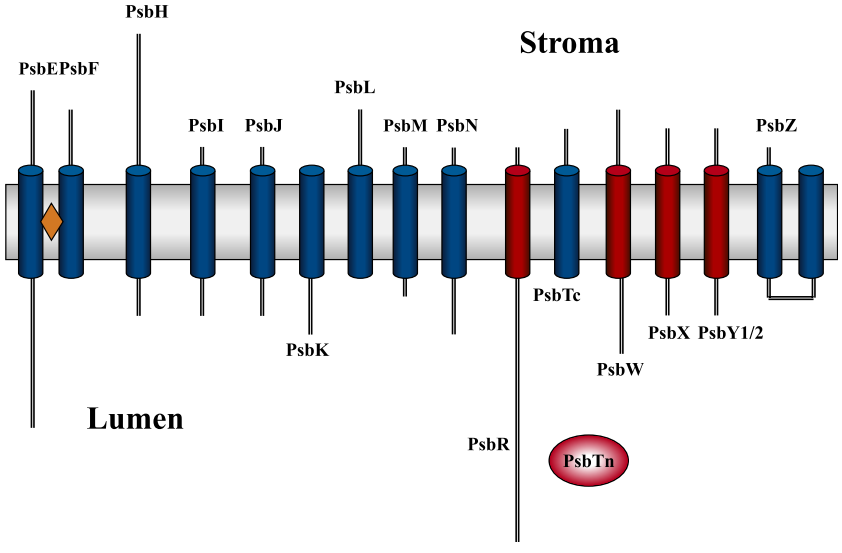


Fig. 5. All 16 low molecular mass subunits of the PSII complex from Arabidopsis. Blue helices represent chloroplast-encoded, and red nuclear-encoded subunits. Names of the proteins are placed near the N-terminus of the protein and the lengths of the N- and C-terminal tails are drawn in proportion to each other. Note that PsbN protein is probably not a PSII protein and that PsbY subunits in higher plants are present in two copies. Note also that PsbR, PsbTn and PsbW are not present in cyanobacteria.

the Sec-dependent pathway (see, for instance Ref. [131] and references therein). However, the PsbW, PsbX and PsbY subunits are all targeted to the membrane by an alternative pathway often referred to as the ''spontaneous'' pathway. The name refers to the finding that the proteins do not need a pH gradient or any ATP to be integrated into the thylakoid membrane. Of the chloroplast-encoded proteins, all but two have their N-terminus at the stromal side of the membrane. This is probably due to the fact that they are all cotranslationally inserted into the membrane. However, it is not clear at the moment why the PsbK and PsbTc proteins have an opposite orientation. Notably, PsbZ has a processing site in the loop between the two helices on the lumenal side. If it is processed at this site it would become two single transmembrane proteins in a manner similar to that of the PsbY protein [123]. Evidence that conflicts with this interpretation has been provided by mass spectrometry analyses, indicating the presence of only one protein with a molecular mass of 6.6 kDa, which is the mass of the intact, uncleaved protein [126] (E.M. Aro, personal communication). One interesting speculation is that PsbZ is synthesized as a double helix protein, and only processed when functional activity is required. Further studies are needed to address this possibility.

### *4.3. The ratio of small subunits to PSII seems to be one*

Another important question that remains to be resolved is the amount of each low molecular mass subunit per PSII complex. Such ratio determinations are challenging, as the long and intense debates on the amount of PsbE/F per PSII have demonstrated [33]. However, with the publication of the X-ray crystal structures of the PSII core from bacteria [10,11], this dispute seems to have been solved, as only one heme group was found to be associated with the small subunits (PsbE and PsbF). The general consensus seems to be that there is only one subunit of each low molecular mass protein per PSII complex. This is of great importance as homo-oligomers of a small subunit could form substructures such as channels or pores through the membrane for ions, water or even oxygen. However, analyses in our laboratory of transmembrane sequences of the low molecular mass subunits by helical-wheel projections gave no evidence to support the hypothesis that these subunits form polar or hydrophilic channels (Shi and Schröder, unpublished data).

### *4.4. Location of the small subunits within the PSII complex*

Various attempts to assign locations to the low molecular mass subunits in the PSII core have been based on chemical cross-linking studies, immunological analyses and some ''informed guesswork'' (see review [132]). Unfortunately, to date, all attempts to work out the location of the small subunits rationally have generated contradictory conformations. Taking the location of the PsbW protein as an example, this protein has been suggested importance for the dimerization of the PSII complex and it is only present in higher plants. Thus, it seems logical to place it in the helix marked by the black arrow in Fig. 1: the helix missing in cyanobacteria and located between the monomers. However, this location of the protein is inconsistent with the finding that the PsbW protein can be chemically cross-linked to Cyt $b_{559}$, which is located on the other side of the complex.

The most detailed structures available at the present time are the two published X-ray crystal structures of cyanobacterial PSII at 3.7- and 3.8-Å resolution. However, although models and pictures based on X-ray structures give the impression of being well-defined ''true structures'' of complexes, it is surprising to note that the location of several small protein subunits differs in two published X-ray structures. According to biochemical studies, both groups place the PsbX close to the cytochrome $b_{559}$. However, one group places the PsbI on the same side as PsbX, while the other places it on the opposite side of cyt559. Similarly, Zouni et al. [10] place the PsbK, PsbL and PsbH in the space between the two monomers, while Kamiya and Shen [11] place PsbH and PsbK on the outside of the monomer and did not assign the PsbL. Nevertheless, both have assigned locations to the PsbE, PsbF, PsbH, PsbI, PsbK and PsbX, but left at least eight α-helices unassigned, giving a total number of 14 transmembrane helices in cyanobacterial PSII. Of the assigned helices, the PsbK is localized quite unambiguously in the 3.7-Å structure, in addition to the PsbE and PsbF [11]. Because there are two proline residues in the middle of the PsbK, its helix adopts a characteristically bent shape [11]. Of the eight unassigned helices seven may belong to PsbJ, PsbL, PsbM, PsbTc, PsbY and PsbZ (two helices). Consequently, there is still one helix in the cyanobacterial crystal structures that lacks a protein candidate. The unambiguous assignment of the low molecular mass subunits in the PSII core complex ''forest'' of α-helices is a challenging task, which will not be fully clarified until higher-resolution crystal structures have been obtained.

## 5. Possible functions of the low molecular mass proteins

It can be argued that every expressed protein should have a function, otherwise the energy costs for its synthesis should be unsustainable. If this is accepted, it poses interesting questions about the possible roles that small proteins (< 10 kDa) could play in a large, multi-subunit complex of perhaps 700 kDa such as PSII. In this section, we explore some functional aspects of this group of proteins and summarize functional suggestions that have been proposed, based on genetically engineered plants and cyanobacteria.

The small proteins are found in several places within the PSII core (see Fig. 1) and are not located at any one specific site, making it unlikely that the low molecular mass subunits

form some kind of separate substructure or pore in PSII. Furthermore, most of their corresponding genes are scattered across the whole genome in Arabidopsis and *Synechocystis* 6803, and no gene fusion of the low molecular mass subunit genes has been observed in any organism. This also indicates that they comprise a very divergent group, for which low molecular mass may be the only common feature.

### 5.1. Sequence homology

With respect to the degrees of similarity in protein sequences between Arabidopsis and *Synechocistis* 6803, PSII small proteins can be classified into four groups. Group I includes the highly conserved subunits PsbE, PsbF, PsbI, PsbJ, PsbK and PsbL, which share near or higher than 70% identity. Group II includes the moderately conserved subunits, PsbH, PsbM, PsbN and PsbTc, sharing about 50% identity. Group III consists of poorly conserved proteins (PsbX, PsbY and PsbZ), displaying less than 30% identity. Proteins in Group IV (PsbR, PsbTn and PsbW) are found only in higher plants and green algae, and are not present in *Synechocistis* 6803. It can be speculated that group I proteins may function in a very similar way in both model organisms, as there seems to be a correlation between the degree of conservation in their primary structure and the effects of the proteins on photosynthetic activity. Except for PsbI, higher plant mutants that lack proteins from group I cannot grow phototrophically. Proteins from Groups II and III might have slightly or even totally different roles in the two organisms, which could explain the large differences observed between the two model organisms. Furthermore, no functional homologs to other known proteins in various protein databases have been found, regardless of the species they cover.

### 5.2. Only two small subunits seem to bind cofactors

The only subunits that have been unambiguously shown to bind any cofactors are PsbE and PsbF, which bind the heme group of cytochrome $b_{559}$. In higher plants, PsbY has been suggested to be involved in binding Mn [118], but this was not found to be the case in *Synechocystis* 6803 [122]. Thus, whether or not this protein has cofactors is unclear. All other low molecular mass subunits have not been reported, so far, to bind any redox cofactors or pigments, and thus are unlikely to take part directly in photosynthetic electron transport. However, more detailed structural X-ray crystal analysis may reveal some connection to pigments or other cofactors.

### 5.3. Do all low molecular mass subunits have the same function?

It seems unlikely that all the small proteins in PSII have the same function. However, at least nine of the subunits (PsbE/F, PsbH, PsbI, PsbJ, PsbK, PsbL, PsbW, and PsbZ) are putatively important for PSII assembly, stabilization and/or dimerization. This function is the most complicated function to assay, as it is difficult to know if detected influences on stability are due to the lack of the specific protein or secondary effects. For instance, when the PsbZ protein is deleted, levels of the CP26 protein also decrease [123], which then raises the question if the effects seen are due to the lack of either one or both of the proteins. Another important consideration is the finding that the stability of the PSII dimer decreases when the degradation and turnover of the PSII core are increased [112]. This again creates a special problem when analyzing PSII stability in various mutants, and emphasizes the importance of avoiding light-stressed plants when analyzing PSII stability.

Another important question that remains to be resolved is why the PSII complex needs so many different small protein subunits for stability. One possible explanation for these findings is that the small subunits may be located at different sites of the large PSII complex, contributing to stability at their specific location, or they may be important at specific stages of assembly. In this way, they could function as specific, separate regulators for the important larger proteins in the complex and thus make regulatory control quicker and more efficient. They could also prevent the misfolding/misassembly of specific proteins in the complex.

The next intriguing issue to consider is how such small protein subunits could have such a dramatic effect on such a large complex as PSII. The interactions between the low molecular mass proteins and other larger proteins of the PSII complex could occur either at the transmembrane α-helical region or at the N- or C-terminal extensions. Experimental data showing that the terminal extensions of the proteins can be of importance for assembly and stability of the PSII complex have been obtained by combinations of import studies and blue-native polyacrylamide gels [113]. Rates of assembly of the PSII-dimer were drastically reduced using a mutated precursor of the PsbW protein in protein import analyses where the negatively charged N-terminal residues were exchanged for positively charged histidines, compared to rates found for "wild type" protein. It is, however, still puzzling that such a small change in charges on the N-terminus of the PsbW subunit could have such a dramatic effect on PSII assembly. Similar considerations apply to the other low molecular mass subunits that have putative importance for assembly and dimerization of the PSII complex.

Surprisingly, several small proteins, especially in cyanobacteria, have been found to have an effect on the acceptor side of PSII (Table 1). However, it is not clear at the moment whether this merely reflects the difficulties of making reliable and reproducible measurements by the highly sensitive fluorescence techniques involved, or that the acceptor side really is more vulnerable and needs unusually high degrees of stabilization, which is provided by several low molecular mass subunits.

### 5.4. Functional analysis using various organisms and mutations

Mutants are frequently used to address questions related to protein function. However, finding the "right" situation, i.e. the appropriate structural unit, growth conditions and stresses in which a protein is indispensable, can be very difficult and time-consuming. In Table 1 we have compiled a list of currently available deletion mutants or antisense lines from prokaryotic and eukaryotic organisms. We also show the reported phenotype of the mutants and suggested functions for the low molecular mass subunits.

In prokaryotes, often *Synechocystis* 6803 in practice, a clone with a deletion or disruption of the gene of interest can be obtained using standard techniques. However, in eukaryotes this technique is not as straightforward as it is in bacteria, so antisense techniques or gene bombardment is often used instead. After the mutant has been obtained, a screen for a changed phenotype and/or missing function is initiated. However, what should be a rather straightforward and direct method for obtaining information on protein function often turns into a long and tedious project to find the conditions in which the deleted protein is unambiguously needed. As seen in Table 1, there are cyanobacterial mutants for all low molecular mass subunits, while in eukaryotes four types of mutants have not yet been reported. As described in Table 1, deletion of the small PSII subunits in prokaryotes has only minor effects (except for the PsbE, PsbF and Psb proteins). However, in eukaryotes, deletions or reductions in the amounts of the small proteins often lead to more severe failures in photosynthesis. This reflects the fact that cyanobacteria are more flexible with respect to genetic and environmental changes, compared to higher plants. It has also been suggested that the transfer of genes from the chloroplast genome to the nucleus that has occurred in higher plants probably made the chloroplast less flexible and therefore more sensitive. The differences in phenotypes arising from mutations of the "same" protein in various organisms have added to the uncertainties concerning the function of the small proteins. It seems that only the deletion of the PsbL and PsbE protein results in the same severe effect on PSII in all organisms so far examined.

### 5.5. Low molecular mass proteins in other multi-enzyme complexes

Photosynthetic reaction centers are generally classified into two groups: types I and II. It is broadly accepted that all photosynthetic reaction centers are structurally similar and have evolved from a common ancestor. The structure of PSI, a trimeric complex, has been solved at 2.5-Å resolution in the thermophilic cyanobacterium *S. elongatus* [7]. Therefore, comparison of PSII with PSI may provide some valuable indications, especially about the functions of small proteins in PSII. The cyanobacterial PSI contains 12 proteins, 96 chlorophyll *a* molecules, 22 carotenoids, three [4Fe4S] clusters, two phylloquinones and four lipids [7]. Of 12 protein subunits, half are small proteins, with molecular masses less than 10 kDa. PsaC (8.9 kDa) and PsaE (8.0 kDa) are two peripheral proteins. The former subunit carries two FeS clusters, $F_A$ and $F_B$, and the latter is involved in anchoring ferredoxin and also in cyclic electron transport [133]. PsaI and PsaM are single-transmembrane-span proteins with molecular masses of 4.3 and 3.4 kDa, respectively. Both of these subunits are located close to the threefold axis and interact with carotenoid molecules. PsaM is also involved in the coordination of one Chl*a* [133]. PsaJ and PsaX are single-transmembrane-span subunits and are located far from the threefold axis in contrast to PsaI and PsaM. The PsaJ protein binds three Chl*a* and interacts with carotenoid molecules [7]. The PsaX protein is unique to the thermophilic cyanobacteria [133]. On the basis of these findings, the functions of the small proteins in PSI can be placed in the following categories: (a) binding or stabilizing electron transport cofactors, such as FeS clusters $F_A$ and $F_B$; (b) light harvesting and energy transfer; (c) docking, assembling and trimerizing subunits; (d) photoprotection and (e) adaptation to unique environments. The strong similarities in the structures between the two photosystems, and a great deal of biochemical and structural data, suggest that small PSII proteins may have similar functions to those listed for PSI proteins. Although there is no evidence to prove that any of the small PSII proteins, except Cyt $b_{559}$, bind any cofactors, the possibility cannot be ruled out that they may be directly or indirectly involved in energy transfer.

To see if the presence of low molecular mass proteins is a specific PSII feature, we performed a search for low molecular proteins in other protein complexes located in plant chloroplasts and mitochondria. Interestingly, this revealed that most protein complexes contained between one to seven small subunits. As percentages of the total amount of protein in the complexes, only cytochrome *c* oxidase (complex IV of the respiratory chain in the mitochondria) contained a similar amount (60%) to the 50% found in PSII. However, in terms of numbers, PSII has 17, which is by far the highest numerical content of small subunits found. An interesting suggestion is the possibility that the need for low molecular mass proteins in large protein complexes may be of special importance for complexes where oxidative stress occurs. In such situations, the protein turnover is likely to be high, which accords well with the finding that the majority of low molecular mass subunits is involved in protein assembly and turnover.

## 6. New proteins and future perspectives

As shown in Table 1, mutants of several of the small proteins (mainly higher plant forms) that could yield valuable information have not as yet been produced. Special priority should be given to generating mutants of PsbM, PsbTn and PsbY. There might also be additional small

proteins that have not yet been identified, as suggested by Kashino et al. [14]. The finding of additional PSII proteins would create problems with respect to naming the proteins. So far, protein names have been given in alphabetical order, but after the finding of PsbZ, no further names of this kind can be used. The name PsbG is still available, as this protein later proved to be a component of the NADPH-quinone oxido-reductase and is now called NghK. A similar fate is likely to befall the PsbN protein. However, to avoid confusion, Kashino et al. [14] recently suggested that names based on numbers should be introduced. After PsbZ, which is the 26th protein of PSII, the next protein found should be called Psb27, then Psb28 and so on. We support this suggestion, especially as there would then be no limits for naming further proteins.

## 7. Conclusions

Of the 16 proteins discussed in this review, one (PsbN) has been questioned whether it is a PSII component, but two new proteins (Psb27 and Psb28) have been recently identified. Thus, at the present time, 17 small protein subunits are putatively associated with PSII. Of these, 12 (in higher plants) or 14 (in cyanobacteria) proteins seem to be present in the PSII core. The small subunits are not located in a specific area, but instead are found at many different sites of the PSII complex. The exact assignment of their location within the PSII core demands improved crystal structure analyses, with higher resolution than 3.7 Å. Only two subunits, PsbE and PsbF, bind a cofactor. The majority of the single α helix subunits in PSII seems to be involved in stabilization, assembly or dimerization of the PSII complex. The high amount of small proteins is likely to increase the structural and functional flexibility and stability of PSII. The small proteins may facilitate dynamic conformational changes of PSII (i.e. help the PSII complex to shuttle between stromal lamellae and grana thylakoids, to degrade and replace damaged D1 protein, and to disassemble and reassemble faster and more efficiently). The large number of small proteins in PSII compared to other protein complexes may be an adaptive feature related to the oxygen evolution performed by PSII, reflecting the vulnerability of the complex to high light intensities and oxidative stress, and the need for flexible turnover of the complex, or at least parts of it.

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