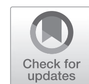

Chapter 16

Evolution and Function of the Extrinsic Subunits of Photosystem II

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Summary 429
I. Introduction 430
II. Localization of Extrinsic Subunits in Photosystem II Structures 431
III. Functions of Each Extrinsic Subunit 432
A. PsbO 432
B. PsbV 433
C. PsbU 434
D. PsbP 435
E. PsbQ 436
F. Psb31 438
IV. Molecular Evolution of PsbP and PsbQ Family Proteins 439
V. Concluding Remarks 440
Acknowledgements 441
References 441

Summary

Photosystem II (PS II) catalyzes photosynthetic water oxidation and is composed of more than 20 subunits, including membrane-intrinsic and extrinsic proteins. PS II extrinsic proteins shield the catalytic Mn_4CaO_5 cluster from the outside bulk solution and stabilize the binding of inorganic cofactors, such as Ca^{2+} and Cl^-, in the oxygen-evolving center of PS II. Among the PS II extrinsic proteins, PsbO is commonly found in all oxygenic organisms, while PsbP and PsbQ are specific to green plants, including vascular plants and green algae, and PsbU, PsbV, CyanoQ, and CyanoP exist in cyanobacteria. Additionally, red algae and

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diatoms have unique PS II extrinsic proteins, such as PsbQ′ and Psb31. Recent structural studies revealed the structure and binding manner of each extrinsic subunit in PS II from different species. Furthermore, various functions of PsbP- and PsbQ-homologs in photosynthetic electron transfer have been identified, indicating that gene duplication and successive functional diversification occurred during the evolution of PS II extrinsic proteins. This chapter focuses on recent results on the structural and functional studies of PS II extrinsic proteins, and discusses their evolutionary changes during the development of PS II.

I. Introduction

Photosystem II (PS II) converts light energy into the electrochemical potential energy required to split water into H^+, electrons, and molecular oxygen (Dau et al. 2012; Cox and Messinger 2013; Vinyard et al. 2013). The PS II core complex is composed of more than 20 subunits, including CP47, CP43, D1, D2, Cyt b_{559} α- and β-subunits, and PsbI, with numerous small subunits that stabilize the reaction (Pagliano et al. 2013; Shen 2015). X-ray structural analysis of the PS II dimeric complex from cyanobacteria at atomic resolution revealed the location of most subunits, pigments, and redox cofactors (Ferreira et al. 2004; Guskov et al. 2009; Umena et al. 2011; Suga et al. 2015) (Chap. 1). Light excitation of the primary donor P680, the special pair chlorophylls in PS II, results in electron transfer to a nearby pheophytin followed by electron transfer to the acceptor quinones (Q_A and Q_B). The resulting cation radical of $P680^+$ receives electrons from the Mn_4CaO_5 cluster via a redox-active tyrosine of D1, Y_Z. The Mn_4CaO_5 cluster converts two water molecules into one molecular oxygen and four protons through a light-driven cycle consisting of five intermediates known as the S_i states (i = 0–4). Among them, the S_1 state is the most dark-stable, and flash illumination advances each S_i state (i = 0–3) to the next S_{i+1} state. Molecular oxygen is released during the S_3–S_4–S_0 transition after the transient S_4 state. Recent studies using a femtosecond X-ray free laser reported the transient structure of intermediate S-states, revealing the nearly complete mechanism of water oxidation in PS II (Suga et al. 2017, 2019; Kern et al. 2018) (Chap. 1).

The mechanism of water oxidation and the basic subunit structure of the PS II core are mostly conserved across oxygenic photosynthetic organisms ranging from cyanobacteria to vascular plants, whereas several peripheral PS II subunits differ (Nelson and Yocum 2006). Particularly, the composition of the extrinsic subunits of PS II surrounding the catalytic Mn_4CaO_5 cluster has undergone a large evolutionary change (Enami et al. 2008). Green eukaryotes, such as vascular plants and green algae, contain a set of three extrinsic proteins, PsbO, PsbP, and PsbQ, which bind to the lumenal surface of PS II (Kuwabara and Murata 1982; Ghanotakis et al. 1984a). In cyanobacterial PS II, PsbV and PsbU are present rather than PsbP and PsbQ (Shen et al. 1992; Shen and Inoue 1993). Furthermore, cyanobacteria contain PsbP and PsbQ homologs designated as

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Abbreviations: Cryo-EM – Cryo-electron microscopy; Cyt – Cytochrome; FTIR – Fourier transform infrad spectroscopy; LHC – Light-harvesting complex; MD – Molecular dynamics; Mn_4CaO_5 – Inorganic center cluster of PS II oxygen evolving complex; NDH – NADH dehydrogenase-like; OEC – Oxygen-evolving complex of PS II; P680 – Primary electron donor of PS II; PPD – PsbP domain; PPL – PsbP-like; PQL – PsbQ-like; PS – Photosystem; Q_A, Q_B – Quinonoe electron acceptors of PS II; Y_Z – Tyrosine electron donor of PS II

CyanoP and CyanoQ, respectively (Kashino et al. 2002; Thornton et al. 2004). Additionally, red algae and diatoms possess PsbQ′, a 20-kDa homolog of CyanoQ, bound to PS II as an extrinsic subunit in addition to PsbO, PsbU, and PsbV (Ohta et al. 2003; Nagao et al. 2010a). Diatoms further possess Psb31 as an additional, specific extrinsic subunit (Okumura et al. 2008). High-resolution structures of individual PS II extrinsic subunits from eukaryotes have been reported (Calderone et al. 2003; Ifuku et al. 2004; Balsera et al. 2005; Kopecky et al. 2012; Nagao et al. 2013; Michoux et al. 2014; Cao et al. 2015); however, their binding sites and topologies were unclear until recently because crystallographic information derived from prokaryotic cyanobacterial PS II cannot be fully applied to eukaryotic PS II.

The X-ray structure of red algal PS II and cryo-electron microscopy (cryo-EM) structure of PS II supercomplex from vascular plants were recently reported (Ago et al. 2016; Wei et al. 2016; Su et al. 2017). It is now possible to describe the structural basis of the binding and function of extrinsic proteins in eukaryotic PS II. Extensive reviews have been published on the structure and interaction of each PS II extrinsic proteins in PS II (Enami et al. 2008; Ifuku et al. 2008, 2011; Bricker et al. 2012; Ifuku 2015; Ifuku and Noguchi 2016; Roose et al. 2016). Therefore, this chapter focus on their evolutional aspects.

II. Localization of Extrinsic Subunits in Photosystem II Structures

Figure 16.1 shows the X-ray and cryo-EM structures of the PS II dimer from cyanobacteria, red algae, and vascular (green) plants, viewed horizontally from the thylakoid lumenal side. The positions of PsbO, PsbU, and PsbV are similar between cyanobacterial and red algal PS II, except that PsbQ′ binds to one of the PS II monomers to interact with the outer surface of CP43 in red algal PS II (Ago et al. 2016). Binding of CyanoQ is not included in the current X-ray structure of cyanobacterial PS II, whereas its individual structure has been reported (Jackson et al. 2010). The study using a chemical crosslinker suggested that CyanoQ forms a dimer at the interface between monomers in the dimeric PS II complex (Liu et al. 2014). Binding of multiple copies of CyanoQ to the PS II assembly intermediates has also been reported (Liu et al. 2015). Unlike eukaryotic PsbQ (PsbQ′), CyanoQ is a lipoprotein with a lipid modification on its N-terminus (Juneau et al. 2016). Therefore, the manner of interaction with PS II may differ between PsbQ and CyanoQ.

In the cryo-EM structure of green plant PS II, PsbP specifically replaces the binding site of PsbV in the structure of cyanobacterial and red algal PS II (Wei et al. 2016; Su et al. 2017). This is consistent with previous *in vitro* and *in vivo* studies indicating that

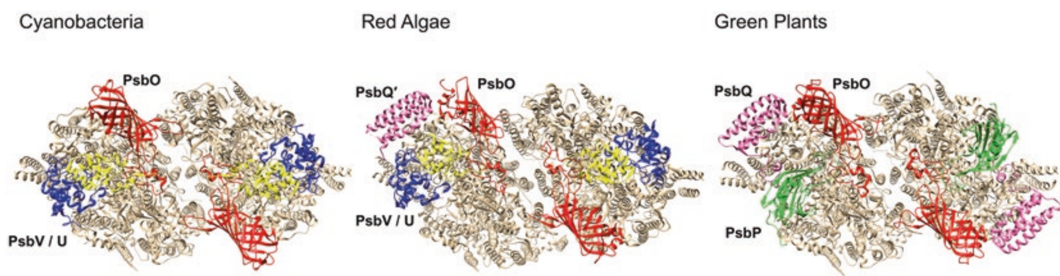

Fig. 16.1. Binding sites of extrinsic subunits in the PS II structure from cyanobacteria (*Thermosynechococcus vulcanus*, PDB ID: 3WU2), red algae (*Cyanidium caldarium*, 4YUU), and green plants (*Spinacia oleracea*, 3JCU). PsbO, red; PsbP, green; PsbQ′ and PsbQ, pink; PsbV, blue; PsbU, yellow

both PsbP and PsbV are required to retain Ca^{2+} and Cl^- ions in the oxygen-evolving center of PS II (Shen et al. 1992, 1995; Shen and Inoue 1993). The binding site of PsbQ is similar to that of PsbQ′ in red algal PS II, suggesting functional conservation between the green and red lineages (Ago et al. 2016). In fact, phylogenetic analysis indicated that PsbQ in green algae and *Euglena gracilis* is more closely related to PsbQ′ in red algae and diatoms (Yabuta et al. 2010). In this chapter, we use the term "PsbQ′" in red algal and diatom PSII; however, the term "PsbQ" may be more appropriate to avoid confusion in the future.

The above structural comparison suggests that the most drastic change in PS II at the branching point of the red and green lineages was the development of PsbP from CyanoP in cyanobacteria (Ifuku 2015). CyanoP is absent from the current X-ray structures of cyanobacterial PS II, whereas its individual structure has been reported (Michoux et al. 2010; Jackson et al. 2012). Recent studies reported that CyanoP interacts with premature PS II subcomplexes during the early stage of its assembly (Cormann et al. 2014; Knoppová et al. 2016). This suggests that CyanoP may function as an assembly factor in cyanobacterial PS II, and PsbP, which functions as an essential extrinsic subunit of PS II in green plants, developed from such an assembly factor for PS II. The molecular evolution of PsbP and PsbQ family proteins is discussed in a later section.

III. Functions of Each Extrinsic Subunit

A. PsbO

In all oxyphototrophs, PsbO, also known as OEC33 because of its apparent molecular mass of 33 kDa, or manganese-stabilizing protein, plays essential roles in stabilizing the Mn_4CaO_5 cluster and preventing destructive reduction of the cluster from exogenous reductants (Ghanotakis et al. 1984b; Kuwabara et al. 1985). PsbO along with other extrinsic subunits interacts with the extensive lumenal domains of D1 (PsbA), D2 (PsbD), CP43 (PsbC), and CP47 (PsbB) to optimize the structure for the water-oxidizing reaction, conceptually known as the oxygen-evolving complex, or oxygen-evolving center (OEC), or water-oxidizing center. The structure, interactions, and functions of PsbO have been extensively summarized in previous reviews (Enami et al. 2008; Bricker et al. 2012).

Fourier transform infrared (FTIR) spectroscopy marked a turning point in the functional analyses of extrinsic subunits in PS II (Ifuku and Noguchi 2016). Nagao et al. (2015) reported the FTIR measurements of S_2-minus-S_1 difference spectra using PS II core complexes from *Thermosynechococcus elongatus* reconstituted with its extrinsic proteins, PsbO, PsbV, and PsbU. Under a low-$CaCl_2$ condition, PsbO is essential for inducing normal spectral changes during the $S_1 \rightarrow S_2$ transition, indicating its significant role in stabilizing the Mn_4CaO_5 cluster. Even at a high-$CaCl_2$ concentration, protein conformational changes in the OEC are induced by removal of all extrinsic proteins, and these changes are largely recovered by the binding of PsbO, with further recovery observed by PsbV and then PsbU in a stepwise manner. Therefore, binding of PsbO mainly supports the conformation of the OEC in cyanobacterial PS II. The recoveries of the OEC protein conformation were consistent with those of oxygen-evolving activity recovered by the binding of respective extrinsic proteins (Shen and Inoue 1993), confirming structural coupling between the activity and protein conformation of the OEC.

The X-ray structures and theoretical calculations suggest that extrinsic proteins play another important role by forming access channels for substrate water to the Mn_4CaO_5 cluster and exit channels for the products (oxygen and protons) (Bondar and Dau 2012; Vassiliev et al. 2013; Linke and Ho

2014). These channels involve numerous water molecules forming hydrogen-bond networks, connecting the OEC towards the protein bulk surface at the lumenal side. As shown in Fig. 16.2, four channels have been proposed (Nagao et al. 2017c), and PsbO participates in the "Cl-1 path" starting from D1-Asp61 to PsbO through the Cl-1 site and the D1-Glu65/D2-Glu312/D1-Arg334 triad. Molecular dynamics (MD) simulation suggested that the absence of PsbO is correlated with the rapid loss of Cl-1 coordinated by D2-Lys317 (Guerra et al. 2018). At the end of the extended hydrogen-bond network, PsbO-Asp224 is part of a cluster of carboxylate groups, which may accept protons on the protein surface (Shutova et al. 2007; Lorch et al. 2015; Bommer et al. 2016). This Cl-1 path is thought to be a major pathway for protons or substrate water and is conserved in PS II-light-harvesting complex II (LHCII) structures from vascular plants, recently resolved by cryo-EM (Wei et al. 2016; Su et al. 2017). These results support the general importance of PsbO in the water-oxidizing reaction in oxyphototrophs.

B. PsbV

PsbV was found in PS II core complexes of the cyanobacterium *Thermosynechococcus vulcanus* (Shen et al. 1992; Shen and Inoue 1993) and was subsequently observed in functional PS II complexes in various red-lineage oxyphototrophs but not in the green lineages (Enami et al. 2008). PsbV is a *c*-type cytochrome (Cyt), known as Cyt c_{550}, with a molecular weight of approximately 15 kDa. The basic function of PsbV is to maintain Ca^{2+} and Cl^{-} retention (Shen et al. 1992, 1995; Shen and Inoue 1993); this function is conserved in red algal and diatom PsbV (Enami et al. 1998; Nagao et al. 2010a). The redox function for Cyt c_{550} in PS II has been proposed, but has not been demonstrated experimentally (Guerrero et al. 2011). The physiological functions and structural organization of PsbV are summarized in previous reviews (Enami et al. 2008; Bricker et al. 2012).

As described above, the binding of PsbV together with PsbO to extrinsic protein-depleted PS II induces the recovery of pro-

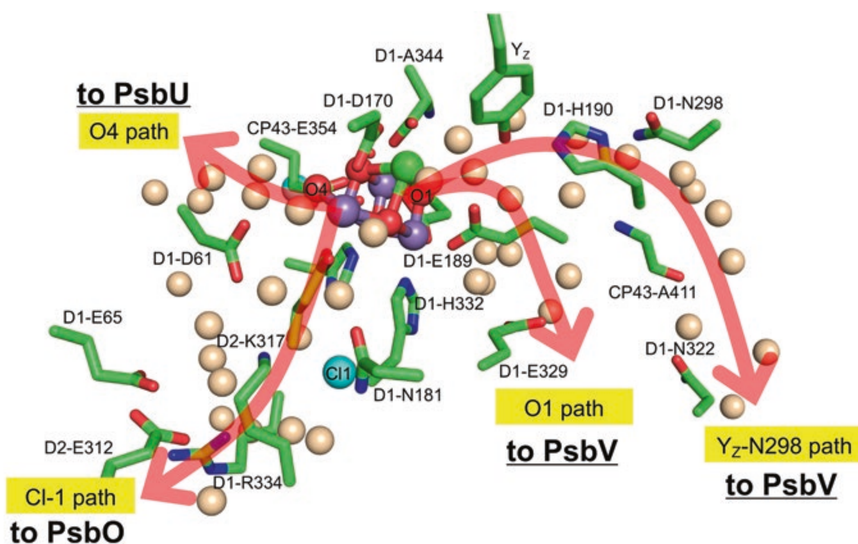

Fig. 16.2. Hydrogen-bond networks around the OEC. The picture was drawn using a high-resolution PS II core structure (PDB ID: 3ARC). Wheat and cyan colored spheres represent water molecules and chloride ions, respectively. Arrows indicate putative proton exit channels, which were designated "Cl-1 path", "O4 path", "O1 path", and "Y_Z-N298 path" in this work

tein conformations around the OEC in the $S_1 \rightarrow S_2$ transition of the Mn_4CaO_5 cluster (Nagao et al. 2015). PsbV induced the recovery of specific amide I bands, suggesting that it affects different regions of polypeptide chains from those affected by PsbO. In the high-resolution PS II structure (Umena et al. 2011), PsbV is associated with the C-terminal helix of the D2 protein including D2-Lys317, which is a ligand of Cl-1 ion, and with the C-terminal helix near D1-His332 and C-terminal loop between D1-His337 and D1-Asp342 of the D1 protein. Eventually, PsbV binding to the PS II cores appears to stabilize the structure of hydrogen-bond networks involved in the Cl-1 path (Fig. 16.2).

PsbV has also been suggested to be connected to the hydrogen-bond network near Y_Z. Y_Z forms a hydrogen-bonded triad with D1-His190 and D1-Asn298, which is connected to a hydrogen-bond network leading to PsbV through D1-Asn322 (Y_Z-N298 path) (Nagao et al. 2017c) (Fig. 16.2). MD simulations suggest that the tight hydrogen-bond network of this pathway is advantageous for proton transfer (Sakashita et al. 2017b; Ogata et al. 2013). Furthermore, a large water cluster interacting with the Y_Z/D1-His190/D1-Asn298 triad also forms another hydrogen-bond network through O1 and D1-Glu329, leading to PsbV (O1 path). MD simulation showed that this channel contains numerous mobile water molecules (Sakashita et al. 2017b). FTIR analysis of the D1-N298A mutant suggested that the Y_Z-N298 path functions as the proton-exit pathway in $S_2 \rightarrow S_3$ and $S_3 \rightarrow S_0$ transitions (Nagao et al. 2017c). These observations indicate that the protein conformational changes of the OEC upon association of PsbV with the cores induce a better structural arrangement for the proton-release events of $S_2 \rightarrow S_3$ and $S_3 \rightarrow S_0$ transitions. Thermoluminescence analysis suggested large contributions of PsbV to the $S_2 \rightarrow S_3$ and $S_3 \rightarrow S_0$ transitions (Shen and Inoue 1993). The further recovery of oxygen-evolving activity observed by PsbV binding plus PsbO compared to the activity by the sole binding of PsbO (Shen and Inoue 1993) may be attributed to the fine-tuning of the OEC structures, particularly the Cl-1 and Y_Z-N298 proton-exit channels. Based on these observations, together with the similar function and location of PsbV in the PS II cores of a primitive red alga *Cyanidium caldarium* (Enami et al. 1995, 1998; Uno et al. 2013; Ago et al. 2016), the contribution of PsbV to the OEC structure has been largely conserved in the red lineages. Notably, red algal PsbV plays a major role in the recovery of the secondary structure in the OEC to a larger extent than the cyanobacterial PsbV as revealed by FTIR measurements (Uno et al. 2013). Differences in the contribution to OEC recovery between cyanobacteria and red algae PsbV is an interesting point indicating the evolutional diversity of PS II.

C. PsbU

PsbU has a molecular weight of approximately 12 kDa and was first found in PS II core complexes isolated from *T. vulcanus* (Shen et al. 1992) followed by that in the red-lineage oxyphototrophs (Enami et al. 1995, 2008). The basic function of PsbU is similar to that of PsbV, i.e., PsbU binding maintains Ca^{2+} and Cl^- retention in PS II of cyanobacteria, red algae, and diatoms (Shen and Inoue 1993; Enami et al. 1998; Nagao et al. 2010a). The physiological functions and structural organization of PsbU have been summarized in previous reviews (Enami et al. 2008; Bricker et al. 2012).

Similar to the functional examination of PsbV, the role of PsbU in the OEC structures was also investigated by FTIR measurements (Nagao et al. 2015). The binding of PsbU together with PsbO and PsbV to extrinsic protein-depleted PS II recovers the secondary structures of the polypeptide main chains in the OEC (Nagao et al. 2015).

In the PS II structure, PsbU contacts with the C-terminal loop between D1-His337 and D1-Asp342 of the D1 protein, suggesting that PsbU also stabilizes the Cl-1 hydrogen-bond network. Additionally, the map of hydrogen-bond networks suggests that a distinct water chain in the O4 path is linked to PsbU (Takaoka et al. 2016) (Fig. 16.2). No studies have been conducted on the spectroscopic analyses of S-state transitions using site-directed mutants of amino acid residues along the O4 path; therefore, it is difficult to experimentally assess the contribution of the O4 path to the S-state transitions. However, theoretical calculation proposed that the O4 path functions as a proton-exit pathway in the $S_0 \rightarrow S_1$ transition (Saito et al. 2015; Sakashita et al. 2017b). These observations indicate that the proton-release event in the $S_0 \rightarrow S_1$ transition is promoted by protein conformational changes of the OEC induced by PsbU binding. The maximum recovery of oxygen-evolving activity by PsbU plus PsbO/V binding compared to the activity of PsbO/V binding (Shen and Inoue 1993) may be related with the fine-tuning of OEC structures, particularly the Cl-1 and O4 proton-exit channels. Given the similar function and location of PsbU in the PS II cores of a primitive red alga *C. caldarium* (Enami et al. 1995, 1998; Uno et al. 2013; Ago et al. 2016) with that of the cyanobacterial PS II, the contribution of PsbU to the OEC structure is largely conserved in the red lineages.

D. PsbP

PsbP, also known as OEC23 or OEC24 because of its apparent molecular mass of 23–24 kDa, is specifically found in green plant PS II (Åkerlund et al. 1982). Like PsbV and PsbU in cyanobacterial PS II, PsbP maintains the required levels of Ca^{2+} and Cl^- in PS II of green lineage organisms (Ghanotakis et al. 1984a; Miyao and Murata 1986). A lack of PsbP largely diminishes photoautotrophy in *Arabidopsis thaliana* (Yi et al. 2007) and *Nicotiana tabacum* (Ifuku et al. 2005b). Therefore, the development of the PsbP protein as an extrinsic subunit is a crucial event in PS II function during evolution from the red lineage to green lineage organisms. The structure and function of PsbP have been intensively reviewed previously (Ifuku et al. 2008, 2011; Ifuku and Noguchi 2016; Roose et al. 2016).

The importance of PsbP in structural coupling with the OEC has been investigated by *in vitro* reconstitution (Ifuku and Sato 2001; Ifuku et al. 2005a) and FTIR analysis: PS II membranes depleted of PsbP and PsbQ by NaCl washing showed clear changes in amide I bands in S_2-minus-S_1 FTIR difference spectra, reflecting conformational changes in polypeptide main chains (Tomita et al. 2009). Unlike cyanobacterial PS II, further depletion of PsbO did not induce additional changes, and the original amide I features were recovered by rebinding of PsbP to NaCl-washed PS II membranes. These results indicate that the PsbP protein mainly contributes to the protein conformation during the $S_1 \rightarrow S_2$ transition in green plant PS II. Various mutations or truncations have been introduced in the PsbP structure and their effects were examined by FTIR (Tomita et al. 2009; Kakiuchi et al. 2012; Ido et al. 2012; Nishimura et al. 2014; Ifuku and Noguchi 2016), and the results obtained revealed the manner of interactions of PsbP with PS II core subunits. Subsequent FTIR measurement of NO_3^--substituted PS II membranes indicated that PsbP binding perturbs the protein conformation around Cl^- ion(s) in the OEC (Kondo and Noguchi 2018). Chemical cross-linking data and cryo-EM structures suggested that PsbP forms multiple interactions with the D1, D2, and CP43 subunits, indicating that both the Cl-1 and Cl-2 sites are affected by PsbP binding (Ido et al. 2012, 2014). Notably, the O1 path and the O4-PsbU path in cyanobacterial PS II appear to be structurally conserved as the channels proceeding along PsbP toward

the protein bulk surface in the cryo-EM structure of plant PS II (Sakashita et al. 2017a). Therefore, binding of PsbP may play a crucial role in maintaining the hydrogen-bond network both for H^+ export and water uptake in the OEC.

In addition to optimizing the water-oxidizing reaction in PS II, it has been reported that a lack of PsbP perturbs electron transport at the reducing side of PS II (Ido et al. 2009). Removal of PsbP and PsbQ with 2 M NaCl significantly slowed the rate of electron transfer from Q_A^- to Q_B (Roose et al. 2010). However, the mechanism of this trans-membrane effect remains unknown. Related to this, Nishimura et al. (2016) reported that N-terminal amino-acid residues of PsbP interacting with PsbE of Cyt b_{559} modulate the redox potential of the heme of Cyt b_{559} located at the stroma side. Even a synthetic pN15 peptide consisting of the 15 N-terminal residues of PsbP altered the structure of Cyt b_{559} in a transmembrane manner and triggered the redox potential change of the heme in Cyt b_{559}, converting it into its high-potential form (Nishimura et al. 2016). The interaction of PsbP with Cyt b_{559} was confirmed by the recently determined cryo-EM structure (Wei et al. 2016) (Fig. 16.3). Cyt b_{559} is known to be involved in alternative or secondary electron flow to suppress photoinhibition: its high potential form donates electrons to $P680^+$, whereas its low potential form oxidizes the reducing side of PS II (Takagi et al. 2019). Interestingly, the pN15 peptide alone reduces the oxygen-evolving activity of PS II and inhibits the $S_1 \rightarrow S_2$ transition in the OEC as suggested by thermoluminescence and FTIR analyses (Nishimura et al. 2016). These data suggest that PsbP forms multiple interactions with PS II and each has a distinct role in regulating electron transfer within PS II. Therefore, binding of PsbP is important for balancing the oxidizing and reducing reactions of PS II in a trans-membrane manner.

E. PsbQ

PsbQ, previously referred to as OEC16 or OEC18 because of its apparent molecular

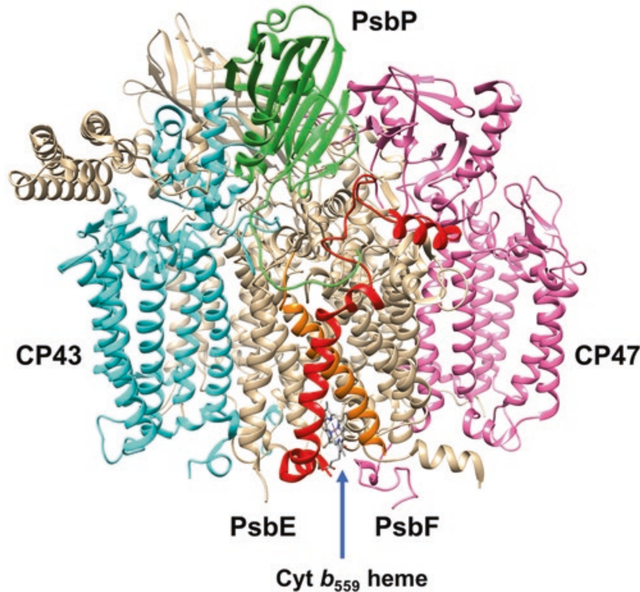

Fig. 16.3. Interactions of PsbP with PS II core subunits in spinach PS II structure (PDB ID: 3JCU). CP43, cyan; CP47, pink; PsbE, red; PsbF, orange; PsbP, green

mass of 16–18 kDa, was originally found in green plant PS II (Åkerlund et al. 1982). Release-reconstitution experiments showed that a lack of PsbQ reduces the oxygen-evolving activity of PS II membranes under low Cl^- conditions (Akabori et al. 1984; Miyao and Murata 1985), whereas PsbQ is not essential for photoautotrophic growth in both *A. thaliana* and *N. tabacum* (Ifuku et al. 2005b; Yi et al. 2006). Cryo-EM structures suggested that PsbQ is located at the interface between CP43 and the monomer-type light-harvesting protein CP26 (Wei et al. 2016; Su et al. 2017). This location is consistent with the fact that PsbQ is required for stabilizing the PS II-LHCII supercomplex (Yi et al. 2006; Allahverdiyeva et al. 2013). FTIR analysis did not suggest a specific function for PsbQ; however, binding of PsbQ partly restored the function of the Δ15-PsbP protein, an N-terminal truncated mutant of PsbP lacking the function to induce conformational changes and activate oxygen evolution of OEC (Kakiuchi et al. 2012). Therefore, PsbQ may play an auxiliary role in supporting PsbP binding and function in vascular plants. The interaction between PsbP and PsbQ was confirmed in the cryo-EM structure of the plant PS II-LHCII supercomplex (Wei et al. 2016). Modification of PsbP binding in the absence of PsbQ was also suggested by pulsed electron-electron double resonance analysis using spin-labeled proteins (Asada et al. 2018).

PsbQ′ was found in the PS II core complexes isolated from the primitive red alga *C. caldarium* (Enami et al. 1995, 1998) and showed low sequence similarity to PsbQ in vascular plants with a molecular weight of approximately 20 kDa (Ohta et al. 2003). This protein was found in highly purified red algal PS II cores (Adachi et al. 2009) and its structure was revealed by the X-ray crystal structure of the red algal PS II core complexes (Ago et al. 2016). The structure of PsbQ′ is composed of a four-helix bundle domain, which is similar to the structure of plant PsbQ (Calderone et al. 2003; Balsera et al. 2005). PsbQ′ is located near CP43, similar to the position of plant PsbQ (Wei et al. 2016; Su et al. 2017). Diatom PS II core also possesses PsbQ′ with a low sequence similarity (Nagao et al. 2007; Okumura et al. 2008; Nagao et al. 2010b), indicating that the diatom PsbQ′ is also located near CP43. Release-reconstitution experiments in red algal PS II showed that rebinding of only PsbQ′ without other extrinsic subunits showed no recovery of oxygen-evolving activity, whereas PsbQ′ significantly contributed to the full binding of PsbV and PsbU (Enami et al. 1998). Similarly, the complete association of diatom PsbV and PsbU also requires the binding of PsbQ′ (Nagao et al. 2010a). Interestingly, conformational recovery of the OEC in the $S_1 \rightarrow S_2$ transition of the Mn_4CaO_5 cluster was not observed in FTIR measurements of the binding of PsbQ′ to PS II (Uno et al. 2013). This is consistent with the binding site of PsbQ′, which is rather far from the Mn_4CaO_5 cluster in the PS II structure compared to those of PsbO, PsbV, and PsbU (Ago et al. 2016).

Genome editing has enabled deletion of *PsbQ′* genes in a primitive red alga *Cyanidioschyzon merolae* (Zienkiewicz et al. 2018). There are two *PsbQ′* genes encoded in the genome of *C. merolae*, and each Δ*psbQ′1* and Δ*psbQ′2* mutant showed a lower growth rate and oxygen-evolving activity compared to wild-type cells. This is likely because of the partial lack of PsbV from isolated PS II cores; therefore, impaired photosynthetic ability may be an indirect effect of PsbQ′ on oxygen-evolving reactions, consistent with the *in vitro* release-reconstitution experiments (Enami et al. 1998; Uno et al. 2013). The main role of PsbQ′ in the oxygen-evolving reactions appears to maintain the protein environments for full binding of PsbV and PsbU. This function of PsbQ′ in red algae is analogous to that of PsbQ for PsbP in vascular plants (Kakiuchi et al. 2012).

Notably, CyanoQ also stabilizes the binding of PsbV in cyanobacterial PS II (Summerfield et al. 2005; Kashino et al. 2006), although a different binding manner has been proposed for CyanoQ (Liu et al. 2014, 2015).

Enami and coworkers examined the functional preservations of extrinsic subunits by comparing the recovery of oxygen-evolving activity using reconstituted PS II samples, particularly in cross-reconstitution experiments, in which, red-algal and plant PsbO were bound to spinach and red-algal PS II, respectively (Enami et al. 2000, 2003; Suzuki et al. 2005). Their results suggested that PsbQ′ is exchangeable with PsbQ in plant PS II, whereas PsbQ is not exchangeable with PsbQ′ in red algal PS II (Enami et al. 2000). Therefore, binding and function of PsbQ and PsbQ′ are preserved to some extent in eukaryotic oxyphototrophs. It has also been reported that red-algal PsbQ′ regulates the redox-potential of Q_A in cyanobacterial PS II (Yamada et al. 2018). Because such a trans-membrane effect has not been reported for PsbQ binding, there may be some functional differences between these proteins.

F. Psb31

Psb31 was identified in PS II complexes isolated from the marine diatom *Chaetoceros gracilis* (Nagao et al. 2007, 2010b). This protein is encoded in the nuclear genome with a molecular weight of approximately 13 kDa and has a homologue in numerous chromophyte algae and red algae, but not in green-lineage oxyphototrophs (Okumura et al. 2008). The electrostatic interactions of Psb31 with PS II cores, independent of the other extrinsic subunits, were observed by cross-linking and release-reconstitution experiments (Nagao et al. 2010a). Cross-linking studies further suggested that the binding partners of Psb31 are PsbE and/or PsbH according to mass spectrometry and immunological detection (Okumura et al. 2008) (Fig. 16.4). The structure of Psb31 is

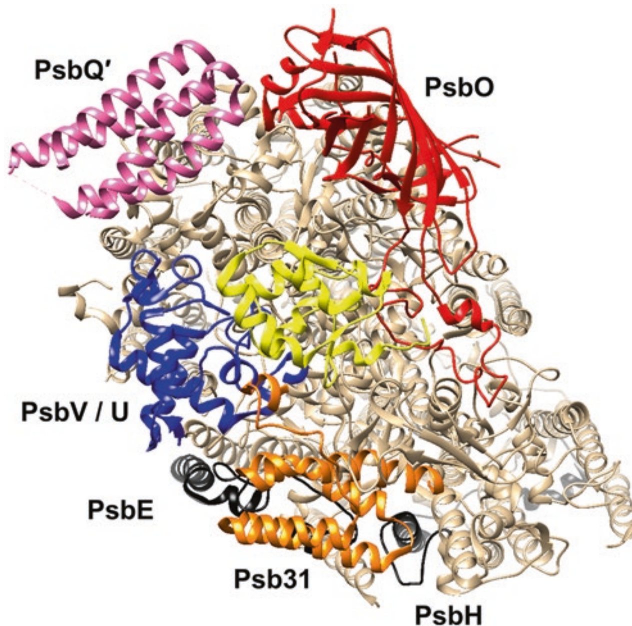

Fig. 16.4. Putative model of diatom PS II core complexes. The model was built using red algal PS II core (PDB ID: 4YUU) and diatom Psb31 (PDB ID: 4K7B). PsbO, red; PsbQ′, pink; PsbV, blue; PsbU, yellow; PsbH and PsbE, black; and Psb31, orange

composed of a four-helix bundle domain including the N-terminus, which is similar to the structures of PsbQ and PsbQ′, and a flexible C-terminal domain as revealed by X-ray crystallography (Nagao et al. 2013). Chemical modification analysis showed that the positively charged amino acids on the Psb31 surface, but not the negative charges, contribute to electrostatic interactions with the cores (Nagao et al. 2017b). Because Lys residues are mainly changed to non-charged Lys by chemical modifications, each of the 10 Lys residues in Psb31 was replaced with Ala (Nagao et al. 2017a, b). The study revealed that involvement of the 10 Lys in the electrostatic interactions can be classified into three groups: Lys39/Lys54/Lys57 associate strongly with the PS II core, Lys33/Lys56/Lys69 associate moderately with the core, and Lys24/Lys76/Lys80/Lys117 do not interact with the core directly. The important Lys residues for PS II binding are well-conserved among diatom species but not among other chromophytes, suggesting high conservation of the Lys residues at the binding sites only in the diatom species.

Psb31 plays a unique role in oxygen-evolving reactions. Release-reconstitution experiments showed that binding of Psb31 alone induces the recovery of oxygen-evolving activity (Nagao et al. 2010a). This recovery is a unique feature of diatom PS II, as the recovery of oxygen-evolutoin was not observed in extrinsic subunit-reconstituted PS II samples without PsbO from cyanobacterial, red algae, green algae, and plants (Enami et al. 2008). In chemical modification and site-directed mutagenesis studies, we found that the flexible C-terminal domain of Psb31 may be elongated near the OEC, resulting in the regulation of oxygen-evolving reactions (Nagao et al. 2017a, b). The structure of diatom PS II in complex with its light-harvesting antenna, fucoxanthin Chl *a*/*c*-binding proteins (FCPII), has been analyzed by cryo-EM recently (Nagao et al. 2019); however, Psb31 was not visible in this structure. Detailed function of Psb31 in the oxygen-evolving reaction remains unclear, and thus further structural, FTIR, and genetic studies of Psb31 are needed.

IV. Molecular Evolution of PsbP and PsbQ Family Proteins

During the acquisition of PsbP and PsbQ in PS II of the green lineage organisms, gene duplication likely occurred in parallel, as multiple homologs for PsbP and PsbQ are found in the genome of green plants. Two PsbP-like proteins (PPL1, 2), seven PsbP-domain proteins (PPD1–7), and three PsbQ-like proteins (PQL1–3) have been found in *A. thaliana* and *Oriza sativa* (Ifuku et al. 2010). Their structures and functions were summarized elsewhere (Ifuku et al. 2008, 2010, 2011; Bricker et al. 2013; Ifuku 2014). Briefly, PPL1 is most closely related to CyanoP, while PsbP and the other PsbP homologs are paralogs of PPL1. PPL and PPD proteins should have distinct functions from PsbP, as no PPL and PPD proteins can compensate for the loss of PsbP (Ifuku et al. 2005b; Yi et al. 2009). Genetic studies using *A. thaliana* mutants reported that PPL1 functions in efficient PS II repair, while PPL2 is a subunit of the chloroplast NADH dehydrogenase-like (NDH) complex involved in photosystem I (PSI) cyclic electron transport and chlororespiration (Ishihara et al. 2007). PPD1 protein is required for PSI accumulation (Liu et al. 2012; Roose et al. 2014). PPD2 is involved in singlet oxygen-dependent signaling in *Chlamydomonas reinhartdii* (Brzezowski et al. 2012). PPD5 was linked to plant development via the plant hormone strigolactone (Roose et al. 2011). PPD6 was identified as a putative lumenal target of thioredoxin, but its function is currently unknown (Hall et al. 2012). All three PQL proteins are required for the activity of the chloroplast NDH complex in *A. thaliana*

(Yabuta et al. 2010). These facts suggest that functional diversification also occurred during evolution of the PsbP and PsbQ family.

Phylogenetic analysis based on structural modeling suggested that PsbP homologs in green plants are classified into eight families from Family A to H (Sato 2010). Family A contains PsbP in PS II, Family B – G contains PPD proteins, and Family H consists of subfamilies H1 and H2, including PPL proteins in green plants and CyanoP-type protein in red algae and cyanobacteria, respectively. The H1 and H2 families are very similar in structure and are the simplest of all PsbP families, suggesting that the PsbP superfamily originated from CyanoP and that the PPL protein likely emerged first. Functional and sequence similarity suggest that PPL1, but not PPL2, is a direct descendant of CyanoP, although PPL1 appears to have developed its specific function in the PS II assembly (Che et al. 2020). After branching of PPL1, various families including PsbP, PPL2, and PPD diverged. Red algae and diatom contain genes for several PsbP family proteins in addition to H2-type PsbP (Sato 2010). This suggests that if we do not consider horizontal gene transfer, a small degree of diversification of the PsbP family likely also occurred in the red lineage; however, the A-type PsbP for PS II is developed at a later stage.

Unlike the PsbP protein family, phylogenetic analysis indicated that PsbQ and PsbQ′ are direct descendants of CyanoQ (De Las Rivas et al. 2004; De Las Rivas and Roman 2005). As described above, green algal PsbQ is more closely related to red algal PsbQ′ than to vascular plant PsbQ, suggesting that functional differentiation of PsbQ homologs continued after the red and green lineages had branched. Although sequence conservation among PsbQ family proteins is very low, the four-helices bundled structure was largely preserved. The dispensability of PsbQ family proteins for photoautotrophy may facilitate a high evolutionary rate, resulting in lower sequence similarity among the PsbQ protein family and subsequent diversification of the PQL protein in the chloroplast NDH complex (Yabuta et al. 2010). Interestingly, PPL2 and PQLs are found specifically in angiosperms that have chloroplast NDH activity but not in mosses and liverworts with other NDH genes (Ueda et al. 2012). Therefore, diversification of NDH-type PPL2 and PQLs likely occurred rather recently in evolution.

V. Concluding Remarks

Figure 16.5 shows changes in PS II extrinsic proteins occurred during the evolution of oxyphototrophs. As described in this chapter, structural, biochemical, biophysical, and genetic studies are needed to reconsider historical concepts regarding the evolution of the extrinsic subunits of PS II. PsbO clearly exerts the important function of stabilizing the Mn_4CaO_5 cluster in all species; however, its function has not been fully conserved during evolution. Particularly, FTIR studies suggested that the function of regulating the OEC conformation was transferred to PsbP and PsbV in the green and red lineage organisms, respectively. The cryo-EM structure of plant PS II-LHCII complexes indicates that PsbP replaced the function of PsbV and PsbU to maintain the hydrogen-bond network both for H^+ export and water uptake in the OEC. The binding of PsbP also regulates electron transfer at the reducing side of PS II from the thylakoid lumenal side in a long distance, trans-membrane manner. The function and manner of interaction of PsbQ (PsbQ′) with the PS II core appear to be largely conserved between the green and red lineage organisms. Therefore, the development of PsbP is a crucial event at the branching point of the green and red lin-

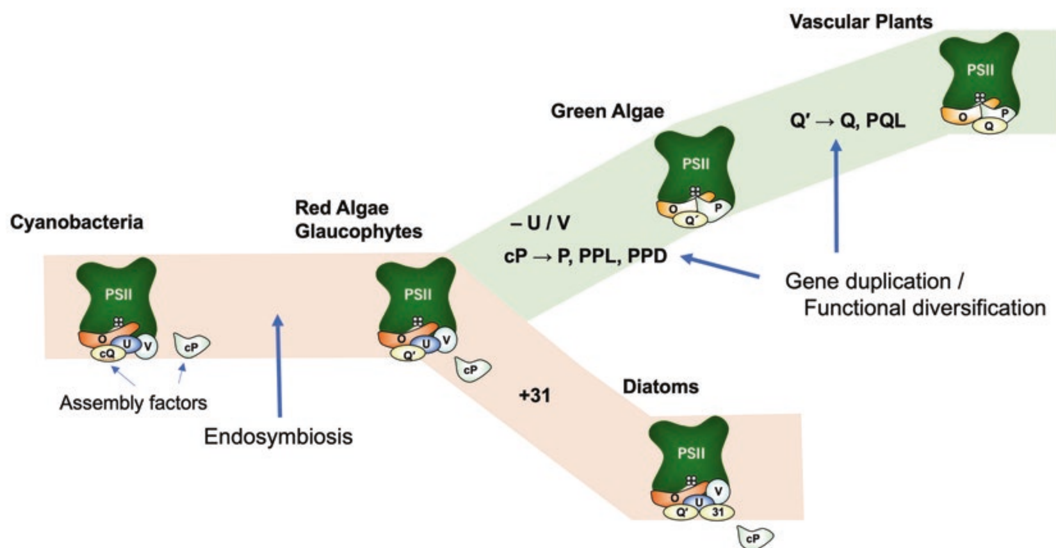

Fig. 16.5. Evolutionary changes in extrinsic proteins of PS II. Only PsbO (O) has been retained in all oxy-phototrophs. In the green lineage, PsbV (V) and PsbU (U) were lost and replaced with PsbP (P) which evolved from CyanoP (cP). In red algae, PsbQ′ (Q′) developed from CyanoQ. PsbQ′ is also used in green algae and subsequently evolved to PsbQ in vascular plants. CyanoP/Q are reported to function as assembly factors for PS II. During the molecular evolution of PsbP and PsbQ, the number of PsbP and PsbQ homologs, such as PsbP-like (PPL), PsbP-domain (PPD), and PsbQ-like (PQL) proteins, were developed by gene duplication and functional diversification. Psb31 (31) has arisen in diatoms. These models are designed to show differences in the PS II extrinsic subunit but do not show the exact location and interaction of these extrinsic subunits within PS II

eages, involving gene duplication and functional diversification. Unlike other extrinsic subunits, only the gene for PsbV is encoded in the plastid genome in the red algae and diatom, indicating the difficulty in replacing it. A recent study reported that most isolates of the marine cyanobacterium *Prochlorococcus* naturally do not contain PsbV and PsbU, but they contain PsbO and evolve oxygen (Partensky et al. 2018); however, the mechanism compensating for their absence remains unknown. One possibility is that CyanoP, commonly found in marine picocyanobacteria, functions as a major extrinsic subunit in these species, which may be relevant to the exchange process of PS II extrinsic subunits. Alternatively, some *Prochlorococcus*-specific proteins may replace PsbU and PsbV. In fact, diatom has developed Psb31 as a unique PS II extrinsic subunit, which partially substitutes for the function of PsbO. It is expected that issues will be solved in the future studies.

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References

Adachi H, Umena Y, Enami I, Henmi T, Kamiya N, Shen JR (2009) Towards structural elucidation of eukaryotic photosystem II: purification, crystallization and preliminary X-ray diffraction analysis of photosystem II from a red alga. Biochim Biophys Acta 1787:121–128

Ago H, Adachi H, Umena Y, Tashiro T, Kawakami K, Kamiya N, Tian L, …, Shen JR (2016) Novel features of eukaryotic photosystem II revealed by its crystal structure analysis from a red alga. J Biol Chem 291:5676–5687

Akabori K, Imaoka A, Toyoshima Y (1984) The role of lipids and 17-kDa protein in enhancing the recovery

of O_2 evolution in cholate-treated thylakoid membranes. FEBS Lett 173:36–40

Åkerlund HE, Jansson C, Andersson B (1982) Reconstitution of photosynthetic water splitting in inside-out thylakoid vesicles and identification of a participating polypeptide. Biochim Biophys Acta 681:1–10

Allahverdiyeva Y, Suorsa M, Rossi F, Pavesi A, Kater MM, Antonacci A, Tadini L, …, Pesaresi P (2013) *Arabidopsis* plants lacking PsbQ and PsbR subunits of the oxygen-evolving complex show altered PSII super-complex organization and short-term adaptive mechanisms. Plant J 75:671–684

Asada M, Nishimura T, Ifuku K, Mino H (2018) Location of the extrinsic subunit PsbP in photosystem II studied by pulsed electron-electron double resonance. Biochim Biophys Acta 1859:394–399

Balsera M, Arellano JB, Revuelta JL, de las Rivas J, Hermoso JA (2005) The 1.49 Å resolution crystal structure of PsbQ from photosystem II of *Spinacia oleracea* reveals a PPII structure in the N-terminal region. J Mol Biol 350:1051–1060

Bommer M, Bondar AN, Zouni A, Dobbek H, Dau H (2016) Crystallographic and computational analysis of the barrel part of the PsbO protein of photosystem II: carboxylate-water clusters as putative proton transfer relays and structural switches. Biochemistry 55:4626–4635

Bondar AN, Dau H (2012) Extended protein/water H-bond networks in photosynthetic water oxidation. Biochim Biophys Acta 1817:1177–1190

Bricker TM, Roose JL, Fagerlund RD, Frankel LK, Eaton-Rye JJ (2012) The extrinsic proteins of photosystem II. Biochim Biophys Acta 1817:121–142

Bricker TM, Roose JL, Zhang P, Frankel LK (2013) The PsbP family of proteins. Photosynth Res 116:235–250

Brzezowski P, Wilson KE, Gray GR (2012) The PSBP2 protein of *Chlamydomonas reinhardtii* is required for singlet oxygen-dependent signaling. Planta 236:1289–1303

Calderone V, Trabucco M, Vujicić A, Battistutta R, Giacometti GM, Andreucci F, Barbato R, Zanotti G (2003) Crystal structure of the PsbQ protein of photosystem II from higher plants. EMBO Rep 4:900–905

Che Y, Kusama S, Matsui S, Suorsa M, Nakano T, Aro EM, Ifuku K (2020) Arabidopsis PsbP-like protein 1 facilitates the assembly of the photosystem II supercomplexes and optimizes plant fitness under fluctuating light. Plant Cell Physiol 61:1168–1180

Cao P, Xie Y, Li M, Pan X, Zhang H, Zhao X, Su X, …, Chang W (2015) Crystal structure analysis of extrinsic PsbP protein of photosystem II reveals a manganese-induced conformational change. Mol Plant 8:664–666

Cormann KU, Bartsch M, Rögner M, Nowaczyk MM (2014) Localization of the CyanoP binding site on photosystem II by surface plasmon resonance spectroscopy. Front Plant Sci 5:595

Cox N, Messinger J (2013) Reflections on substrate water and dioxygen formation. Biochim Biophys Acta 1827:1020–1030

Dau H, Zaharieva I, Haumann M (2012) Recent developments in research on water oxidation by photosystem II. Curr Opin Chem Biol 16:3–10

De Las Rivas J, Roman A (2005) Structure and evolution of the extrinsic proteins that stabilize the oxygen-evolving engine. Photochem Photobiol Sci 4:1003–1010

De Las Rivas J, Balsera M, Barber J (2004) Evolution of oxygenic photosynthesis: genome-wide analysis of the OEC extrinsic proteins. Trends Plant Sci 9:18–25

Enami I, Murayama H, Ohta H, Kamo M, Nakazato K, Shen JR (1995) Isolation and characterization of a photosystem II complex from the red alga *Cyanidium caldarium*: association of cytochrome *c*-550 and a 12 kDa protein with the complex. Biochim Biophys Acta 1232:208–216

Enami I, Kikuchi S, Fukuda T, Ohta H, Shen JR (1998) Binding and functional properties of four extrinsic proteins of photosystem II from a red alga, *Cyanidium caldarium*, as studied by release-reconstitution experiments. Biochemistry 37:2787–2793

Enami I, Yoshihara S, Tohri A, Okumura A, Ohta H, Shen JR (2000) Cross-reconstitution of various extrinsic proteins and photosystem II complexes from cyanobacteria, red algae and higher plants. Plant Cell Physiol 41:1354–1364

Enami I, Iwai M, Akiyama A, Suzuki T, Okumura A, Katoh T, Tada O, …, Shen JR (2003) Comparison of binding and functional properties of two extrinsic components, Cyt *c*550 and a 12 kDa protein, in cyanobacterial PSII with those in red algal PSII. Plant Cell Physiol 44:820–827

Enami I, Okumura A, Nagao R, Suzuki T, Iwai M, Shen JR (2008) Structures and functions of the extrinsic proteins of photosystem II from different species. Photosynth Res 98:349–363

Ferreira KN, Iverson TM, Maghlaoui K, Barber J, Iwata S (2004) Architecture of the photosynthetic oxygen-evolving center. Science 303:1831–1838

Ghanotakis DF, Topper JN, Babcock GT, Yocum CF (1984a) Water-soluble 17 and 23 kDa polypeptides restore oxygen evolution activity by creating a high-

affinity binding site for Ca^{2+} on the oxidizing side of photosystem II. FEBS Lett 170:169–173

Ghanotakis DF, Topper JN, Youcum CF (1984b) Structural organization of the oxidizing side of photosystem II. Exogenous reductants reduce and destroy the Mn-complex in photosystems II membranes depleted of the 17 and 23 kDa polypeptides. Biochim Biophys Acta 767:524–531

Guerra F, Siemers M, Mielack C, Bondar A-N (2018) Dynamics of long-distance hydrogen-bond networks in photosystem II. J Phys Chem B 122:4625–4641

Guerrero F, Sedoud A, Kirilovsky D, Rutherford AW, Ortega JM, Roncel M (2011) A high redox potential form of cytochrome c_{550} in photosystem II from *Thermosynechococcus elongatus*. J Biol Chem 286:5985–5994

Guskov A, Kern J, Gabdulkhakov A, Broser M, Zouni A, Saenger W (2009) Cyanobacterial photosystem II at 2.9-Å resolution and the role of quinones, lipids, channels and chloride. Nat Struct Mol Biol 16:334–342

Hall M, Kieselbach T, Sauer UH, Schröder WP (2012) Purification, crystallization and preliminary X-ray analysis of PPD6, a PsbP-domain protein from *Arabidopsis thaliana*. Acta Crystallogr Sect F 68:278–280

Ido K, Ifuku K, Yamamoto Y, Ishihara S, Murakami A, Takabe K, Miyake C, Sato F (2009) Knockdown of the PsbP protein does not prevent assembly of the dimeric PSII core complex but impairs accumulation of photosystem II supercomplexes in tobacco. Biochim Biophys Acta 1787:873–881

Ido K, Kakiuchi S, Uno C, Nishimura T, Fukao Y, Noguchi T, Sato F, Ifuku K (2012) The conserved His-144 in the PsbP protein is important for the interaction between the PsbP N-terminus and the Cyt b_{559} subunit of photosystem II. J Biol Chem 287:26377–26387

Ido K, Nield J, Fukao Y, Nishimura T, Sato F, Ifuku K (2014) Cross-linking evidence for multiple interactions of the PsbP and PsbQ proteins in a higher plant photosystem II supercomplex. J Biol Chem 289:20150–20157

Ifuku K (2014) The PsbP and PsbQ family proteins in the photosynthetic machinery of chloroplasts. Plant Physiol Biochem 81:108–114

Ifuku K (2015) Localization and functional characterization of the extrinsic subunits of photosystem II: an update. Biosci Biotech Biochem 79:1223–1231

Ifuku K, Noguchi T (2016) Structural coupling of extrinsic proteins with the oxygen-evolving center in photosystem II. Front Plant Sci 7:84

Ifuku K, Sato F (2001) Importance of the N-terminal sequence of the extrinsic 23 kDa polypeptide in photosystem II in ion retention in oxygen evolution. Biochim Biophys Acta 1546:196–204

Ifuku K, Nakatsu T, Kato H, Sato F (2004) Crystal structure of the PsbP protein of photosystem II from *Nicotiana tabacum*. EMBO Rep 5:362–367

Ifuku K, Nakatsu T, Shimamoto R, Yamamoto Y, Ishihara S, Kato H, Sato F (2005a) Structure and function of the PsbP protein of photosystem II from higher plants. Photosynth Res 84:251–255

Ifuku K, Yamamoto Y, Ono TA, Ishihara S, Sato F (2005b) PsbP protein, but not PsbQ protein, is essential for the regulation and stabilization of photosystem II in higher plants. Plant Physiol 139:1175–1184

Ifuku K, Ishihara S, Shimamoto R, Ido K, Sato F (2008) Structure, function, and evolution of the PsbP protein family in higher plants. Photosynth Res 98:427–437

Ifuku K, Ishihara S, Sato F (2010) Molecular functions of oxygen-evolving complex family proteins in photosynthetic electron flow. J Integr Plant Biol 52:723–734

Ifuku K, Ido K, Sato F (2011) Molecular functions of PsbP and PsbQ proteins in the photosystem II supercomplex. J Photochem Photobiol B 104:158–164

Ishihara S, Takabayashi A, Ido K, Endo T, Ifuku K, Sato F (2007) Distinct functions for the two PsbP-like proteins PPL1 and PPL2 in the chloroplast thylakoid lumen of *Arabidopsis*. Plant Physiol 145:668–679

Jackson SA, Fagerlund RD, Wilbanks SM, Eaton-Rye JJ (2010) Crystal structure of PsbQ from *Synechocystis* sp. PCC 6803 at 1.8 Å: implications for binding and function in cyanobacterial photosystem II. Biochemistry 49:2765–2767

Jackson SA, Hind MG, Eaton-Rye JJ (2012) Solution structure of CyanoP from *Synechocystis* sp. PCC 6803: new insights on the structural basis for functional specialization among PsbP family proteins. Biochim Biophys Acta 1817:1331–1338

Juneau AD, Frankel LK, Bricker TM, Roose JL (2016) N-terminal lipid modification is required for the stable accumulation of CyanoQ in *Synechocystis* sp. PCC 6803. PLoS One 11:e0163646

Kakiuchi S, Uno C, Ido K, Nishimura T, Noguchi T, Ifuku K, Sato F (2012) The PsbQ protein stabilizes the functional binding of the PsbP protein to photosystem II in higher plants. Biochim Biophys Acta 1817:1346–1351

Kashino Y, Lauber WM, Carroll JA, Wang Q, Whitmarsh J, Satoh K, Pakrasi HB (2002) Proteomic analysis of a highly active photosystem II preparation from the cyanobacterium *Synechocystis* sp. PCC 6803 reveals the presence of novel polypeptides. Biochemistry 41:8004–8012

Kashino Y, Inoue-Kashino N, Roose JL, Pakrasi HB (2006) Absence of the PsbQ protein results in destabilization of the PsbV protein and decreased oxygen evolution activity in cyanobacterial photosystem II. J Biol Chem 281:20834–20841

Kern J, Chatterjee R, Young ID, Fuller FD, Lassalle L, Ibrahim M, Gul S, …, Yachandra VK (2018) Structures of the intermediates of Kok's photosynthetic water oxidation clock. Nature 563:421–425

Knoppová J, Yu J, Konik P, Nixon PJ, Komenda J (2016) CyanoP is involved in the early steps of photosystem II assembly in the cyanobacterium *Synechocystis* sp. PCC 6803. Plant Cell Physiol 57:1921–1931

Kondo J, Noguchi T (2018) PsbP-induced protein conformational changes around cl− ions in the water oxidizing center of photosystem II. Photosynthetica 56:178–184

Kopecky V Jr, Kohoutova J, Lapkouski M, Hofbauerova K, Sovova Z, Ettrichova O, González-Pérez S, …, Ettrich R (2012) Raman spectroscopy adds complementary detail to the high-resolution X-ray cystal structure of photosynthetic PsbP from *Spinacia oleracea*. PLoS One 7:e46694

Kuwabara T, Murata N (1982) Inactivation of photosynthetic oxygen evolution and concomitant release of three polypeptides in the photosystem II particles of spinach chloroplasts. Plant Cell Physiol 23:533–539

Kuwabara T, Miyao M, Murata T, Murata N (1985) The function of 33-kDa protein in the photosynthetic oxygen-evolution system studied by reconstitution experiments. Biochim Biophys Acta 806:283–289

Linke K, Ho FM (2014) Water in photosystem II: structural, functional and mechanistic considerations. Biochim Biophys Acta 1837:14–32

Liu J, Yang H, Lu Q, Wen X, Chen F, Peng L, Zhang L, Lu C (2012) PsbP-domain protein1, a nuclear-encoded thylakoid lumenal protein, is essential for photosystem I assembly in *Arabidopsis*. Plant Cell 24:4992–5006

Liu H, Zhang H, Weisz DA, Vidavsky I, Gross ML, Pakrasi HB (2014) MS-based cross-linking analysis reveals the location of the PsbQ protein in cyanobacterial photosystem II. Proc Natl Acad Sci USA 111:4638–4643

Liu H, Weisz DA, Pakrasi HB (2015) Multiple copies of the PsbQ protein in a cyanobacterial photosystem II assembly intermediate complex. Photosynth Res 126:375–383

Lorch S, Capponi S, Pieront F, Bondar AN (2015) Dynamic carboxylate/water networks on the surface of the PsbO subunit of photosystem II. J Phys Chem B 119:12172–12181

Michoux F, Takasaka K, Boehm M, Nixon PJ, Murray JW (2010) Structure of CyanoP at 2.8 Å: implications for the evolution and function of the PsbP subunit of photosystem II. Biochemistry 49:7411–7413

Michoux F, Boehm M, Bialek W, Takasaka K, Maghlaoui K, Barber J, Murray JW, Nixon PJ (2014) Crystal structure of CyanoQ from the thermophilic cyanobacterium *Thermosynechococcus elongatus* and detection in isolated photosystem II complexes. Photosynth Res 122:57–67

Miyao M, Murata N (1985) The Cl^- effect on photosynthetic oxygen evolution: interaction of Cl^- with 18-kDa, 24-kDa and 33-kDa proteins. FEBS Lett 180:303–308

Miyao M, Murata N (1986) Light-dependent inactivation of photosynthetic oxygen evolution during NaCl treatment of photosystem II particles: the role of the 24-kDa protein. Photosynth Res 10:489–496

Nagao R, Ishii A, Tada O, Suzuki T, Dohmae N, Okumura A, Iwai M, …, Enami I (2007) Isolation and characterization of oxygen-evolving thylakoid membranes and photosystem II particles from a marine diatom *Chaetoceros gracilis*. Biochim Biophys Acta 1767:1353–1362

Nagao R, Moriguchi A, Tomo T, Niikura A, Nakajima S, Suzuki T, Okumura A, …, Enami I (2010a) Binding and functional properties of five extrinsic proteins in oxygen-evolving photosystem II from a marine centric diatom, *Chaetoceros gracilis*. J Biol Chem 285: 29191–29199

Nagao R, Tomo T, Noguchi E, Nakajima S, Suzuki T, Okumura A, Kashino Y, …, Enami I (2010b) Purification and characterization of a stable oxygen-evolving photosystem II complex from a marine centric diatom, *Chaetoceros gracilis.* Biochim Biophys Acta 1797:160–166

Nagao R, Suga M, Niikura A, Okumura A, Koua FH, Suzuki T, Tomo T, …, Shen JR (2013) Crystal structure of Psb31, a novel extrinsic protein of photosystem II from a marine centric diatom and implications for its binding and function. Biochemistry 52:6646–6652

Nagao R, Tomo T, Noguchi T (2015) Effects of extrinsic proteins on the protein conformation of the oxygen-evolving center in cyanobacterial photosystem II as revealed by Fourier transform infrared spectroscopy. Biochemistry 54:2022–2031

Nagao R, Suzuki T, Dohmae N, Shen JR, Tomo T (2017a) Functional role of Lys residues of Psb31 in electrostatic interactions with diatom photosystem II. FEBS Lett 591:3259–3264

Nagao R, Suzuki T, Okumura A, Kihira T, Toda A, Dohmae N, Nakazato K, Tomo T (2017b) Electrostatic interaction of positive charges on the

surface of Psb31 with photosystem II in the diatom *Chaetoceros gracilis*. Biochim Biophys Acta 1858:779–785

Nagao R, Ueoka-Nakanishi H, Noguchi T (2017c) D1-Asn-298 in photosystem II is involved in a hydrogen-bond network near the redox-active tyrosine Y_Z for proton exit during water oxidation. J Biol Chem 292:20046–20057

Nagao R, Kato K, Suzuki T, Ifuku K, Uchiyama I, Kashino Y, Dohmae N, …, Akita F (2019) Structural basis for energy harvesting and dissipation in a diatom PSII-FCPII. Nature Plants 5:890–901

Nelson N, Yocum CF (2006) Structure and function of photosystems I and II. Ann Rev Plant Biol 57:521–565

Nishimura T, Uno C, Ido K, Nagao R, Noguchi T, Sato F, Ifuku K (2014) Identification of the basic amino acid residues on the PsbP protein involved in the electrostatic interaction with photosystem II. Biochim Biophys Acta 1837:1447–1453

Nishimura T, Nagao R, Noguchi T, Nield J, Sato F, Ifuku K (2016) The N-terminal sequence of the extrinsic PsbP protein modulates the redox potential of Cyt b_{559} in photosystem II. Sci Rep 6:21490

Ogata K, Yuki T, Hatakeyama M, Uchida W, Nakamura S (2013) All-atom molecular dynamics simulation of photosystem II embedded in thylakoid membrane. J Am Chem Soc 135:15670–15673

Ohta H, Suzuki T, Ueno M, Okumura A, Yoshihara S, Shen JR, Enami I (2003) Extrinsic proteins of photosystem II: an intermediate member of PsbQ protein family in red algal PSII. Eur J Biochem 270:4156–4163

Okumura A, Nagao R, Suzuki T, Yamagoe S, Iwai M, Nakazato K, Enami I (2008) A novel protein in photosystem II of a diatom *Chaetoceros gracilis* is one of the extrinsic proteins located on lumenal side and directly associates with PSII core components. Biochim Biophys Acta 1777:1545–1551

Pagliano C, Saracco G, Barber J (2013) Structural, functional and auxiliary proteins of photosystem II. Photosynth Res 116:167–188

Partensky F, Mella-Flores D, Six C, Garczarek L, Czjzek M, Marie D, Kotabová E, …, Prášil O (2018) Comparison of photosynthetic performances of marine picocyanobacteria with different configurations of the oxygen-evolving complex. Photosynth Res 138:57–71

Roose JL, Frankel LK, Bricker TM (2010) Documentation of significant electron transport defects on the reducing side of photosystem II upon removal of the PsbP and PsbQ extrinsic proteins. Biochemistry 49:36–41

Roose JL, Frankel LK, Bricker TM (2011) Developmental defects in mutants of the PsbP domain protein 5 in *Arabidopsis thaliana*. PLoS One 6:e28624

Roose JL, Frankel LK, Bricker TM (2014) The PsbP-domain protein 1 functions in the assembly of lumenal domains in photosystem I. J Biol Chem 289:23776–23785

Roose JL, Frankel LK, Mummadisetti MP, Bricker TM (2016) The extrinsic proteins of photosystem II: update. Planta 243:889–908

Saito K, Rutherford AW, Ishikita H (2015) Energetics of proton release on the first oxidation step in the water-oxidizing enzyme. Nat Commun 6:8488

Sakashita N, Watanabe HC, Ikeda T, Ishikita H (2017a) Structurally conserved channels in cyanobacterial and plant photosystem II. Photosynth Res 133:75–85

Sakashita N, Watanabe HC, Ikeda T, Saito K, Ishikita H (2017b) Origins of water molecules in the photosystem II crystal tructure. Biochemistry 56:3049–3057

Sato N (2010) Phylogenomic and structural modeling analyses of the PsbP superfamily reveal multiple small segment additions in the evolution of photosystem II-associated PsbP protein in green plants. Mol Phylogenet Evol 56:176–186

Shen JR (2015) The structure of photosystem II and the mechanism of water oxidation in photosynthesis. Ann Rev Plant Biol 66:23–48

Shen JR, Inoue Y (1993) Binding and functional properties of two new extrinsic components, cytochrome *c*-550 and a 12-kDa protein, in cyanobacterial photosystem II. Biochemistry 32:1825–1832

Shen JR, Ikeuch M, Inoue Y (1992) Stoichiometric association of extrinsic cytochrome *c*-550 and 12 kDa protein with a highly purified oxygen-evolving photosystem II core complex from *Synechococcus vulcanus*. FEBS Lett 301:145–149

Shen JR, Burnap RL, Inoue Y (1995) An independent role of cytochrome *c*-550 in cyanobacterial photosystem II as revealed by double-deletion mutagenesis of the *psbO* and *psbV* genes in *Synechocystis* sp. PCC 6803. Biochemistry 34:12661–12668

Shutova T, Klimov VV, Andersson B, Samuelsson G (2007) A cluster of carboxylic groups in PsbO protein is involved in proton transfer from the water oxidizing complex of photosystem II. Biochim Biophys Acta 1767:434–440

Su X, Ma J, Wei X, Cao P, Zhu D, Chang W, Liu Z, …, Li M (2017) Structure and assembly mechanism of plant $C_2S_2M_2$ -type PSII-LHCII supercomplex. Science 357:815–820

Suga M, Akita F, Hirata K, Ueno G, Murakami H, Nakajima Y, Shimizu T, …, Shen JR (2015) Native

structure of photosystem II at 1.95 Å resolution viewed by femtosecond X-ray pulses. Nature 517:99–103

Suga M, Akita F, Sugahara M, Kubo M, Nakajima Y, Nakane T, Yamashita K, …, Shen JR (2017) Light-induced structural changes and the site of O=O bond formation in PSII caught by XFEL. Nature 543:131–135

Suga M, Akita F, Yamashita K, Nakajima Y, Ueno G, Li H, Yamane T, …, Shen J-R (2019) An open-cubane oxyl/oxo mechanism for O=O bond formation in PSII revealed by XFEL. Science 366:334–338

Summerfield TC, Shand JA, Bentley FK, Eaton-Rye JJ (2005) PsbQ (Sll1638) in *Synechocystis* sp. PCC 6803 is required for photosystem II activity in specific mutants and in nutrient-limiting conditions. Biochemistry 44:805–815

Suzuki T, Ohta H, Enami I (2005) Cross-reconstitution of the extrinsic proteins and photosystem II complexes from *Chlamydomonas reinhardtii* and *Spinacia oleracea*. Photosynth Res 84:239–244

Takagi D, Ifuku K, Nishimura T, Miyake C (2019) Antimycin A inhibits cytochrome b_{559}-mediated cyclic electron flow within photosystem II. Photosynth Res 139:487–498

Takaoka T, Sakashita N, Saito K, Ishikita H (2016) pK_a of a proton-conducting water chain in photosystem II. J Phys Chem Lett 7:1925–1932

Thornton LE, Ohkawa H, Roose JL, Kashino Y, Keren N, Pakrasi HB (2004) Homologs of plant PsbP and PsbQ proteins are necessary for regulation of photosystem II activity in the cyanobacterium *Synechocystis* 6803. Plant Cell 16:2164–2175

Tomita M, Ifuku K, Sato F, Noguchi T (2009) FTIR evidence that the PsbP extrinsic protein induces protein conformational changes around the oxygen-evolving Mn cluster in photosystem II. Biochemistry 48:6318–6325

Ueda M, Kuniyoshi T, Yamamoto H, Sugimoto K, Ishizaki K, Kohchi T, Nishimura Y, Shikanai T (2012) Composition and physiological function of the chloroplast NADH dehydrogenase-like complex in *Marchantia polymorpha*. Plant J 72:683–693

Umena Y, Kawakami K, Shen JR, Kamiya N (2011) Crystal structure of oxygen-evolving photosystem II at a resolution of 1.9 Å. Nature 473:55–60

Uno C, Nagao R, Suzuki H, Tomo T, Noguchi T (2013) Structural coupling of extrinsic proteins with the oxygen-evolving center in red algal photosystem II as revealed by light-induced FTIR difference spectroscopy. Biochemistry 52:5705–5707

Vassiliev S, Zaraiskaya T, Bruce D (2013) Molecular dynamics simulations reveal highly permeable oxygen exit channels shared with water uptake channels in photosystem II. Biochim Biophys Acta 1827:1148–1155

Vinyard DJ, Ananyev GM, Dismukes GC (2013) Photosystem II: the reaction center of oxygenic photosynthesis. Annu Rev Biochem 82:77–606

Wei X, Su X, Cao P, Liu X, Chang W, Li M, Zhang X, Liu Z (2016) Structure of spinach photosystem II–LHCII supercomplex at 3.2 Å resolution. Nature 534:69–74

Yabuta S, Ifuku K, Takabayashi A, Ishihara S, Ido K, Ishikawa N, Endo T, Sato F (2010) Three PsbQ-like proteins are required for the function of the chloroplast NAD(P)H dehydrogenase complex in *Arabidopsis*. Plant Cell Physiol 51:866–876

Yamada M, Nagao R, Iwai M, Arai Y, Makita A, Ohta H, Tomo T (2018) The PsbQ′ protein affects the redox potential of the Q_A in photosystem II. Photosynthetica 56:185–191

Yi X, Hargett SR, Frankel LK, Bricker TM (2006) The PsbQ protein is required in *Arabidopsis* for photosystem II assembly/stability and photoautotrophy under low light conditions. J Biol Chem 281:26260–26267

Yi X, Hargett SR, Liu H, Frankel LK, Bricker TM (2007) The PsbP protein is required for photosystem II complex assembly/stability and photoautotrophy in *Arabidopsis thaliana*. J Biol Chem 282:24833–24841

Yi X, Hargett SR, Frankel LK, Bricker TM (2009) The PsbP protein, but not the PsbQ protein, is required for normal thylakoid architecture in *Arabidopsis thaliana*. FEBS Lett 583:2142–2147

Zienkiewicz M, Krupnik T, Drożak A, Wasilewska W, Golke A, Romanowska E (2018) Deletion of *psbQ′* gene in *Cyanidioschyzon merolae* reveals the function of extrinsic PsbQ′ in PSII. Plant Mol Biol 96:135–149