Review

The extrinsic proteins of Photosystem II

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ABSTRACT

In this review we examine the structure and function of the extrinsic proteins of Photosystem II. These proteins include PsbO, present in all oxygenic organisms, the PsbP and PsbQ proteins, which are found in higher plants and eukaryotic algae, and the PsbU, PsbV, CyanoQ, and CyanoP proteins, which are found in the cyanobacteria. These proteins serve to optimize oxygen evolution at physiological calcium and chloride concentrations. They also shield the Mn₄CaO₅ cluster from exogenous reductants. Numerous biochemical, genetic and structural studies have been used to probe the structure and function of these proteins within the photosystem. We will discuss the most recent proposed functional roles for these components, their structures (as deduced from biochemical and X-ray crystallographic studies) and the locations of their proposed binding domains within the Photosystem II complex. This article is part of a Special Issue entitled: Photosystem II.

1. Introduction

Photosystem II (PSII) functions as a light energy-driven water-plastoquinone oxidoreductase. In higher plants, eukaryotic algae and cyanobacteria, light is trapped by and funneled through light-harvesting pigment–protein complexes (light-harvesting complex II or phycobilisomes) to the reaction center of PS II, which contains a specialized pigment complex, P₇₃₀. After excitation, P₇₃₀ becomes photooxidized and donates an electron to the primary acceptor of PS II, a protein-bound pheophytin. This charge separation is then further stabilized by the transfer of this electron, first to Qₐ and then to Qₐ, protein-bound plastoquinones. The accumulation of two reducing equivalents on Qₐ leads to its protonation and the formation of plastoquinol, which is then released from PS II. Concomitant to these electron transfers, P₇₃₀ is reduced by Y₂, a tyrosyl residue (161Y) protein, PsbR, is also present in higher plants and green algae. In cyanobacteria and cyanobacteria also contain a PsbP homologue (CyanoP); the stoichiometry of this component remains controversial [13,14]. Interestingly, while the green algae contain the same extrinsic components present in higher plants (PsbO, PsbP, PsbQ and PsbR), the red algae contain PsbO, PsbP, PsbQ (a 20 kDa homologue of CyanoQ), PsbU and PsbV [15,16]; PsbR is not present in the red algae.

In this review, we will examine the structural and functional properties of these extrinsic protein components which function in support of oxygen evolution. We will principally focus on findings
obtained during the last decade, which has witnessed an explosive growth in our understanding of the roles played by these proteins in the photosystem. Organizationally, we will first examine the PsbO, PsbP and PsbQ proteins. These components (or their homologues) are present in virtually all oxygenic organisms. The enigmatic PsbR protein, which is not present in cyanobacteria or red algae, will also be briefly examined. The cyanobacterial PsbU and PsbV proteins along with the cyanobacterial homologues of PsbQ (CyanQ) and PsbP (CyanP) will then be discussed. It should be noted here that we will not examine the extended PsbP and PsbQ families of proteins which are found in higher plants. The numerous members of these protein families may play other important roles within PS II, the Ndh dehydrogenase complex, and possibly other systems (for reviews, see [17–19]).

1.1. PsbO structure

Sequence alignments for the PsbO protein have been presented elsewhere [20,21] and indicate that PsbO is conserved across the higher plants, algal and cyanobacterial clades. Our understanding of the structural association of the PsbO protein within PS II comes principally from the crystal structures of the photosystem which have been obtained from the thermophilic cyanobacteria. These studies have, in large measure, confirmed earlier investigations which indicated that the PsbO component, in solution, exhibited a high degree of β-sheet secondary structure [22,23] and that the protein was significantly elongated [24,25]. It should be noted that no crystal structures for the higher plant protein either as an isolated subunit or associated with the photosystem are currently available.

PsbO, in association with PS II from Thermosynechococcus vulcanus [5] (henceforth T. vulcanus), is shown in Fig. 1 in orange. This protein is located on the luminal face of the complex and interacts with a variety of other PS II subunits including CP43, CP47, D1, D2 and PsbU. Since the PsbO protein can be removed from PS II membranes by treatment with 1 M CaCl₂, it is hypothesized that charge-pair interactions provide the principal binding energy of PsbO to the photosystem. Within this context, it should be noted that PsbO remains firmly attached to PS II membranes even in the presence of 2 M NaCl. The higher charge density of calcium (or other divalent cations) appears to be required to disrupt the interaction of PsbO with the PS II core components. The PsbO protein can also be removed from PS II membranes by treatment with 2.6 M urea + 200 mM NaCl [9]. In this instance, the urea is hypothesized to weaken inter- or intramolecular hydrogen bond interactions sufficiently to allow release of the PsbO component by the 200 mM NaCl.

To identify possible salt-bridge interactions of surfaces within the PS II crystal structure, we utilized the program KFC [26], which uses shape and residue properties to identify contact domains on multimeric proteins predicted to contribute to the highest binding energy. We then examined the residues within the contact domains for possible charge-pair interactions. Additionally, we used the program CAPTURE [27] to identify possibly relevant cation–n interactions. The results from this analysis are shown in Table 1. CP43, CP47, D1 and D2 all appear to possess residues which could contribute to the binding of PsbO.

Only a small subset of these residues, principally on the CP47 subunit, has been examined in more detail. Within the T. vulcanus crystal structure [5], a charge-pair “complex” appears to be formed involving the residues 384R:CP47_160D:PsbO_422R:CP47_179E:PsbO_423K:CP47 (Fig. 2). Please note that 384R:CP47 appears to interact with the C-terminal carboxyl group of the D2 protein (152L:D2). These residues are partially buried, which would increase the relative strength of the charge-pair interactions. The basic residue pairs of CP47, 384R:385R and 422R:423R, have been examined by site-directed mutagenesis in Synechocystis sp. PCC 6803 (henceforth Synechocystis 6803). Positive charges at these positions are absolutely conserved across all CP47 proteins. While alteration of the 322R:423R pair to neutral residues had a relatively small effect on oxygen evolution [28], modification of residues 384R:385R to either neutral (384G:385G) [29] or negatively charged (384E:385E) residues [28] had a dramatic effect. Individual site-directed modification at either residue 384R or 385R also yielded mutants with significantly decreased oxygen evolution capability [28]. The double mutants evolved oxygen at rates comparable to that reported for the ΔpsbO mutant. Later studies, indicating that permeabilized thylakoids isolated from the CP47 384E385E mutant bound the PsbO protein very weakly, and that the ΔpsbO:384E385E triple mutant exhibited a phenotype very similar to the ΔpsbO mutant, provided strong evidence that 384R:385R of CP47 provided an important binding site for PsbO to PS II [30]. It should be noted that detergent-permeabilized thylakoids isolated from the CP47 384G:385G mutant bound the PsbO protein significantly more strongly than the CP47 384E385E mutant [30], indicating that other, yet unidentified residues also contribute to the association of PsbO with PS II. Neither of the PsbO residues (160D and 179E) predicted from the KFC analysis described above to participate

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1. The numbering of the specific amino acid residues in the various PS II proteins is problematic as various authors have used numbering derived from the immature translated gene sequence (with or without removal of the N-terminal methionyl residue) or from the fully processed, mature protein, from a variety of different organisms. Except where indicated, we have used the numbering system from the T. vulcanus crystal structure of Umena et al. [5].

2. His-tagged PS II particles containing mutations at residues 384R:385R of CP47 also lacked the PsbO subunit (C. Putnam-Evans and T. M. Bricker, unpublished).
in these charge-pair interactions with CP47 has been examined by site-directed mutagenesis. PsbO also may participate in an inter-monomer interaction with CP47, possibly stabilizing the PS II dimer [20]. KFC analysis indicates that 59 K of PsbO could form a putative charge-pair interaction with 307E of CP47. Such an interaction might explain the loss of PS II dimers in ΔpsbO mutants [31]. An alternative hypothesis, however, is that increased photodamage occurring in the absence of PsbO in the ΔpsbO mutant accelerates the rate of monomer formation as a prelude to the repair of the photosystem.

Two residues in CP43 (362R and 381K) are also predicted to participate in charge-pair interactions with PsbO (Table 1). The residues 362R and 381K lie within the Synechocystis 6803 deletion mutants ΔL364/D370, respectively [32]. These mutants did not assemble functional PS II reaction centers. Since the observed phenotype was much more extreme than that observed in the ΔpsbO mutant, the loss of assembly of the photosystem cannot be ascribed to the possible loss of PsbO binding alone. The severe phenotype may be the result of the removal of ten and seven residues, respectively in these two deletion strains. The effects of alteration of the individual CP43 residues 362R and 381K have not been directly probed by site-directed mutagenesis. The putative 1D:Psbo–362R:CP43 interaction is particularly interesting, however. A variety of N-terminal modifications of higher plant PsbO have been shown to affect PsbO binding to the photosystem. Eaton-Rye and Murata [33], using spinach PS II membrane, demonstrated that proteolytic removal of the N-terminal 16–18 amino acid residues from PsbO abolished its ability to bind to PS II. Popelkova et al. [34] identified two N-terminal domains of 7TYD10E and 15TYL18E in the spinach PsbO protein which the authors demonstrated were required to bind the two copies of the PsbO protein per PS II monomer (see below) in higher plants. One of these domains is missing at the N-terminus of the cyanobacterial protein. Sequence alignment of higher plant and cyanobacterial PsbO sequences [21] indicates that the cyanobacteria uniformly exhibit a nine–ten amino acid deletion at the N-terminus (the 7TYD10E domain lies within this deletion). These results may explain a major discrepancy between site-directed mutagenesis studies performed in higher plants and cyanobacteria. In spinach, site-directed modification of 1D to either 3N or 3K had neither an effect on the binding of the modified PsbO protein to PsbO-depleted PS II membranes nor the restoration of oxygen-evolving activity [35]. In Synechocystis 6803, however, modification of 1D to 3K weakened the binding of PsbO to thylakoid membranes and resulted in a moderate loss of oxygen-evolving activity [30]. The findings of Popelkova et al. [34] demonstrate that the 1D residue is not equivalent in the higher plant and cyanobacterial systems; in spinach, this residue lies in the first of two binding domains, while in cyanobacteria it lies in the sole binding domain present in these organisms, which is equivalent to the second binding domain found in higher plants (please note that the 3A residue in Synechocystis 6803 is equivalent to the 3D residue in the T. vulcanus crystal structure [5]). The putative interacting residue, 362R:CP43, has not been examined by site-directed mutagenesis.

Finally, a number of residues in the D1 and D2 proteins are predicted to interact via charge-pair interactions with PsbO (Table 1). No site-directed mutagenesis experiments examining the residues in D1 and D2 with respect to PsbO binding have been reported. 32E of the D2 protein is of particular interest, however. Its putative binding partner is 160K of PsbO, the modification of which dramatically altered the binding properties of the mutated PsbO protein in Thermosynechococcus elongatus5 [henceforth, T. elongatus] [36]. Other residues in this vicinity have also been implicated in the functional association of PsbO with the photosystem. Examination of the crystal structure indicates that the backbone carboxyl of 158D of PsbO may be hydrogen-bound to 314R of D1 (3.0 Å). This latter residue was modified in Synechocystis 6803 in an attempt to determine its involvement in extrinsic protein binding [37]. While no evidence was found indicating this role, the resultant mutant did exhibit a variety of PS II defects including altered S2 state properties and altered oxygen release kinetics. Modification of 158D of PsbO yielded a mutant6 (D159N) which exhibited a 30% loss of steady-state oxygen evolution and loss of photautotrophy in a ΔpsbB background, neither of which could be directly attributed to loss of PsbO from the photosystem [38]. These authors hypothesized that 158D formed an intramolecular charge-pair with 162R. Examination of the T. vulcanus crystal structure [5] indicates that this is plausible, as these residues are separated by 2.7 Å.

Many of the results discussed above apply directly only to cyanobacterial PS II. It must be noted that while the PS II crystal structures have provided important insights into the structure of the photosystem, some questions remain. None of the current structures contain the CyanoQ or the Cyanop subunits. The former is almost certainly a stoichiometric component of the photosystem [12,13], while the latter also may be present in stoichiometric quantities [14], although this is controversial [13,39]. The observation that loss of CyanoQ destabilizes the binding of PsbV [40] also raises questions concerning the overall integrity of the observed subunit composition in the cyanobacterial structures currently available.

A number of studies, obtained principally by biochemical examination of higher plant PS II, appear inconsistent with current cyanobacterial crystal structures. Given the absence of a higher plant crystal structure it is unclear if these differences are due to real differences between the cyanobacterial and higher plant photosystem, possible limitations in the current cyanobacterial structures (particularly with regard to subunit composition), or investigator error. Numerous crosslinking studies have indicated that in higher plants [35,41,42], green algae [43] and cyanobacteria [44] the PsbO protein can be crosslinked to CP47 using the 0-length crosslinker 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC). EDC crosslinks amino groups which are interacting with carboxylates via charge-pair interactions [45] and which are exposed to the bulk solvent. Odom et al. [46] mapped the crosslinked domain between these two components and reported that residues within the N-terminal trypptic peptide 1E–76 K of PsbO were crosslinked to residues within the domain 362E–440D of CP47. Examination of the T. vulcanus [5] crystal structure indicates that the only possible crosslinking candidates for this interaction are 307E of CP47 and the residue 158K of PsbO in spinach. The latter residue corresponds to 314K in T. vulcanus. Interestingly, this would be the result of inter-monomer crosslinking. However, these

5 In the original paper, Motoki et al. [36] examined “... a thermophilic cyanobacteria, Thermosynechococcus elongatus. This organism is now named Thermosynechococcus elongatus. Thermosynechococcus elongatus is now the genus and species name used for Synechococcus sp. PCC 7942.”
6 Synechocystis 6803 numbering.
residues are separated by nearly 10 Å, thus precluding these residues from forming the charge-pair interaction required for EDC crosslinking.

Frankel and Bricker [47] reported that upon removal of the PsbO component from spinach PS II membranes, the domains 308K–312K and 389K–411K of CP47 became susceptible to modification with the amino group-reactive reagent NHS-biotin. Possible biotinylated residues include 304K, 308K and 312K in the first domain and 389K, 411K and 415K in the second. Four of these CP47 residues are conserved in T. vulcanus (308K, 312K, 389K, and 411K). None of these residues appears closely associated with the PsbO protein in the T. vulcanus structure. Interestingly, residues 308K, 389K, and possibly 411K are surface-exposed residues. If the structure of higher plant PS II is similar to that observed for cyanobacteria, these residues should have been labeled with NHS-biotin. No biotinylation, however, of any residues on CP47 was observed in the presence of PsbO in the higher plant system [47].

Enami et al. [48] used the crosslinker hexamethylene-diisocyanate (HMDI) to crosslink PS II components in spinach NaCl-washed n-heptylthioglucoside-solubilized spinach PS II particles. HMDI can react with a variety of functional groups and can crosslink proteins which are within 11 Å [49]. Individual crosslinked products were observed which contained both the PsbO protein and either PsbI or PsbE (α-subunit of cytochrome b559). Examination of this result within the context of the T. vulcanus crystal structure yields interesting observations. The closest approach of PsbO with PsbI is 8–10 Å, a distance which is consistent with the crosslinking result [48]. The nearest approach of PsbE with PsbO, however, is over 30 Å, a distance which cannot be spanned with HMDI. One possible explanation for this result could be inter-dimer crosslinking. However, this is unlikely since the experiment was performed on solubilized PS II particles and, consequently, the effective local PS II concentration would be much lower than would be observed in PS II membranes.

2. Stoichiometry: cyanobacterial vs. higher plant

In all cyanobacterial PS II crystal structures published to date, one copy of the PsbO protein is present per monomer. This has led many workers to assume that one copy of PsbO is present in all oxygenic organisms [50,51]. In higher plants, however, persistent biochemical observations indicate that two copies of PsbO are present per monomer of the photosystem. These studies have been reviewed extensively elsewhere [21,52,53]. Briefly, this evidence falls into three main categories: protein quantification experiments, rebinding studies and mutant characterization. Direct immuno-quantification using internal PsbO protein standards indicates that two copies of PsbO are present per monomer in both PS II membranes and Β- dodecyl maltoside-solubilized PS II particles [54]. The number of PsbO copies bound is independent of the presence or absence of an intact Mn₄CaO₅ cluster, although the Kₐ for both PsbO copies is modulated by the intactness of the metal cluster [55]. Earlier, protein purification efforts had indicated that it was possible to isolate 1.7 mol of the PsbO component per mole PS II from PS II membranes; these membranes contain only strongly bound PsbO [56]. Rebinding studies from a number of laboratories examining higher plant PsbO binding to PS II membranes after extraction by CaCl₂ or NaCl-urea treatment demonstrated that binding of PsbO saturates at two copies of PsbO per PS II monomer [55,57–59]. The binding of native PsbO to PS II exhibits cooperativity with a Hill coefficient of 2.0 [55] and in some cases sigmoidal binding was evident [60]. Certain mutated [57,61] and chemically modified PsbO proteins [62] exhibit markedly sigmoidal binding isotherms. These are critical observations. The observed sigmoidicity necessitates cooperative binding which, by definition, requires at least two binding components. Finally, analysis of N-terminal truncation mutants has identified two binding domains, both of which must be present to observe normal PsbO binding stoichiometry [34,63]. One of these domains is not conserved in cyanobacterial sequences, perhaps explaining the apparent lack of a second copy of PsbO in these organisms. Functional studies indicate that the two copies of PsbO perform different functions within the photosystem. The first bound PsbO stabilizes the Mn₄CaO₅ and lowers the chloride requirement for oxygen evolution while the second PsbO further lowers the chloride requirement and optimizes oxygen evolution capacity [64,65].

If higher plants do contain two copies of the PsbO component, where is the second copy bound? At least two models are possible. First, the second copy could be bound symmetrically with respect to the first copy, on the luminal surface of the PS II complex. This would necessitate the second copy associating principally with the CP47 protein in a similar manner as the first copy associates principally with CP43 in the cyanobacterial crystal structure. In this model, the second copy of PsbO would be quite distant from the Mn₄CaO₅ cluster. A second possibility is that the two PsbO copies form a homodimer. Three lines of circumstantial evidence support this hypothesis. First, during crystallization experiments of the spinach protein, only PsbO dimers are observed in the microcrystals which formed [66]. Second, single particle analysis of PS II supercomplexes has interpreted the density arising from the PsbO subunit as being agolobar and/or being present as a dimer [67]. Finally, re-examination of the chemical modification data presented by Frankel and Bricker [68] indicated that the majority of the PsbO residues which were labeled when PsbO was in solution but which were not labeled when PsbO was associated with NaCl-washed PS II membranes are solvent-exposed in the

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Footnote 7: It should be noted that in the only reconstitution study performed with cyanobacterial mutated PsbO proteins, sigmoidal binding was also observed for some mutants [36].
T. vulcanus crystal structure. These residues may define the interaction domain of the second copy of the PsbO protein with higher plant PS II. It should be noted that these models are not necessarily exclusive. A homodimer of PsbO would almost certainly also interact with the CP47 present in the other PS II monomer. If this were the case, it could explain a number of the discrepant results described above [46,47]. Clearly, resolution of this question of PsbO stoichiometry in higher plants ultimately will require crystal structures of intact higher plant PS II.

While the cyanobacterial and higher plant PsbO proteins are quite similar, some differences have been noted. In addition to the N-terminal truncation which is present in all cyanobacteria (see above), several loop domains are also different. These features have been examined in detail elsewhere [20,53]. The most striking of these differences is the presence of a fourteen amino acid residue "cyano-loop" [20] which is present in all cyanobacteria. In T. vulcanus this loop extends from 129T to 141F while the analogous loop in spinach is quite small (130G–141F). The functional significance of this difference is unclear; however, it has been noted that the cyano-loop effectively compensates for the ubiquitous cyanobacterial N-terminal truncation, so that all PsbO proteins are approximately the same length. It has been suggested that this may have functional implications [53,69].

3. PsbO Function

Functionally, what parameters of the oxygen-evolving process are affected by the biochemical or genetic removal of PsbO? Numerous investigators have examined this question using higher plant PS II membranes which were depleted of the PsbO component by either CaCl₂ [70] or NaCl-urea [9] treatment. These preparations, while completely lacking the PsbO protein, retain the Mn₄CaO₅ cluster and the ability to evolve oxygen, albeit at very low rates [11]. It should be noted that these studies were typically performed at high chloride and calcium concentrations to maintain the intactness of the Mn₄CaO₅ cluster (see below) and to compensate for the absence of the PsbO and PsbQ subunits which are also removed by these treatments. Ono and Inoue [71], using thermoluminescence as a probe for S-state transitions, observed that the S₂ → S₃ → S₂ transition was significantly slowed. Miyao et al. [72] performed flash oxygen yield measurements on PsbO-depleted PS II membranes. These studies indicated that the S₂ lifetime of the PsbO-depleted samples increased five-fold and that the lifetime of the S₃ state also increased. Additionally, the S₂ → S₃ transition was slowed and the S₃ → S₄ transition was retarded two- to threefold. Similar results have been obtained in vivo. An Arabidopsis thaliana (hereafter Arabidopsis) mutant which lacks a functional PsbO-1 protein [59,73], the isoform responsible for normal PS II activity, exhibited long-lived S₂ and S₃ states [88]; this phenotype can be chemically complemented by the addition of calcium and a calcium ionophore but not by the addition of chloride, alone. The mechanism(s) by which the PsbO protein could modulate the chloride and calcium requirements for oxygen evolution, however, remain unclear.

The PsbO component was earlier termed the 'manganese-stabilizing protein' based on the observation that removal of this component from higher plant PS II membranes leads to a destabilization of the Mn₄CaO₅ cluster at low chloride concentrations. In the absence of added chloride, two of the four manganese become paramagnetically uncoupled [89] and are eventually lost to the bulk media [90,91]. In the presence of high chloride concentrations (>100 mM) the Mn₄CaO₅ cluster remains intact and oxygen can be evolved even in the complete absence of the PsbO component [11,90], although at significantly lower rates (20–40% of control). It has recently been reported that intermediate chloride concentrations (10–20 mM) preserve the integrity of the Mn₄CaO₅ cluster to varying degrees, and this is modulated in the higher plant system by the number of the PsbO subunits associated with each monomer [64]. While the PsbO protein stabilizes the active site Mn₄CaO₅ cluster, EXAFS experiments performed on PS II membranes depleted of PsbO indicated that essentially no change occurred in the structure of the metal cluster upon removal of PsbO [92]. This result strongly indicates that the PsbO protein does not provide any ligands directly to the Mn₄CaO₅ cluster. Early investigations also suggested that PsbO was directly involved in calcium binding at the oxygen-evolving site of PS II [93–95]; later studies indicated that PsbO was not involved in this process (see Seidler, [96], for an in-depth discussion). Seidler and Rutherford [97], for instance, demonstrated that in the absence of the PsbO protein, the high affinity binding site for calcium associated with the oxygen-evolving site was still present. These latter findings have been confirmed by the various cyanobacterial PS II crystal structures which indicate that the PsbO protein does not provide any ligands to either the calcium or manganese ions in the Mn₄CaO₅ cluster [57,8,98].

Putative low affinity calcium-binding sites, distinct from the active site metal cluster may, however, be present on the PsbO protein, although the functional significance of these is unclear. Murray and Barber [99] identified a calcium-binding site with the calcium ligands being ⁹⁵Se, ¹¹⁴C and ⁷⁷H, which was also observed by Gusakov et al. [8]. It has been speculated that this low affinity site may be associated with a putative proton exit pathway leading from the Mn₄CaO₅ cluster to the surface of the complex [7,20]. The authors note, however, that with the exception of ¹¹⁴C, these residues are rather poorly conserved.
The mechanism by which the PsbO protein modulates the chloride (and possibly calcium) requirements for the photosystem is unclear. Several conserved residues on the PsbO protein have been modified by site-directed mutagenesis in spinach in an attempt to clarify this process. Modification of residues 151R and 161R in spinach, yielding the R151G, R151D, and R161G variants [106], lower the binding affinity of PsbO for PS II. When PsbO-depleted PS II membranes were saturated with two copies of the mutated PsbO proteins per monomer, normal sequestration of chloride was not observed and oxygen was evolved at only 20–40% of control rates. The $K_d$ for chloride was 1.5–2.5 mM vs. 0.4 mM for NaCl-washed PS II membranes, which retain PsbO. No alteration for the affinity for calcium was observed. Mutations at position 153D in spinach PsbO (D157N, D157K and D157E) are particularly interesting [61]. PsbO proteins with these mutations exhibit essentially normal binding characteristics when reconstituted onto PsbO-depleted PS II membranes. Nevertheless, all of these altered proteins exhibit lower affinity for chloride ($K_d$ of 1.5–1.6 mM), no alteration in the affinity for calcium, and an inability to fully reconstitute steady-state oxygen evolution, with only about 50% of the control activity being observed. Interestingly, the D157E protein performs as poorly as the D157N and D157K variants. Additionally, fluorescence experiments indicated that the presence of these PsbO variants resulted in slow charge recombination in the presence of DCMU ($Q_{b} → Q_{a}$ electron transfer was not affected), indicative of a more stable $S_0$ state. Flash oxygen yield experiments indicated that after dark incubation a higher proportion of the PS II reaction centers were in the $S_0$ state [107]. Consequently, these results are similar to, but not as extreme as, results obtained from PS II membranes which lack the PsbO component or have been depleted of chloride. Popelkova and Yocum have subsequently hypothesized [53] that these residues (151D, 157D and 161R) along with the additional conserved PsbO residues 190K, 224D and 226D, interact to properly fold a domain important for chloride retention. Examination of the interactions among the analogous residues (152R, 158P, 163R, 168R, 222D and 224D) in the $T. vulcanus$ crystal structure [5] in large measure appears to confirm their hypothesis. PsbO residues 153D and 163R appear to form a charge-pair, while 152A appears to hydrogen bond with the backbone carbonyl of 64R of D1. 222D of PsbO may participate in three relevant interactions. First, its carboxylate may hydrogen bond with the backbone amide of 310G of D2; its backbone amide appears to hydrogen bond with the carboxylate of PsbO residue 222D, while its backbone carbonyl forms a hydrogen bond with the backbone amide of PsbO residue 226G. The backbone carboxyl of this latter residue appears to hydrogen bond with both 189R and 189R of PsbO. It should be noted that the domain 155R–162R of PsbO closely approaches both $Cl^{-} 1$ (10.1 Å) and $Cl^{-} 2$ (12.2 Å) in the $T. vulgaris$ crystal structure [5]. One can speculate that biochemical or genetic removal of the PsbO protein or site-directed modification of residues in the 152R–162R domain could lead to conformational changes in intervening residues, such as residues 317K of D2 and 334R of D1 (including residues in the vicinity of 78R which is hydrogen-bonded to 120R of PsbO) at the $Cl^{-} 1$ site and/or 336A–338N of D1 at the $Cl^{-} 2$ site, which would destabilize the bound chloride and result in the multitude of chloride-related defects described above.

Within PS II, access channel(s) must exist which allow the substrate water to reach the Mn$_4$CaO$_5$ cluster and exit channels must exit which allow the products, protons and molecular oxygen, to leave the active site. A number of in silico studies have sought to identify such channels and to determine the roles played by the various PS II subunits in their formation [20,108–110]. Recently, Gabdulkhakov et al. [111] have combined in silico studies using noble gas derivatization and dimethyl sulfoxide co-crystallization to identify nine possible channels in $T. elongatus$; the channels designated A1 and A2 are putative water entrance channels, channels B1 and B2 are putative oxygen egress channels and channels C–G are putative proton exit channels. A number of PsbO residues were predicted to be associated with water channel A2 and the proton exit channels C–E and G. The locations of these channels were calculated
using the 2.9 Å structure [8]. It will be most interesting to see similar analysis performed on the higher resolution T. vulcanus structure [5]. Determination of the actual functions performed by these putative channels will require additional experimentation.

4. PsbO as a GTPase

Evidence has been presented that the PsbO protein binds GTP with high affinity [112] and functions as a GTPase [113]. In this role, it has been hypothesized to act to control the phosphorylation state of the D1 protein [114]. Since the phosphorylation level of the D1 component appears to be coupled with its efficient PS II turnover [115,116], it was proposed that the PsbO protein may be an important regulatory component of this process [117]. In spinach, this proposed function is apparently performed by the single PsbO gene product found in this organism which also facilitates the oxygen-evolving process, as described above. The mechanisms regulating these two functions of the PsbO component are unclear. In Arabidopsis, two genes which encode the PsbO component (psbO-1, At5g66570 and psbO-2, At3g50820) are normally expressed, yielding two different PsbO proteins (PsbO-1 and PsbO-2, respectively). There are 11 amino acid differences between these two isoforms [73]. It has been hypothesized that the two functions ascribed to PsbO, support of oxygen evolution and regulation of PS II turnover, have differentiated between the two PsbO isoforms, with the primary function of the PsbO-1 protein being support of normal oxygen evolution by PS II and with the PsbO-2 protein principally acting to regulate the phosphorylation state and turnover of the D1 protein [114]. This suggestion was based largely on the observation that the PsbO-2 component exhibited substantially higher GTPase activity than the PsbO-1 protein while functioning poorly in support of oxygen evolution [117]. The hypothesis that the PsbO protein plays an important role in PS II turnover has been evaluated critically elsewhere [118].

5. PsbP and PsbQ

In higher plants, the PsbP and PsbQ proteins modulate the calcium and chloride requirement for oxygen evolution. In spinach, these proteins exhibit mature molecular masses of 20.2 kDa and 16.5 kDa, respectively [119]. Sequence alignments of these proteins from a variety of different organisms have been previously published [21]. Other members of the extended PsbP and PsbQ families have been found to function in PS II repair or as components of the chloroplastic NDH dehydrogenase and will not be examined in this review (for an in-depth examination of this topic, see [17–19]).

6. Structures

A number of groups have recently published crystal structures of PsbP from higher plants [120–122]. Fig. 3A illustrates the three-dimensional structure of the PsbP protein from spinach at 1.98 Å resolution (PDB ID: 2VU4, [121,122]). The structures of the spinach and tobacco (PDB ID: 1V2B, [120]) PsbP proteins and the cyanobacterial homologue CyanoP (PDB ID: 2XB3, [123]), which is shown in Fig. 3B, are quite similar although the cyanobacterial protein has very limited sequence homology with its higher plant counterparts [21]. The protein exhibits a two-strand anti-parallel β-sheet near its N-terminus, and a central anti-parallel six-strand β-sheet which is flanked by two α-helical domains. The N-terminus of the protein is disordered and is not resolved in any of the available structures. The protein contains a number of bound zinc ions, the biological consequences of which are unclear (see below). As originally pointed out by Ifuku et al. [120], the protein shares its fold with Mog1p (PDB ID: 1EQ6 [124]), a regulatory protein which interacts with the rna GTPase in yeast [125]. This is quite interesting as the PsbP protein is known to interact with the PsbO component which, as described above, has been suggested to exhibit GTPase activity [112,113].

Several groups have published crystal structures of PsbQ from higher plants [126,127]. Fig. 4A illustrates the three-dimensional structure of the PsbQ protein from spinach at 1.49 Å resolution (PDB ID: 1VYK, [127]). The protein exhibits a four α-helix bundle in its C-terminal domain which is very similar to the fold exhibited by the cyanobacterial homologue CyanoQ (PDB ID: 3LSO, [128]), as shown in Fig. 4B. A two-strand parallel β-sheet is located in the N-terminal domain along with a polyproline type II (PPI) left-handed helix formed by four sequential prolyl residues (Pro–Pro–Pro–Pro) in the spinach protein; these features are absent in CyanoQ. The spinach protein contains a number of bound Zn+2 ions, again the function, if any, of these is unknown (see below). The loop which connects the two β-strands (L–34T–35T) is not resolved in the current structure (probably because it exhibits high mobility) but has been modeled using a molecular dynamic simulation [129].

A variety of studies have examined the association of these components to the photosystem and to each other. Early studies indicated that the PsbO protein was required for PsbP binding and that the PsbP component was required for the association of PsbQ to PS II [130,131]. Relatively few studies have addressed the mechanism of PsbP and PsbQ binding to the photosystem. Ifuku and Sato [132] reported that the binding affinity of a recombinant mutant of the PsbP protein, of which
Additional investigations have attempted to identify the residues on PsbQ which are required for its association with the photosystem. Earlier work indicated that proteolytic removal of the N-terminal 12 amino acid residues of the protein abolished binding to the photosystem [135]. It was unclear, however, if this domain was sufficient to account for the interaction of PsbQ with the photosystem, and the amino acid residues responsible for the interaction were not determined. Meades et al. [136] used EDC coupled with glycine methyl ester to modify lysyl residues on the PsbQ protein. They identified four lysyl residues (90K, 96K, 101K, and 102K) whose modification also abolished high affinity binding of PsbQ to PS II. These residues all clustered on one face of the protein and are in the vicinity of the N-terminus (Fig. 4A).

In C. reinhardtii PS II, evidence is available for the direct interaction of PsbP with PsbQ. Enami and coworkers, using the 0-length crosslinker EDC, identified a crosslinked product formed between PsbP and PsbQ [43]. 176K of the PsbP protein was identified as being crosslinked to 28D of PsbQ. It should be noted that no EDC-crosslinked products have been identified between PsbP and PsbQ in higher plants. This may be due to the fact that 28D is not a conserved residue in higher plant PsbQ. 176K of C. reinhardtii is analogous to residue 114K in spinach, which was identified in the NSP-labeling experiments described above [133]. Additionally, Nagao et al. [43] showed immunologically that PsbP and the α subunit of cytochrome b559 (PsbE) were crosslinked with EDC, although the interacting residues were not identified. If this assignment is correct, then PsbP is bound to the opposite side of the PS II monomer from the PsbO protein. A direct interaction of PsbP with both PsbE and PsbO would require that PsbP occupy a position roughly similar to that occupied by PsbU and PsbV in the cyanobacterial crystal structure. This, however, would appear unlikely since the red algae contain stoichiometric amounts of PsbP, PsbQ, PsbU and PsbV [15,16] and, presumably, the PsbU and PsbV proteins in the red algae are bound to PS II in analogous locations to the PsbU and PsbV proteins in the cyanobacteria.

While most investigators believe that the standard model is correct (PsbO binding to the PS II core, PsbP binding to PsbO and PsbQ binding to PsbP), it should be noted that some results indicate that the association of PsbP and PsbQ with the photosystem may be more complicated and that the accepted model may need modification. Evidence has been presented indicating that green algal PsbP and PsbQ can bind independently of the PsbO component to the photosystem [137,138].

Additionally, three groups working in higher plant systems have reported that the PsbO protein can be removed from PS II while PsbP and PsbQ remain bound to the photosystem. Differential extraction experiments using either a butanol–water phase separation system [139] or HgCl2 [140,141] indicated that PsbO could be removed from PS II preparations without the concomitant extraction of the PsbP and PsbQ proteins. In the latter studies, a moderate chloride concentration inhibited the release of the PsbO component. Unfortunately, none of these investigators demonstrated that the PsbP and PsbQ proteins were bound normally after HgCl2 treatment and could subsequently be extracted by standard procedures (i.e. 1 M NaCl treatment). It is possible that PsbO is required for the initial binding of the PsbP component but that upon binding, conformational changes occur within the intrinsic components of PS II which allow the selective removal of PsbO while PsbP and PsbQ are retained [21]. In other studies, Caffarri et al. [142] used image analysis of detergent-solubilized (ω-ω-dodecyl maltoside) PS II particles from several Arabidopsis mutants which were resolved by sucrose density gradient ultracentrifugation, to examine the association of the light-harvesting antennae proteins with the PS II core complex. They reported that, while PsbO is present in all PS II core-containing particles, PsbQ was present only in supercomplexes which contained Lhcb and that PsbP binding required CP26/LHCII. Unfortunately, the majority of these experiments were performed at pH 7.5, a pH known to destabilize the oxygen-evolving complex. Additionally, the functional integrity of their preparations is difficult to determine since their oxygen evolution experiments also were performed at pH 7.5 and at sub-saturating light intensities. Nevertheless, it is possible that removal of subsets of the light-harvesting apparatus might induce conformational changes in the PS II core proteins which could alter the binding characteristics of either PsbP or PsbQ to the photosystem.
7. PsbP and PsbQ functions

Many early studies have demonstrated that the PsbP and PsbQ proteins function to optimize oxygen evolution at physiological concentrations of calcium and chloride. These findings have been reviewed in detail elsewhere [21,96,143,144]. Briefly, removal of the PsbP and PsbQ proteins by NaCl treatment of PS II membranes results in a marked loss of oxygen evolution activity (to about 25% of control). Much of the lost activity can be restored by reconstitution of the extracted protein(s) to protein-depleted PS II [90,145–147], but only if the calcium and chloride cofactors are present during the reconstitution [90,148,149]. It should be noted that high, non-physiological concentrations of calcium and chloride can support reconstitution onto the PsbP and PsbQ components [90,148,150]. Inhibition of oxygen-evolving activity after extraction of the PsbP and PsbQ proteins is accelerated in the light. This appears to be due to differential binding affinities for both calcium [151,152] and chloride [153,154], which are modulated by the S-state of the oxygen-evolving complex. Depletion of the PsbP and PsbQ components also markedly increases the accessibility of the Mn4CaO5 cluster to exogenous reductants [155].

While phenomenologically the PsbP and PsbQ proteins optimize oxygen evolution under physiological calcium and chloride concentrations, the mechanism(s) of such optimization remain largely unknown. At least two possibilities exist. First, the proteins could serve to stabilize a conformation of PS II required for the normal integration of these ions into the oxygen-evolving complex. This would imply that other components of PS II exist in different conformational states which are dependent on the presence or absence of the PsbP and/or PsbQ proteins. Direct evidence for such conformational changes exists. Removal of these proteins appears to introduce alterations in the function and structure of the PS II complex. Functionally, genetic or biochemical removal of PsbP (with the concomitant removal of PsbQ) leads not only to alterations in oxygen evolution capability but also to alterations in electron transport from QA to QB [156,157]. No reducing-side defects are associated with the removal of PsbQ alone [158], at least in plants grown under normal growth light conditions. Since both the QA and QB sites reside on the stromal side of the membrane and PsbP is bound to the luminal side, these results indicate that transmembrane conformational changes occur upon the removal of PsbP. Image analysis of PS II supercomplexes from higher plants indicates that removal of PsbP and PsbQ induces CP29 to move approximately 12 Å towards the central core of the PS II complex [67]. This result indicates that the structural organization of the core proteins of PS II is altered by the removal of PsbP and PsbQ. The most likely candidates for such conformational changes are the D1 and D2 proteins, which bear the QA and QB-binding sites. Conformational changes have also been observed during PS II binding to PS I in an FTIR study using 12C-labeled PsbP, Tomita and coworkers [159] specifically probed for conformational changes in PS II arising from PsbP binding [160]. Conformational changes occurring in the vicinity of the oxygen-evolving complex were identified with no identifiable changes being observed in the PsbP protein itself. When reconstitution was carried out with an N-terminally truncated PsbP (Δ15-PsbP), the conformational changes were not observed. Previously, it had been shown that the Δ15-PsbP truncated protein, while able to bind to the photosystem, could not restore normal calcium and chloride binding upon reconstitution onto PsbP-depleted PS II membranes [161]. These results indicate that the N-terminal 15 amino acid residues of PsbP are critical in supporting normal calcium and chloride function in the photosystem. Consequently, there is a correlation between the absence of induced conformational changes near the oxygen-evolving complex, the inability to restore oxygen evolution at physiological calcium and/or chloride concentrations and the absence of the N-terminal 15 amino acid residues of PsbP. Unfortunately, these residues are not resolved in the current crystal structures [120–122]. It should be noted that this view is complicated by the observation that PsbQ may act in a coordinated manner with PsbP with respect to calcium sequestration. This hypothesis has been suggested previously, although without direct evidence [152,162]. The N-terminal truncation of 19 amino acid residues from PsbP yields a protein (Δ19-PsbP) which exhibits non-saturating binding characteristics and the complete loss of the ability to lower the calcium requirement for oxygen evolution [122]. If, however, native PsbQ protein is subsequently reconstituted onto Δ19-PsbP protein-containing PS II membranes, much of the oxygen evolution rate is recovered, even in the absence of added calcium. So, calcium retention can be restored in the presence of a truncated PsbP protein and, presumably, the absence of conformational alterations in the vicinity of the oxygen-evolving site. If the conformational changes observed are directly related to the ability to retain calcium, then similar conformational changes should be observed after reconstitution of PS II with the truncated PsbP protein and PsbQ. The possibility of such conformational changes has not been examined experimentally.

A second possibility is that the PsbP and/or the PsbQ components actively sequester the calcium and chloride cofactors required for active and stable oxygen evolution. While no direct evidence for this possibility exists, some circumstantial evidence is available, at least with respect to the possibility that the PsbP protein can bind divalent cations like calcium. The high resolution crystal structures of both PsbP and Cyanop contain bound zinc ion(s). In tobacco and spinach PsbP [121], the zinc appears to be ligated to 14His and 16Asp, which also corresponds to one of the four zinc-binding sites observed in Cyanop [123]. It is unclear at this time if this association is physiologically relevant or is an artifact. Three possibilities exist. First, the bound zinc could be physiologically relevant. Trace metal analysis of PS II membrane preparations from higher plants indicated that PS II membranes which contained 4 atoms of manganese per PS II also contained 1–2 atoms of zinc per PS II [163,164]. The physiological consequences of the removal of this zinc from PS II have not been thoroughly investigated, although it should be noted that zinc is an inhibitor of PS II activity in both higher plants and cyanobacteria [165,166]. A second possibility is the PsbP protein contains a physiologically relevant divalent cation binding site which, during crystallization [123], becomes occupied by a zinc atom. The most likely ions for such an interaction would be either calcium or manganese. As noted earlier, PsbP appears to function in regulating the calcium and chloride requirements for optimal PS II activity. If, in vivo, a calcium ion was bound to this site it could conceivably donate this ion during formation of the Mn4CaO5 cluster or alter the calcium equilibrium in favor of bound calcium within the active site. Calcium was not present during the crystallization process in any of these studies. In this regard, it should be pointed out that it has been hypothesized that PsbP is a manganese-binding protein which delivers manganese to the oxygen-evolving site during photoactivation. This hypothesis has been critically evaluated elsewhere [118]. Finally, given the high zinc concentrations (5 mM in the case of the spinach protein [121], 200 mM in the case of Cyanop [123]) present during the crystallization of PsbP, it is possible that the zinc binding is purely an artifact of the crystallization process. Analogous arguments can be made concerning the observed bound zinc in the PsbQ and Cyanop Q structures (see below).

Both PsbP and PsbQ may be phosphorylated in Arabidopsis. While no phosphorylation has been reported using standard radiolabeling procedures in any system, two recent proteomic studies have identified putative phosphorylated peptides from these two components after IMAC or TiO2 enrichment for phosphopeptides [167,168]. PsbP-1 and PsbP-2 were reported to contain multiple phosphorylation sites (Thr, Ser, Tyr) of the mature protein. While phosphorylated PsbQ-1 peptides were not identified, phosphorylation of the mature PsbQ-2 protein at Ser of the mature protein was detected. Both PsbP and PsbQ
phosphorylation was detected under both dark and light incubation conditions. The protein kinases and phosphatases associated with the observed phosphorylation have not been identified and the biological role played by the phosphorylation remains undetermined.

Finally, the PsbP and PsbQ proteins appear to function as assembly and/or stability factors for PS II in higher plants. These findings result from RNAi suppression experiments used to examine the effects of the loss of PsbP in tobacco [169] and Arabidopsis [156]. The results obtained in these two experimental systems are quite similar. Moderately strong suppression of PsbP (5–10% wild-type levels) leads to slow photoautotrophic growth and a variety of functional defects associated with both the oxidizing and reducing sides of the photosystem [156,169,170]. The PS II reaction center proteins (CP47, CP43, D1 and D2) accumulate to relatively normal amounts, with only modest decreases being observed and with the accumulated proteins assembling into PS II dimers. LHC II–PS II supercomplexes, however, do not form and the reaction center proteins and the LHC proteins are not phosphorylated [170]. Significant defects in chloroplast and thylakoid architecture are also apparent in these plants [170,171]; however, the mechanism(s) giving rise to these alterations are unknown. Under stronger suppression of the PsbP protein (<1% wild-type levels), a significantly different phenotype is observed. While, as expected, the various PS II functional alterations are exacerbated and the ability to grow photoautotrophically is lost, the mutant’s ability to accumulate the PS II core proteins CP47 and D2 is markedly reduced [156] and the thylakoid membranes are dramatically altered [171]. It is interesting to note that the loss of the reaction center components does not parallel the loss of PsbP; even low levels of PsbP (5–10% of wild type) support the near normal accumulation of these intrinsic components. It is only under severe depletion of PsbP that loss of the reaction center components is observed. In this respect, it is possible to hypothesize that PsbP may possess some “catalytic” function with respect to PS II assembly. The suppression of PsbP expression, while not affecting the accumulation of PsbQ, leads to the complete loss of the PsbQ component, even in plants containing detectable levels of PsbP [156,169]. It is unclear at this time whether the loss of the PsbQ protein is due to decreased synthesis, inefficient transport or increased degradation. It should be noted, however, that large pools of unassembled mature PsbQ, PsbQ and PsbP proteins normally exist in the thylakoid lumen without being degraded either in the presence [172,173] or in the absence [174,175] of assembled and functional PS II reaction centers. Consequently, the loss of the PsbQ component must be the direct result of the loss of expression of the PsbP protein and not a consequence of the reduced levels of PS II reaction centers observed when the PsbP component is strongly suppressed.

The PsbQ protein has also been examined using RNAi suppression techniques in both tobacco [169] and Arabidopsis [158]. Under normal growth light conditions, the complete suppression of PsbQ expression did not affect photoautotrophic growth, PS II assembly, or most PS II functional parameters (oxygen evolution rate, Fv/Fm, etc.) in system. In Arabidopsis some modest defects were observed with respect to S-state distribution and the stability of the oxygen-evolving complex under dark incubation conditions [158]. A major alteration in phenotype was observed, however, when Arabidopsis mutants lacking the PsbQ proteins were placed under low light conditions (5 μmol photons·m⁻²·s⁻¹). After three weeks these plants yellowed and died while wild-type plants were unaffected. The PsbQ-deficient plants lost the ability to assemble functional PS II reaction centers, with major losses of the CP47, CP43 and D2 components being observed. Interestingly the D1 protein was not seriously affected. The functional characteristics of the PS II reaction centers which did assemble were also seriously compromised [158]. A more detailed examination of the assembly characteristics of PsbP- and PsbQ-deficient higher plants and a comparison between the higher plant, C. reinhardtii and cyanobacterial model systems is presented elsewhere [118].

8. PsbR

The PsbR protein is the most enigmatic of the extrinsic proteins associated with higher plant and green algal PS II; genes encoding this component are not found in the genomes of cyanobacteria or red algae. PsbR was first identified in the PS II complex of spinach [176]. Higher plants utilize a poorly understood targeting mechanism for this component. PrePsbR, which is translated on cytosolic ribosomes, has a very short transit sequence (only 41 amino acid residues in both Arabidopsis and spinach) relative to the other nuclear-encoded extrinsic proteins of the PS II complex. For instance, the PsbO-1 protein in Arabidopsis contains an 85 amino acid residue bipartite transit sequence. It has been suggested that the shortened N-terminal sequence acts to target PsbR to the chloroplast stroma while a C-terminal hydrophobic domain of the protein functions as a non-cleavable signal for lumenal transport. This domain would subsequently act as a hydrophobic anchor of the PsbR protein to the luminal side of the thylakoid membrane, although this hypothesis has not been tested experimentally. The hydrophobic nature of the C-terminus of the PsbR protein apparently makes this component relatively insoluble and the protein tends to precipitate during isolation [178]. Interestingly, the protein is removed from PS II membranes by alkaline-Tris washing of PS II membranes [176,178]. This almost certainly indicates that the hydrophobic C-terminal domain is not organized as a transmembrane α-helix since proteins with a single transmembrane helix, such as cytochrome f, are not removed by this treatment.

It is unclear which subunits are the nearest neighbors of PsbR within the PS II complex. Since PsbR is not present in cyanobacterial PS II, no crystallographic information is available. The only experimental structural information is from the analysis of protein crosslinking experiments [179] using 3,3′-dithiobis(sulfosuccinimidylpropionate), a cleavable crosslinker which spans 12 Å. In this study, CP47 formed a crosslinked species with an unidentifiable 10 kDa polypeptide, yielding a 54–56 kDa product. The authors tentatively identified the 10 kDa component as the PsbR protein due to its apparent molecular weight and the disappearance of the crosslinked species when the PS II membranes were Tris-washed prior to the initiation of the crosslinking experiment. PsbR appears to be required for PsbP assembly into the PS II complex. Additionally, PsbJ, a low molecular mass plastid-encoded hydrophobic subunit, appears to be required for the stable assembly of PsbR [180] and, consequently, PsbP [181,182]. One possible model would be that PsbJ serves to anchor the PsbR protein to the PS II complex (possibly by interacting with the hydrophobic C-terminus of PsbR) and that PsbR participates along with PsbO in the formation of a binding domain for PsbP. If this model is correct, PsbR would be positioned at approximately the location of PsbV in cyanobacteria.

In the absence of crystallographic information, I-TASSER was used to predict the three-dimensional structure of the PsbR protein [183,184]. I-TASSER was the best performing structural prediction software platform on the CASP-7, CASP-8 and CASP-9 three-dimensional structural prediction trials. The top two models provided by I-TASSER are shown in Fig. 5. While such models must be considered tentative, in all of the predicted structures examined (top five models) the C-terminal domain is predicted to be organized as a hydrophobic helix–coil–helix motif. The longest helical domain averaged about fourteen amino acid residues in length. It is unlikely that such a structure could span the thylakoid membrane and it is

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9 In cyanobacteria the loss of CyanoP or CyanoQ does not dramatically affect PS II assembly or stability.

10 In this regard it should be noted that the closest approach of PsbV to CP47 in cyanobacteria is less than 12 Å [5]. If PsbR occupies nearly the same position in higher plants, this could be consistent with the crosslinking evidence presented in [179].
possible that this anchors PsbR to the membrane (or to hydrophobic protein subunits such as PsbJ), which would be consistent with its release by alkaline–Tris treatment [176,178].

The function of the PsbR protein in vivo has been investigated using mutant plants in both potato [185] and Arabidopsis [180,186,187]. In these reports, the absence of the PsbR protein was reported to lead to impaired oxygen evolution and Q₅ reoxidation. These defects may be directly related to the inability of the PsbR proteins to bind to PS II in the absence of the PsbR protein. In initial studies, the absence of PsbR did not appear to affect the accumulation of other PS I components. Specifically, in whole leaf extracts, the amounts of immunologically detectable PsbP and PsbQ proteins were not affected [185]. Later studies, however, examining thylakoids [180,186] or PS II membranes [187] isolated from these reports, the absence of PsbR protein was reported to lead to impaired oxygen evolution and QA reduction at pH 10 [201]. Although the mechanistic basis for this pH-dependent growth has proved difficult to pinpoint [202] it does appear consistent with the in vitro binding studies performed with T. vulcanus, since in those reconstruction studies, PsbU bound with twice the affinity to complexes having only PsbV compared with complexes having only PsbO [199]. Gene deletion and gene interruption studies with Synechocystis 6803 found the ΔpsbV mutant and the ΔpsbV:ΔpsbU double mutant had indistinguishable phenotypes, both consistent with PsbU requiring the presence of PsbV to functionally associate with PS II [200,201]. Interestingly, while the ΔpsbV:ΔpsbU double mutant was able to grow phototrophically, the ΔpsbO:ΔpsbU double mutant was not — at least when the cells were grown at pH 7.5. Phototrophy could be restored to the ΔpsbO:ΔpsbU double mutant when cells were grown at pH 10 [201].

9. PsbU

9.1. Structure

The X-ray-derived crystal structure of bound PsbU has been determined in T. vulcanus [5,191] and T. elongatus [7,8,98]. The structure of PsbU from T. vulcanus at a resolution of 1.9 Å is shown in Fig. 1 in blue and possesses six alpha helices connected by five loops with little or no β-sheet structure being recognizable. No functional domains or other structural homology exist to other proteins in the current databases. The interacting partners in the photosystem include CP47, PsbO and PsbV.

PsbU was first identified in the cyanobacterium Phormidium laminosum as an ∼9 kDa polypeptide [192] and was subsequently shown to be a stoichiometric component of cyanobacterial PS II [193]. The first report of this protein in a eukaryotic alga was in isolated PS II preparations from the red alga Cyanidium caldarium (henceforth, C. caldarium) [194]. Unlike PsbV, PsbU is nuclear-encoded in the red algae [195,196]. The PsbU subunit has been identified in most cyanobacteria, with the exception of Prochlorococcus marinus strains MED4 and SS120, although it is present in P. marinus MIT9313. This subunit has also been detected in chlorophyll a/c-containing algae [197,198]. In contrast, PsbU is absent from all chlorophyll a/b-containing eukaryotes. The binding of PsbU to PS II was initially studied in isolated PS II complexes from T. vulcanus [193,199]. The PsbU protein was not able to bind to PS II in the absence of PsbO and PsbV. PsbU was observed to bind at low levels if only one of these polypeptides was present [199].
9.2. Function

Synechocystis 6803 cells lacking PsbU are photoautotrophic when grown under standard laboratory conditions in BG-11 growth medium and assemble PS II centers at a similar level to wild type [200,203,204]. While photoautotrophic growth rates of the mutant and wild type are similar, the ΔpsbU cells have a more stable S2 state [200,204,205], although the consequences of this for the viability of the cells are not immediately obvious. In the absence of PsbU, oxygen evolution rates are reduced by typically 20% or more relative to wild type and photoinactivation is observed during steady-state oxygen evolution measurements. This is consistent with the observation that ΔpsbU mutants are susceptible to photodamage when exposed to high light, exhibiting a more rapid degradation of the D1 protein [200,203,204,206–208].

In addition to the above PS II-specific perturbations, the ΔpsbU mutants are more susceptible to heat stress. Heat-inactivation of PS II is known to involve the loss of manganese from the oxygen-evolving complex [209]. Addition of PsbU to isolated thylakoid membranes from Synechococcus sp. PCC 7002 (henceforth Synechococcus 7002) that had been depleted of this subunit increased the heat stability of oxygen evolution while inactivation of psbU in Synechococcus 7002 prevented the ability of these mutants to acclimate to high temperature. In the absence of PsbU, the cells were unable to acquire cellular thermotolerance [210–212]. A similar result was obtained with a ΔpsbU strain of Synechocystis 6803, although in this cyanobacterium the removal of PsbU only partially prevented acclimation to high temperature, whereas cells lacking PsbO or PsbV failed to develop cellular thermotolerance when grown at moderately high temperatures [206]. These results indicate that the stability of PS II, conferred by the luminal extrinsic proteins, is important for cells to acquire thermotolerance by optimizing specific protein–protein or protein–lipid interactions; however, the mechanism is as yet unidentified.

Interestingly, removal of PsbU in Synechocystis 6803 also resulted in uncoupling of energy transfer from the phycobilisome to the photosystem. ΔpsbU cells exhibited a decreased chlorophyll a variable fluorescence yield when excited with light absorbed by allophycocyanin but not when excited by light absorbed primarily by chlorophyll a [203,207]. Furthermore, picosecond fluorescence decay kinetics revealed changes in both allophycocyanin- and PS II-associated decay components, consistent with a decrease in the coupling of phycobilisomes to PS II and an increase in the number of closed PS II reaction centers in the dark-adapted ΔpsbU mutant [207]. Thus removal of PsbU introduces PS II-specific structural changes on both sides of the thylakoid membrane.

Inactivation of PsbU has also been studied in Synechococcus sp. PCC 7942 (henceforth, S. elongatous) [208]. These experiments demonstrated an increased rate of D1 degradation under both low and high light conditions. In addition, the recombination reaction between the reduced primary quinone acceptor QA and the S2 state in the presence of DCMU was inhibited by up to 5-fold in the mutant, which is perhaps consistent with the long-lived S2 state observed in the ΔpsbU mutant of Synechocystis 6803 [200,204]. However, the thermoluminescence signal in the ΔpsbU mutant of S. elongatous was greatly diminished compared to that observed in Synechocystis 6803 [199]. This result is difficult to interpret and could possibly be a result of uncoupling of the phycobilisome from the reaction center, as reported to be the case in Synechocystis 6803 ΔpsbU cells [207] or irreversible inactivation of PS II in the S. elongatous mutant at the sub-zero temperatures utilized in the thermoluminescence assay. The suggestion that the observed thermoluminescence signal might arise from the loss of additional protein subunits in the mutant seems less likely since oxygen evolution rates were reported to be similar in both the ΔpsbU mutant and wild type [208]. However, the most striking result reported for the ΔpsbU S. elongatous strain was the viability of the mutant cells when challenged by H2O2 or methyl viologen. Compared to wild type, the mutant exhibited greater resistance and appeared to up-regulate antioxidant enzymes. This study suggests that perturbation of PS II activity by removing PsbU may increase the production of reactive oxygen species. Consequently these mutants may be more resistant when challenged by an external oxidative stress because their endogenous protective mechanisms are already activated [208].

The requirements for the chloride and calcium cofactors in PS II are modulated by the extrinsic proteins of the photosystem [213]; the effects of omitting these ions have been investigated in ΔpsbU strains of Synechocystis 6803. Photoautotrophic growth in calcium-limiting medium was similar for wild type and the ΔpsbU mutant; however, under chloride-limiting conditions, growth was retarded in the ΔpsbU strain. Additionally, photoautotrophic growth was abolished for the ΔpsbU strain when both Ca2+ and Cl– were omitted from the growth medium [39,200,204]. In the 1.9 Å resolution structure of PS II from T. vulcanus two chloride ions have been identified in the vicinity of the Mn4CaO5 cluster of the oxygen-evolving complex and may contribute to the maintenance of the coordination environment of the cluster [5]; hence it is possible that structural rearrangements resulting from removing PsbU might affect the integrity of these chloride-binding sites.

The two chloride binding sites identified in PS II from T. vulcanus are positioned at the start of hydrogen bond networks arising from the Mn4CaO5 cluster and extending towards the bulk solution. The network through the Cl–1 site is located in the interface of the D1, D2 and PsbB subunits, while the Cl–2 site is located at the interface of D1, CP43 and PsbB subunits. These channels may function as proton exit channels or water inlet channels and hence removing PsbU may disrupt these processes, which in turn may contribute to the phenotype of the ΔpsbU mutants. The presence of a putative oxygen exit channel in the vicinity of PsbU in T. vulcanus is shown in Fig. 6 and would correspond to the putative B2 oxygen exit channel suggested by Gabdulkhakov et al. [111]. The channel is formed by contributions from the D1, D2, CP43, CP47, PsbB and PsbV subunits with the mouth of the channel composed of residues from PsbB, PsbB and D2.

A similar role for PsbU has been suggested following analysis of the X-ray-derived PS II structure from T. elongatous at 2.9 Å resolution [111]. Theoretical calculations suggested the presence of several channels potentially associated with water access, proton exit and oxygen exit. The two channels suggested to play a role in oxygen exit received experimental support by co-cystalization with the noble gas krypton as well as dimethyl sulfoxide, which were both shown to localize to the putative channels. One of the exit channels was blocked by the C-terminal lysyl residue of PsbU (136K in PsbU from T. elongatous) and the authors proposed that the C-terminus of PsbU might operate in a gating mechanism for this channel. While the lysyl residue at this position is not absolutely conserved, an arginyl residue is frequently present that would preserve the required hydrogen bonding network; however, some PsbU sequences do not retain a positive charge at their C-terminus, and therefore the significance of this proposal requires further experimental investigation. Indeed, mutation of this terminal lysyl residue in vitro employing PS II isolated from the red alga C. caldarium did not produce any detectable phenotype [214].

Although the C-terminal lysyl residue of PsbU is not always present, the adjacent tyrosyl residue (103Y) is conserved in both prokaryotic and eukaryotic organisms. Mutation of this residue dramatically altered the calcium and chloride requirements for oxygen evolution and resembled a PsbU-minus phenotype even though the binding of PsbU was unaffected. Moreover, only phenylalanine can partially substitute for tyrosine at this position suggesting that an aromatic group is required [214,215]. Okumura and co-workers have proposed that 103Y interacts with 340P located near the C-terminus of the D1 protein and it is the disruption of this interaction that results in the elevated requirement for calcium and chloride in the absence of PsbU or upon mutation of the tyrosyl residue [214,215]. It is therefore plausible that the pleiotropic effects
of removing PsbU from PS II manifest themselves through structural changes that include alteration of the chloride-binding site(s) which, in turn, can perturb the environment of the Mn₄CaO₅ cluster, as well as altering additional protein–protein or protein–lipid interactions within the photosystem. Additionally, ¹⁰³⁵Y may indirectly interact with ²²⁰⁸R of CP43. ²²⁰⁸R:CP43 hydrogen bonds with ¹⁰⁸⁶N:PsbU (moreover a second hydrogen bond between these two residues is also apparent via an intervening water: see Fig. 6) and this residue forms a hydrogen bond with ¹⁰³⁵Y:PsbU. Within this context, mutation of the analogous Synechocystis 6803 CP43 lysyl residue (³⁰⁷⁵R) yields a mutant (R305S) which, while growing at a normal rate in complete medium, exhibited a loss of oxygen evolution capability (70% of control), assembled 30% fewer PS II reaction centers and was more susceptible to photoinactivation [216]. Under chloride-limiting conditions this mutant grew very slowly, evolved oxygen at only 20% of the control rate, and a loss of oxygen evolution capability (70% of control), assembled 30% fewer PS II reaction centers and was more susceptible to photoinactivation [216]. Under chloride-limiting conditions this mutant grew very slowly, evolved oxygen at only 20% of the control rate, and exhibited a long-lived S₂ state [217]. PS II particles isolated from this mutant lost the ability to bind PsbV [218] and PsbU (T. M. Bricker and C. Putnam-Evans, unpublished). These results indicate that this CP43 arginyl residue may be involved in the formation of a high-affinity binding site for both PsbU and PsbV.

Isolated PS II from C. caldarium has been used extensively to investigate the role of PsbU and this has established that the binding of PsbU moderates the requirements for calcium and chloride by minimizing the concentrations of these cofactors that are required for maximum activity [¹⁴2¹⁴]. Hence the requirements for calcium and chloride upon removal of PsbU in red algae and cyanobacteria are similar; however, while the PsbQ orthologue (PsbQ'), PsbU and PsbV from C. caldarium are able to bind to T. vulcanus PS II, leading to restoration of oxygen evolution, the cyanobacterial PsbU and PsbV from T. vulcanus only partially rebind to C. caldarium PS II and are unable to restore PS II activity [2¹⁹]. Therefore, while the extrinsic protein environments of red algae and cyanobacteria are similar, they are not identical. Nevertheless, red algal PsbU can functionally bind to PS II from T. vulcanus in the presence of either the cyanobacterial or red algal PsbV proteins, although cyanobacterial PsbU cannot reconstitute red algal PS II even in the presence of cyanobacterial PsbV [²²²].

10. PsbV

PsbV, also referred to as cytochrome c₅₅₃, is a PS II luminal extrinsic subunit found in many cyanobacteria and red algae. It is a c-type monoheme cytochrome with a molecular weight of approximately 15 kDa and an acidic isoelectric point. The existence of a low-potential cytochrome in cyanobacterial species has long been known [²²¹], but PsbV was first identified as a stoichiometric PS II component in isolated core complexes from T. vulcanus [²²²]. Since then, PsbV has been the focus of extensive research aimed at understanding its contribution to PS II oxygen-evolving activity given the unusually low midpoint potential (Eₗₘ) of its heme group.

10.1. Structure

A number of structural studies have been performed on soluble PsbV from different cyanobacteria including Synechocystis 6803, Arthrospira maxima, and T. elongatus [2²³–2²⁵]. The PsbV heme is covalently bound to two cysteinyl residues (¹⁷²C and ¹⁴⁶C) and non-covalently coordinated by two histidyl axial ligands (¹⁴¹H and ¹⁵²H) [²²³–²²⁵]. This bis-histidyl ligation scheme is somewhat unusual for c-type cytochromes. However, the rest of the protein structure is quite typical of other cytochromes, with a hydrophobic inner core of the protein forming a pocket for the prosthetic group and generally protecting it from the solvent, with the exception of an exposed edge including rings C and D along with the D propionate group.

PsbV has also been structurally resolved as a component of PS II crystal structures (see Fig. 1, green subunit) from thermophilic cyanobacteria [⁵,⁷,⁸,⁹,²¹⁹], and the soluble and PS II-bound PsbV protein structures are quite similar. Within the context of cyanobacterial PS II, the PsbV protein is located near lumenal loop regions of the CP43 (residues 386–397 and 4¹²–4¹⁷) and D₁ (residues 3⁰⁵–3¹⁵) proteins [⁵]. The ³²⁰⁸R residue of CP43 also comes to within 3 Å of residues ⁴⁹⁸ and ⁵¹⁸ on PsbV. This is significant because site-directed modification of the analogous residue in Synechocystis 6803 (CP43 ³⁰⁵₉) exhibited markedly reduced PsbV binding to PS II [²¹⁸]. In the current PS II structure calcium and chloride ions can each be observed at the periphery of PsbV, but the closest residues to these ions on PsbV are more than 4 Å away. The C and D rings of the heme group are shielded by large lumenal loop regions of the CP43 protein, but there is a small opening in the protein surface that exposes the propionate oxygen of the D ring. The closest edge of the heme group is approximately 23 Å away from the nearest manganese atom of the Mn₄CaO₅ cluster, which is the nearest redox cofactor to the PsbV heme in PS II [⁵]. Residues of the PsbV protein (¹⁴³⁵Y and ¹⁴²⁵K) have also been implicated in a hydrogen bond network linking Y₂ and the bulk solvent on the lumenal side of the complex. This channel, formed by residues of the D₁, CP43 and PsbV proteins, may serve as a proton exit channel for proton-coupled electron transfer via Y₂ [⁵].

The binding properties of PsbV differ between cyanobacteria and red algae. Reconstitution experiments in T. vulcanus revealed that the PsbV protein could bind to PS II complexes independently of the PsbO protein, but this association did not support oxygen-evolving activity. The presence of both the PsbO and PsbU proteins was necessary for

Fig. 6. The presence of a putative oxygen exit channel in the vicinity of PsbU in T. vulcanus (PDB ID: 3ARC). The channel (gray) was predicted using the CAVER program [²⁵⁵,²⁶⁰]. Waters and a chloride (green) from the PDB ID: 3ARC file that are located in the channel are depicted as spheres. The calcium (yellow), manganese (purple) and oxygens (red) of the Mn₄CaO₅ cluster are shown as spheres. In addition the position of the conserved ¹⁰³⁵Y:PsbU residue is shown. The hydrogen bond network connecting ¹⁰³⁵Y:PsbU (PsbU:Y1⁰³) with ²²¹, but PsbV was first identified as a stoichiometric PS II component in isolated core complexes from T. vulcanus [²²²]. Since then, PsbV has been the focus of extensive research aimed at understanding its contribution to PS II oxygen-evolving activity given the unusually low midpoint potential (Eₗₘ) of its heme group.

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full rebinding and maximal oxygen evolution rates [199]. PsbV was also identified in PS II preparations of the red alga C. caldarium [194,226]. In contrast, PsbV could not bind independently of the other extrinsic proteins in red algae [15]. Cross-reconstitution studies found the red algal PsbV protein to be functionally exchangeable between the cyanobacterial and red algal PS II systems. However, this was not true for the cyanobacterial PsbV protein [220]. Thus, while the PsbV proteins from cyanobacteria and red algae are quite similar (40–60% identical), the binding sites of the PS II membrane components are apparently quite different between the two systems [220].

10.2. Function

Subsequent variant studies in cyanobacteria further solidified the link between the PsbV protein and PS II oxygen evolution. A Synechocystis 6803 mutant lacking PsbV (ΔpsbV) has reduced accumulation of PS II in the thylakoids (60% of that of wild type) and lower oxygen evolution rates (40% relative to wild type) [227]. Consequently, the ΔpsbV mutant exhibits severe growth phenotypes. The doubling time for photoautotrophic growth rates in complete medium. However, in Synechocystis ΔpsbV mutant, the doubling time for photoautotrophic growth of Synechocystis 6803 ΔpsbV mutant was not reported [229]. The authors postulate that the presence of PsbV is +200 mV when bound to PS II, it is possible that electron transfer reactions (ms−1 timescale) could occur between the

Additionally, Synechocystis 6803 ΔpsbV mutant cells heterologously expressing psbV2 contained detectable amounts of heme corresponding to cytochrome c550; however, no heme was detected in cells expressing only psbV2 [231]. While there is no experimental evidence that the heme group of PsbV participates in electron transfer reactions within PS II (see below), it is intriguing that the absence of the heme group in the PsbV2 protein correlates with an inability to fully complement the ΔpsbV mutant phenotype.

10.3. Midpoint potential

The Em of PsbV and its functional significance has been the subject of intense research. Unlike other c-type monoheme cytochromes, which have an Em of +0 mV or greater [232], the Em of soluble PsbV has been reported to be approximately −250 mV in numerous cyanobacterial species [221,230,233,234]. A variety of roles have been proposed for this low-potential cytochrome, including a redox factor in cyclic photosynthetic electron transfer around PS I [235], a ferredoxin electron acceptor for removal of excess electrons under anaerobic conditions [236] and a factor involved in hydrogen metabolism [237]. Because, as discussed above, there is overwhelming evidence that PsbV is associated with PS II function, the negative Em value is a functional mystery, as participation in electron transfer reactions in the vicinity of the PS II oxygen-evolving complex would require a much more positive Em value.

Numerous studies have been directed at determining the factors that govern the Em properties of PsbV. Structural studies and analyses of the heme environment in soluble PsbV suggest that the heme environment of PsbV is quite similar to other c-type cytochromes with much higher Em values [223–225,238]. The Em of soluble PsbV is also pH-dependent, possibly due to the protonation state of a tyrosine residue (probably 82Y, T. elongatus numbering) [239] or the heme propionate group near 45N (T. elongatus numbering) [240]. Notably, mutation of 45N in Synechocystis 6803 PsbV resulted in a small potential increase of 12–20 mV, but the pH dependence of the Em value for this mutant was not reported [241].

More recent studies have focused on determining the Em of PsbV bound to PS II. Because greater solvent exposure of the heme group tends to lower the Em of cytochromes, the Em of PsbV in the context of PS II may be different from that of the soluble protein. Indeed, several groups have reported slightly higher Em values for PsbV (−110 or −80 mV) in the context of PS II [230,239,240]. Furthermore, the Em of the PS II-bound form is pH-independent [239]. Kirilovsky and co-workers [230] characterized the Em of mutants containing site-directed mutations of 82H, the sixth axial ligand to the heme in T. elongatus. The H92M and H92C mutants displayed lower Em values (−140 mV and −164, respectively) relative to wild type in the PS II-bound form (−110 mV). The proteins carrying the H92M and H92C substitutions were more readily lost during PS II purification relative to native PsbV, but H92M and H92C mutants did not display any PS II activity defects at the cellular level. Based on these results, the Em of PsbV is not critical for PS II function under the conditions analyzed to date.

Recently, Guerrero and co-workers have reported an Em for PS II-bound PsbV of +200 mV [242]. This vastly different Em value was determined by reductive titration in the absence of the redox mediators typically used in such titrations. While a much lower Em value (−200 mV) was observed for oxidative titration in the absence of redox mediators, the authors showed that treatment with dithionite, which is used to fully reduce PsbV prior to oxidative titration measurements, resulted in reduction of the Mn4Ca4O4 cluster and release of PsbV. Therefore, the authors postulate that the presence of redox mediators used in the titrations of previous reports partially red the Mn4Ca4O4 cluster of PS II, causing conformational changes which weaken the association of PsbV and lower its midpoint potential. If the Em of PsbV is +200 mV when bound to PS II, it is possible that electron transfer reactions (ms−1 timescale) could occur between the
heme group and the nearest redox-active cofactor, the Mn$_2$CaO$_6$ cluster (~23 Å) [242]. To date, there is no experimental evidence for electron transfer between the heme group of PsbV and the Mn$_2$CaO$_6$ cluster, but these findings will surely stimulate future studies along these lines.

11. CyanoQ

11.1. Structure

The association of a PsbQ orthologue in cyanobacterial PS II was discovered by Pakrasi and co-workers when analyzing the complete protein complement of isolated PS II complexes from Synechocystis 6803 [243]. The cyanobacterial protein, CyanoQ, is related to the authentic PsbQ-1 and PsbQ-2 proteins from Arabidopsis (At4g21280 and At4g05180, respectively) rather than the more divergent members of the PsbQ gene family that have auxiliary functions [18]. The X-ray crystal structure of CyanoQ (Fig. 4B) is composed of a four-helix bundle arranged in an up–down–up–down fold that resembles the PsbQ structure derived from spinach, despite retaining only 17% sequence identity [126–128]. However, the two proteins differ at their N-termini where, in the case of spinach PsbQ, there are two parallel β-strands that are required for binding to the photosystem [127,135]. The absence of this structural motif in CyanoQ suggests the mechanism for binding to cyanobacterial PS II is different from the binding mechanism of PsbQ to plant PS II. In addition, comparison of the electrostatic surfaces of the two proteins reveals CyanoQ possesses a dipole with a positively charged surface comprising helices 2 and 3 and a negatively charged face formed by helices 3 and 4; in contrast, PsbQ has only a positively charged surface formed by helices 2 and 3 [128]. The corresponding helices of the two proteins also differ in length; notably, helix 2 of CyanoQ is split to give a kink by a fully conserved dipeptide composed of 77G and 78P in Synechocystis 6803.

A further unique feature of CyanoQ is a motif for a signal-peptide cleavage site with a lipid-modified cysteinyl residue, 22C, [123,244]. The equivalent lipidated cysteinyl is conserved in virtually all cyanobacteria. However, CyanoQ is not present in Gloeobacter violaceus or marine Prochlorococcus strains [13,20], and some strains of Synechococcus either lack a lipidation site or possess a modified lipobox [245]. Furthermore, there is no lipobox associated with the PsbQ-like proteins found in red and brown algae [128].

Both PsbQ and CyanoQ bind zinc; however, although both proteins crystallized with two bound zinc atoms, the binding sites were at different locations in the spinach and cyanobacterial structures. While PsbQ required the presence of zinc for crystallization, the structure of CyanoQ was solved both in the presence and absence of zinc [128]. In the CyanoQ structure, one of the zinc atoms was coordinated by 76H and 116D, which are located above a cavity on the molecular face between helices 2 and 3, and the size of the cavity increased in volume from 208 Å$^3$ to 249 Å$^3$ with zinc bound. Moreover, 76H and 116D and the majority of residues that make up the cavity are highly conserved. Therefore this feature might reflect a specific cation-mediated binding site and mechanism of interaction with another PS II subunit [128,245]. In contrast, the second zinc-binding site in the CyanoQ structure appears to be a crystallization artifact, as the ligands are not conserved.

Direct evidence for the binding of CyanoQ to PS II was obtained by isolating PS II complexes using a histidine tag placed on the C-terminus of the protein [12]. The isolation of PS II complexes has routinely been achieved by placing a histidine tag on one of the core antenna chlorophyll a-binding proteins, CP47 or CP43 [246,247]. The PS II complexes isolated using histidine-tagged CyanoQ were found to have a higher rate of oxygen evolution compared to PS II complexes isolated with the histidine tag on CP47. It has been suggested that this arises because the population of PS II isolated when the tag is on CP47 will include some inactive PS II centers that are undergoing assembly and repair [12,248].

The PS II crystal structures from T. elongatus and T. vulcanus do not contain bound CyanoQ [5,7,98]. However, to investigate the binding of CyanoQ to PS II, in silico docking studies have been undertaken using the T. elongatus 3.0-Å resolution structure (PDB ID: 2AXT), positioning CyanoQ next to PsbV and below CP43 [245]. The result of in silico docking studies of CyanoQ to the 1.9-Å resolution structure from T. vulcanus (PDB ID: 3ARC) is presented in Fig. 7 and was carried out as described in Fagerlund and Eaton-Rye [245]. The CyanoQ structure from T. elongatus was used to model the CyanoQ protein, as no sequence information is available for CyanoQ from T. vulcanus. The proximity of CyanoQ to the mouth of the putative B1 oxygen exit channel proposed by Gabdulkhakov et al. [111] is evident. In Fig. 7 CyanoQ sits immediately below the exit of the channel with its putative cation-mediated binding site facing PsbV.

11.2. Function

Synechocystis cells lacking CyanoQ have been shown to exhibit reduced oxygen-evolving activity compared to wild type under CaCl$_2$-depleted conditions [13], but photoautotrophic growth of ΔcyanoQ cells under either calcium-limiting or chloride-limiting conditions was only slightly, or not at all, retarded compared to wild type [13,39]. However, upon limitation of calcium, chloride and iron from the growth medium, ΔcyanoQ cells could not grow photoautotrophically [39]. Thus, CyanoQ is not absolutely required for optimal PS II activity unless additional stress conditions exist, a characteristic common to the cyanobacterial PS II extrinsic proteins.

The physiological role of CyanoQ has also been investigated through the phenotypic analysis of double mutants lacking other PS II extrinsic proteins [39]. Under calcium-limiting conditions, the photoautotrophic doubling time of the ΔpsbU:ΔcyanoQ mutant was extended by 30 h compared with ΔpsbU cells, whereas, photoautotrophic growth of the ΔpsbU:ΔcyanoQ strain showed little change under chloride-limiting conditions. However, the photoautotrophic doubling time of the ΔpsbO:ΔcyanoQ mutant in chloride-limiting media was extended by 10 h compared with ΔpsbO cells. In calcium-limiting conditions, strains lacking PsbO are not photoautotrophic. The double mutant lacking PsbV was not photoautotrophic in nutrient-replete conditions; however, the obligate photoheterotrophic phenotype of the ΔpsbV:ΔcyanoQ strain was restored to photoautotrophy by growing the cells at pH 10 [39]. As noted elsewhere a similar pH dependence has been observed with the ΔpsbO:ΔpsbU double mutant [201,202,249] although the molecular mechanism underlying this pH effect is currently not known.

The absence of CyanoQ has a more pronounced effect on PS II activity and stability at the level of isolated PS II complexes. PS II complexes from ΔcyanoQ had greater oxygen-evolving activity in the presence of 20 mM CaCl$_2$ compared to 5 mM CaCl$_2$, whereas the rates observed with PS II complexes isolated from wild type were similar at both CaCl$_2$ concentrations [13,40]. Furthermore, PS II complexes isolated from cells lacking CyanoQ were found to contain lower levels of PsbV than PS II complexes isolated from wild type [12,40]. Removal of PsbV is known to destabilize PS II and introduce a dependence on calcium and chloride for photoautotrophy (see section on the role of the PsbV protein). Hence, while the absence of CyanoQ is associated with an enhanced requirement for calcium and chloride (particularly in the double mutants lacking either PsbO or PsbU and in isolated PS II complexes) the reduced level of PsbV in the absence of CyanoQ is sufficient to maintain PS II activity that is similar to that seen in wild type in Mg-11 growth medium. Additionally, PsbII complexes lacking CyanoQ are more sensitive to the reducing agent hydroxylamine (NH$_2$OH) than wild type [40]. This implies that while the water oxidation machinery is largely protected from hydroxylamine in wild type, it appears to be partially...
exposed in the ΔCyanoQ mutant; consequently, the PS II complexes in this strain are more easily inactivated.

12. CyanoP

12.1. Structure

A PsbP orthologue was first identified in cyanobacteria in isolated PS II complexes from Synechocystis 6803 [243]. Subsequently, it has been shown through phylogenetic studies that PsbP-like proteins are found in all oxygenic photosynthetic organisms [18,250,251]. These PsbP-like proteins represent an extended family with eleven homologues identified in Arabidopsis belonging to three distinct groups: PS II-specific or authentic PsbP (two homologues), PsbP-like (two homologues), and those containing a “PsbP-like domain” (seven homologues) [123,251]. The homologue in Synechocystis 6803, referred to as CyanoP [123,248], is most closely related to the PsbP-like PPL1 protein of Arabidopsis that has been suggested to play a role in the repair of photodamaged PS II [252]. The relationship between the PsbP-like proteins and CyanoP has also been supported by phylogenetic studies [251].

CyanoP, like CyanQ, possesses a lipobox for signal-peptide cleavage and lipidation at a Cys [13]. Lipidation of CyanoP has not yet been demonstrated, but CyanoP was not released from the thylakoid membrane by salt washes that release other PS II extrinsic proteins, consistent with lipid-mediated membrane attachment. Also consistent with lipidation, the N-terminus of the protein was blocked when sequenced by Edman degradation [14]. To date CyanoP has not been found in any X-ray-derived crystal structures of cyanobacterial PS II; however, a functional relationship with PS II has been inferred from the observation that CyanoP was not present in mutant strains of Synechocystis 6803 that lack PS II; hence it seems expression of CyanoP requires the presence of PS II [14].

The crystal structure of CyanoP from T. elongatus has been solved to 2.8 Å and is an antiparallel β-sheet sandwiched between α-helices (Fig. 3B) (PDB ID: 2XB3, [123]). This structure is similar to the structures solved for the tobacco and spinach PsbP proteins, despite low (~20%) sequence identity [120,121,123]. CyanoP was crystallized in the presence of zinc and it was shown the protein coordinated five zinc ions. Seven distinct contacts were made with the metal ion as a result of the protein’s crystallization symmetry. It is possible that some of these sites may be artifacts of the crystallization process; however, two are highly conserved and the binding of zinc at one of these two sites (zinc ion 4 in [123]) is at an equivalent position to the zinc ion located in the PsbP structure from spinach. Moreover, conserved residues from both of the conserved zinc-binding sites have been previously identified as potential ligands that may interact with manganese in tobacco PsbP [253] (however, see [118] for a critical analysis of this hypothesis). The physiological significance of a divalent metal-binding site on CyanoP is unknown, although conservation of coordinating residues across phyla suggests it may have an as of yet unidentified role.

The docking of CyanoP to the PS II structure from T. elongatus (PDB ID: 2AXT) has been modeled and positions CyanoP below D2 and away from the other extrinsic proteins. In addition, a putative interaction between the C-terminus of the α-subunit of cytochrome b59 (PsbE) and a cavity on the CyanoP surface was identified [245]. In silico docking to the T. vulcanus PS II structure (PDB ID: 3ARC) yielded similar results (Fig. 8). However, an additional interaction with a helix incorporating 277S to 294S of loop E of CP47 was predicted. A Synechocystis 6803 mutant, the CP47Δ(A277-E283) strain, containing a 7 amino acid deletion in this region of loop E, has been reported to have impaired photoautotrophic growth and impaired oxygen evolution and reduced levels of assembled PS II centers [254]. Intriguingly, the CAVER program [255] predicted the presence of a proton exit channel leading directly from YD (160T of D2) to the bulk phase at the docking site of CyanoP (Fig. 7). The required hydrogen bond network for this protein pathway may be provided by waters and the side chain of180R:D2. This channel is composed of residues from CP47, D1, PsbV and PsbE and the mouth comprises residues from D1, PsbV, PsbE and D2.

13.2. Function

Synechocystis 6803 strains lacking CyanoP typically exhibited photoautotrophic growth rates in BG-11 that are similar to wild type; even under chloride- or calcium-limiting conditions only small effects on photoautotrophic growth have been observed [13,14,256]. However, ΔCyanoP cells grown in CaCl2-depleted medium were observed to exhibit a rate of oxygen evolution 63% of that found for the wild type grown under similar conditions [13]. Furthermore, oxygen evolution measurements of PS II complexes purified via a histidine tag on CP47
revealed that complexes isolated from ΔcyanoP cells had reduced oxygen-evolving activity compared to isolated PS II from wild type, particularly in the presence of reduced levels of calcium, suggesting CyanoP was necessary for optimal PS II activity in these preparations [13].

Since it has been suggested that PsbU and PsbV might have similar functions to PsbP and PsbQ in plants and green algae [199,257], the possibility that CyanoP is required when either PsbU or PsbV is absent was investigated by the construction of two double mutants, ΔpsbU:ΔcyanoP and ΔpsbV:ΔcyanoP [256]. The genetic deletion of CyanoP in combination with these other extrinsic components had little or no additional affects on photoautotrophic growth in normal as well as chloride- or calcium-limiting medium. A similar result was obtained with both the ΔpsbQ:ΔcyanoP and ΔcyanoQ:ΔcyanoP strains. Likewise, no specific effects on oxygen evolution attributable to the removal of CyanoP were observed in any of the double mutants. Moreover, there was no additive effect of removing CyanoP in the ΔpsbQ:ΔpsbU:ΔcyanoP triple mutant compared to the corresponding double mutant strains [256].

Since results obtained with mutants lacking CyanoP produced small effects and these results varied among different laboratories, a comparative study of the three available ΔcyanoP strains was carried out [258]. Competitive growth of the wild type with the mutants indicated a selective advantage for the presence of CyanoP. Furthermore, flash-induced oxygen yield experiments displayed a decreased level of oxygen produced on the third flash, increased level on the fourth flash, and an earlier dampening of the oscillations relative to the wild type in ΔcyanoP cells. Additionally, thermoluminescence measurements demonstrated an apparent enhanced recombination between the S3 state of the oxygen-evolving complex and the reduced secondary plastoquinone electron acceptor Qb in the ΔcyanoP strains. These data indicate that the ΔcyanoP mutants exhibit a major $S_0Q_b^{-}\rightarrow Q_b^{-}$ recombination in addition to $S_0Q_b^{\text{red}}$ recombination, whereas the wild type predominately exhibits the thermoluminescence band corresponding to $S_0Q_b^{\text{red}}$ recombination. These results may implicate equilibration between $Y_D$ (160Y of D2) (or some other unspecified redox-active species) and the S3 state favoring, for example, $Y_D\rightarrow Y_O$ over $Y_D\rightarrow S_2$ in PS II centers lacking CyanoP, thereby pointing to a role for CyanoP in the stabilization of charge separation in PS II [258]. It is therefore of interest that a putative proton exit channel linking $Y_D$ to a possible docking site for CyanoP (Fig. 8) has been detected; however, the existence of this channel requires experimental verification.

Additionally, immunodetection of CyanoP in Synechocystis 6803 thylakoid membranes has indicated that CyanoP is present at a similar level to the PsbO protein [14], although this finding is controversial [13]. Thus, oxygen flash yield experiments, thermoluminescence and western blotting suggest CyanoP is stoichiometrically present in PS II [14] in contrast to an earlier finding that it is associated only with ~3% of PS II complexes in the thylakoid membrane [13]. As detergent is required during the isolation of PS II complexes, it is possible that CyanoP is lost during complex purification [13,14,258]. However, despite the uncertainty regarding the stoichiometry of CyanoP, the role of this subunit is not essential under a wide range of conditions and it appears likely that the selective advantage conferred by the protein is linked to a regulatory role that remains to be fully elucidated.

13. Conclusions

As is evident in this review, over the last thirty years an enormous amount of effort has been focused on the elucidation of the structure and function of the extrinsic proteins of PS II. Much progress has clearly been made, but many questions remain unanswered. While phenomenologically the functions of these proteins have generally been identified, the mechanisms by which they influence PS II oxygen evolution remains elusive. While the structure of cyanobacterial PS II progresses towards atomic resolution (i.e., 1.2 Å), the organization of the CyanoP and CyanoQ subunits within the photosystem remains unclear. Experimental investigations into the structural and possible regulatory roles played by the extrinsic components in the numerous channels present in PS II must also be forthcoming. Additionally, while structures for isolated higher plant PsbP and PsbQ are available, no progress is evident in the elucidation of the overall structure of the higher plant photosystem. Clearly, much work remains.

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