ResearchGate

See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/50213572

Auxiliary functions of the PsbO, PsbP and PsbQ proteins of higher plant Photosystem II: A critical analysis

ARTICLE *in* JOURNAL OF PHOTOCHEMISTRY AND PHOTOBIOLOGY. B, BIOLOGY · FEBRUARY 2011

Impact Factor: 2.96 · DOI: 10.1016/j.jphotobiol.2011.01.025 · Source: PubMed

CITATIONS
27

READS
30

2 AUTHORS, INCLUDING:

Terry M Bricker
Louisiana State University
93 PUBLICATIONS **2,615** CITATIONS

SEE PROFILE

Available from: Terry M Bricker
Retrieved on: 24 January 2016

Journal of Photochemistry and Photobiology B: Biology 104 (2011) 165–178

Contents lists available at ScienceDirect

Journal of Photochemistry and Photobiology B: Biology

journal homepage: www.elsevier.com/locate/jphotobiol

Short Review

Auxiliary functions of the PsbO, PsbP and PsbQ proteins of higher plant Photosystem II: A critical analysis

Terry M. Bricker *, Laurie K. Frankel

Department of Biological Sciences, Division of Biochemistry and Molecular Biology, Louisiana State University, Baton Rouge, LA 70803, United States

ARTICLE INFO

Article history:
Available online 25 February 2011

Keywords:
Photosystem II
GTPase
Thylakoid organization
PsbO
PsbP
PsbQ

ABSTRACT

Numerous studies over the last 25 years have established that the extrinsic PsbO, PsbP and PsbQ proteins of Photosystem II play critically important roles in maintaining optimal manganese, calcium and chloride concentrations at the active site of Photosystem II. Chemical or genetic removal of these components induces multiple and profound defects in Photosystem II function and oxygen-evolving complex stability. Recently, a number of studies have indicated possible additional roles for these proteins within the photosystem. These include putative enzymatic activities, regulation of reaction center protein turnover, modulation of thylakoid membrane architecture, the mediation of PS II assembly/stability, and effects on the reducing side of the photosystem. In this review we will critically examine the findings which support these auxiliary functions and suggest additional lines of investigations which could clarify the nature of the functional interactions of these proteins with the photosystem.

© 2011 Elsevier B.V. All rights reserved.

Contents

1. Introduction

Photosystem II (PS II) functions as a light-driven, water-plastoquinone oxidoreductase. In higher plants and cyanobacteria at least six intrinsic proteins appear to be required for O_2 evolution [1–3]. These are CP47, CP43, D1, D2, and the α and β subunits of cytochrome b_{559}. Deletion of these subunits uniformly results in the complete loss of functional PS II and assembly [4]. At least eleven other intrinsic membrane protein subunits are present, although the roles for many of these remain obscure. Additionally, in higher plants, three extrinsic proteins, with molecular masses of 26.5 kDa (PsbO), 20.2 kDa (PsbP) and 16.5 kDa (PsbQ) are also required for maximal rates of O_2 evolution under physiological inorganic cofactor concentrations. These extrinsic proteins have been the subject of extensive investigations by numerous laboratories over the last 25 years. Removal of these components from PS II-enriched membranes [5] by differential chemical treatment (for a full discussion, see [6]) or modification by mutagenesis [7–14] followed by biochemical and biophysical analysis have unequivocally demonstrated their role in support of oxygen evolution and, specifically, the modulation of the inorganic cofactor (manganese, calcium and chloride) requirements for photosynthesis. Other studies have provided moderate to high resolution structures of

* Corresponding author. Tel.: +1 225 578 1555; fax: +1 225 578 7258.
E-mail address: btbric@lsu.edu (T.M. Bricker).

1011-1344/$ - see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jphotobiol.2011.01.025

these extrinsic proteins either associated with the PS II reaction center (PsbO as associated with cyanobacterial PS II [15,16] or as purified proteins PsbP [17] and PsbQ [18]). These studies have been the subject of numerous recent reviews ([6,19–21] Bricker and Eaton-Rye, in preparation) and are not the focal point of this communication.

Recently, studies from a number of laboratories have indicated that the PsbO, PsbP and PsbQ proteins may perform alternative functions within the photosystem. The PsbO component has been suggested to exhibit carbonic anhydrase [22,23] and/or GTPase activity [24,25], the PsbP protein has been implicated in binding the manganese required for photoactivation [26,27] and, along with PsbQ, participation in granal stack formation [28–31]. Additionally, functional defects on the reducing side of the photosystem have been attributed to PsbP removal [32,33]. Finally, all three of the extrinsic proteins appear to be required for the accumulation of PS II reaction centers in higher plants either under normal growth conditions (PsbO and PsbP, [11,12,14]) or during growth at low light intensities (PsbQ, [13]). Consequently, they may act as assembly/stability factors for the photosystem. In this review we will critically examine the evidence for these putative auxiliary functions of the extrinsic proteins and suggest experimental paths to clarify these possible roles in PS II.

2. PsbO as a carbonic anhydrase

A number of hypotheses have been presented to suggest that bicarbonate is required for normal PS II function. Involvement of bicarbonate on both reducing- and oxidizing-sides of the photosystem have been suggested (for a review in support of these hypotheses see [34]). While the function of bicarbonate on the reducing side of the photosystem is reasonably well established, its functioning on the oxidizing-side of the photosystem is quite controversial [35–39]. It is not the role of this review to examine the assertion that bicarbonate functions on the oxidizing-side of PS II, but rather to examine the specific hypothesis that the PsbO protein functions as a carbonic anhydrase.

Several reports have indicated the PsbO protein may exhibit carbonic anhydrase-like activity [22,23,40,41]. Initial studies, performed in maize, demonstrated that a protein fraction which was denoted CA_{ext} was removed from PS II membranes by $CaCl_2$ washing. Immunoblotting experiments performed with an antibody raised against the *Chlamydomonas* lumenal CAH3 carbonic anhydrase identified a protein from the $CaCl_2$ fraction which migrated during SDS–PAGE at the same location as the PsbO component [22]. More extensive studies were performed with recombinant pea prePsbO protein expressed in *Escherichia coli* and the native PsbO protein purified from pea PS II membranes [40]. The recombinant prePsbO protein and the native component both exhibited a manganese-dependent carbonic anhydrase activity. The activity was heat-stable, which is a known feature of the PsbO protein [42]. The removal of the native PsbO protein from PS II membranes by increasing $CaCl_2$ washing concentrations also removed the carbonic anhydrase activity. Additionally, antibodies raised against either pea PsbO or CAH3 carbonic anhydrase from *Chlamydomonas* inhibited the carbonic anhydrase activity of purified PsbO and recombinant prePsbO. Analysis of the cation requirement for the observed carbonic anhydrase activity indicated that $Mn^{2+} > Ca^{2+} > Zn^{2+} = Co^{2+} = Mg^{2+} = Na^{+}$, with the latter four ions exhibiting essentially no activity. The observed carbonic anhydrase activity was strongly pH dependent, being significant only at pH < 6.5 [41]. Finally, reconstitution with either purified pea PsbO or recombinant pea prePsbO protein to $CaCl_2$-washed pea PS II membranes restored both oxygen evolution and carbonic anhydrase activity. It should be noted, however, that while the observed reconstitution of purified PsbO protein onto $CaCl_2$-washed membranes exhibited saturating binding kinetics with respect to protein binding and the restoration of oxygen evolution, the carbonic anhydrase activity did not exhibit similar binding kinetics. If the carbonic anhydrase activity was directly associated with the PsbO protein, these should exhibit identical binding isotherms.

In a series of similar experiments, Shitov et al. [23] also extracted the extrinsic proteins from pea PS II membranes and isolated a fraction enriched in the PsbO protein which exhibited a manganese-dependent carbonic anhydrase activity (these authors also reported that the PsbP and PsbQ proteins isolated by salt-washing PS II membranes exhibited carbonic anhydrase activity). With respect to the PsbO component, these authors determined that the carbonic anhydrase activity was manganese-dependent (K_d 670 μM) and was sensitive to dithiothreitol. Metal analysis of the PS II membranes which they used as a source of the extrinsic proteins indicated that they contained 4.24 ± 0.2 atoms of Mn and 1.25 ± 0.2 atoms of Zn per 250 chl.

Taken at face value, these studies indicate that the PsbO protein exhibits a novel carbonic anhydrase activity with extremely unusual characteristics. Three possibilities exist which could account for these data. First, the PsbO protein indeed functionally exhibits carbonic anhydrase activity. If this is the case, then the PsbO protein is a truly unique carbonic anhydrase with extraordinarily novel properties. Most notably it is a manganese-requiring enzyme. No other manganese-requiring carbonic anhydrases have been identified, all others being zinc-containing proteins (with the singular exception of a recently identified cadmium-containing enzyme [43,44]). These form six evolutionarily unrelated families (the α, β, γ, δ, ε and the newly proposed ζ-carbonic anhydrases). α-, and γ-, and δ-carbonic anhydrases posses a four-coordinate metal center with three histidyl ligands and a bound reactive hydroxyl ion. β-, ε and ζ-carbonic anhydrases possess a four-coordinate metal center with two cystinyl , one histidyl residue and a bound reactive hydroxyl. The ζ-carbonic anhydrases can ligate cadmium, cobalt or zinc at their active sites. The PsbO protein contains no histidyl residues but does contain two cystinyl residues, which are organized as a disulfide bond [45]. These have been suggested to be under regulatory redox control [46–48]. Consequently, other residues must coordinate the metal cofactor. Since manganese is classified a "hard" ion it would usually be coordinated to hard ligands, such as the carboxylates which are plentiful in the PsbO protein.

A second possibility is that the detected carbonic anhydrase activity associated with the PsbO component was derived from a contaminating "authentic" carbonic anhydrase. This is a particularly insidious problem since carbonic anhydrases typically exhibit extremely high k_{cats}. Very small amounts of a contaminating carbonic anhydrase could yield the rather low observed carbonic anhydrase specific activity reported for the PsbO protein. Exacerbating this problem is the fact that carbonic anhydrase is one of the most abundant enzymes in C3 plants, being second only to ribulose-1,5-bisphosphate carboxylase/oxygenase in abundance [49]. Two carbonic anhydrases (Can and CynT) [50] are also present in *E. coli*, raising the possibility that even the recombinant prePsbO protein could be contaminated with *E. coli* carbonic anhydrase(s). Finally, if antibodies were raised against either isolated plant PsbO or recombinant PsbO protein which were contaminated with "authentic carbonic anhydrases", it is possible that anti-"authentic carbonic anhydrase" antibodies are present in these preparations. These could give rise to antibody inhibition effects noted above. Within this context it is important to note that the CAH3 antibody, which was used in a number of studies described above, was unequivocally demonstrated to react not only with the CAH3 carbonic anhydrase but also with an unknown pro-

tein exhibiting an apparent molecular mass of about 33 kDa [51] on polyacrylamide gels. This is very similar to the apparent molecular mass exhibited by the PsbO protein during SDS–PAGE analysis. One solution to this difficult problem would be the use of either monoclonal reagents or polyclonal antisera directed against synthetic PsbO peptides. Both of these antibody types are freely available [52,53].

Finally, it is possible that the PsbO protein could artifactually bind manganese and that this protein-bound manganese could exhibit limited carbonic anhydrase activity. Such binding could be associated with a putative calcium-binding site identified on the PsbO protein from the cyanobacterium *Synechococcus elongatus* which is distinct from the calcium-binding site that is associated with the $Mn_4Ca_1Cl_{1-2}$ metal center [54,55]. Interestingly, Mn^{2+}-substituted human carbonic anhydrase 1 exhibits a small amount of residual carbonic anhydrase activity (7–10% of the normal zinc-containing enzyme [56]). Within this context it should be noted that Shitov et al. found [23] four manganese per PS II reaction center in their PS II membranes. This is the value which has been observed by numerous other investigators. All four of these manganese are associated with the Mn_4–Ca_1–Cl_{1-2} cluster at the heart of the photosystem in the cyanobacterial crystal structure [15,16,55]. Consequently, two possibilities exist. First, if a manganese-dependent carbonic anhydrase is present in the photosystem, then it must not be associated with manganese in isolated PS II membranes. However, the carbonic anhydrase activity in these PS II membranes was not stimulated by added manganese. Alternatively, if a carbonic anhydrase exists in these PS II membranes and it contains bound manganese, then the functional manganese cluster in PS II from higher plants must contain less than four manganese. This seems extraordinarily unlikely.

Other investigators also have examined the possibility that the PsbO protein exhibits carbonic anhydrase activity and have come to different conclusions. Recent experiments by McConnell et al. [57] indicate that the association of carbonic anhydrase activity with PS II is highly variable. They demonstrated that individual PS II membrane preparations exhibited strikingly different levels of carbonic anhydrase activity while simultaneously exhibiting rather stable oxygen–evolving activity. This uncoupling of oxygen evolution activity from carbonic anhydrase activity was further investigated in inhibitor studies. The carbonic anhydrase activity observed was not affected by the PS II inhibitor DCMU, which fully inhibited oxygen evolution, and the observed oxygen evolution activity was not affected by ethoxyzolamide, which nearly completely inhibited the carbonic anhydrase activity. This experiment would seem to eliminate the possibility of any tight coupling between oxygen evolution activity and carbonic anhydrase activity. Finally, these authors demonstrated that the carbonic anhydrase activity associated with PS II membranes was lost upon further purification of an oxygen-evolving PS II core complex which contained the PsbO component and exhibited high rates of oxygen evolution. If the PsbO protein were a carbonic anhydrase it would be expected that this activity would be enhanced in parallel to enhancement of the oxygen evolution rate in these core preparations. These results seem to clearly indicate that the PsbO protein is not a carbonic anhydrase. At this juncture, it would appear that the proponents of the hypothesis that the PsbO protein is a carbonic anhydrase must address these serious objections for the hypothesis to remain viable.

3. PsbO as a GTPase

Evidence has been presented that the PsbO protein binds GTP with high affinity [24] and functions as a GTPase [25]. In this role, it has been hypothesized to act to control the phosphorylation state of the D1 protein [58]. Since the phosphorylation level of the D1 component appears to be coupled with its efficient turnover [59,60], it was proposed that the PsbO protein may be an important regulatory component of this process [61]. It must be noted that a tight coupling between the D1 phosphorylation state and D1 turnover has recently been called into serious question [62,63].

In initial experiments [24], it was demonstrated that in thylakoids and PS II core complexes a protein with an apparent molecular mass of 33 kDa became labeled with the photoaffinity label [α-^{32}P]8-azido-guanosine-5-triphosphate ([α-^{32}P]8-N_3GTP) but not [α-^{32}P]8-azido-adenosine-5-triphosphate. The observed labeling was Mg-dependent, a typical requirement for GTP-binding proteins and GTPases. In thylakoids, only samples which were pre-illuminated with white light became subsequently affinity labeled upon exposure to ultraviolet illumination. In the PS II core complex, photoaffinity labeling was markedly increased after exposure to white light. Intriguingly, the affinity labeling observed was DCMU-sensitive even though a substantial dark period (3 min) separated the white light illumination from the ultraviolet exposure. The affinity labeled component was released from the PS II core complex by alkaline Tris treatment, cross-reacted with an anti-PsbO antibody, and sequence analysis of the Coomassie–stained protein band yielded the N-terminal sequence of the PsbO component. Unlabeled GTP and GDP inhibited the affinity labeling while unlabeled GMP, ATP and CTP had little effect. Analysis of the labeling indicated that the binding exhibited a K_d of 22.8 μM with one [α-^{32}P]8-N_3GTP bound per PS II dimer. Additionally, purified PsbO protein could also be photoaffinity labeled with [α-^{32}P]-8-N_3GTP with an observed K_d of 8.1 μM. It is quite interesting that PsbO in solution exhibits a higher affinity for GTP than does PsbO which is bound to the photosystem, particularly since GTP hydrolysis was later correlated to release of the bound protein [25]. Finally, trypsin treatment of the affinity labeled PsbO indicated that the label was incorporated into an unidentified N-terminal domain of the protein. These results indicate that the PsbO protein can bind GTP.

Subsequently results were presented which documented structural alterations occurring in the PsbO protein upon GTP binding and indicating that the protein not only binds GTP but also functions as a GTPase [25]. Structural changes in the spinach PsbO protein in solution were examined in the presence of GTP. The addition of 150 μM GTP resulted in significant changes in the far-UV circular dichroism spectra of the protein, leading to a significant loss of β-sheet structure as indicated by markedly reduced positive and negative molar ellipticity at 195 and 205–220 nm, respectively. Fluorescence studies were also performed. The PsbO protein contains a single tryptophan residue near its N-terminus. The fluorescence characteristics of this residue have been used to monitor structural changes induced in the protein by various treatments [64,65] as increasing tryptophan fluorescence is an indicator of a more hydrophobic environment for the residue. Treatment of purified PsbO protein with 15 μM GTP induces a very small but apparently reproducible change in the tryptophan fluorescence characteristics of the protein. A possible binding site was identified by comparison of the PsbO sequence to those of known GTP-binding proteins. A non-canonical form of the P-loop (phosphate-binding) domain, which was 50% similar to that found in many Mg-GTP-binding proteins, was identified in the N-terminal domain of the protein (^{42}PGKYTAK^{49}K). Other important residues of the putative binding site include ^{84}T, an acidic domain (^{86}DEIE^{90}G) possibly involved with interaction with the magnesium ion, and a possible guanine-binding domain (^{136}GKP^{139}E).

Low GTPase activity has been observed for intact PS II, PS II monomers and purified PsbO. The highest activity was observed in PS II membranes and PS II dimers, although data were not presented in the latter case. The activity observed for PS II membranes

at pH 6.0 was 4.5 mmol GDP produced per mole PsbO protein per min. This corresponds to a k_{cat} of 7.5×10^{-5} per second or one GTP hydrolyzed every 3.7 h. This activity was stimulated about 2-fold at pH 7.4. Small GTPases typically exhibit extremely low k_{cats} in the absence of specific GTPase-activating proteins. H-*ras*, for instance, exhibits a k_{cat} of $1–4 \times 10^{-4}$ per second, which is accelerated by a factor of 10^5 by GTPase-activating proteins (for a review, see [66]). No activating proteins have yet been identified for the GTPase activity associated with the PsbO protein although these may not be necessary. D1 lifetimes exhibit a $T_{1/2}$ of about 2 h under either growth light or photoinhibitory conditions [67]. This differs from the observed GTP turnover rate only by a factor of two which may be within the limits of error for these measurements. Consequently, even the low GTPase activity may be sufficient to support the proposed regulation of PS II function and turnover.

These authors also presented evidence that the release of the PsbO protein from PS II exposed to photoinhibitory conditions could be modulated by the presence of GTP. Both NaCl-washed PS II membranes (lacking the PsbP and PsbQ components) and a dimeric LHCII-PS II supercomplex preparation were used in these studies. In the NaCl-washed PS II membranes probed at pH 6.0, 200 μM GTP enhanced the release of the PsbO protein both in the dark and upon illumination. At pH 7.4, there was a marked increase in PsbO protein released in the light both in the presence and absence of GTP. In the presence of GTP, slightly more PsbO was released then in the absence of the nucleotide. A significantly larger effect was observed in the PS II supercomplexes assayed at pH 6.0. In these experiments the presence of GTP was clearly correlated to loss of oxygen evolution, loss of the D1 protein and release of the PsbO protein from the complex upon illumination with increasing amounts of light. Unfortunately, the authors did not provide data documenting an increase in GTPase activity with increasing light intensity.

Several questions remain. First, the observed GTPase activity for the purified PsbO protein was heat-sensitive. After treatment at 95 °C for 10 min all GTPase activity was lost. This is in marked contrast to other properties of the PsbO protein. Lydakis-Simantiris et al. [42] demonstrated that after treatment at 90 °C for 120 min, the PsbO protein was able to regain a largely functional structure upon cooling to 25 °C, could rebind to PS II with normal stoichiometry, and essentially fully support oxygen-evolving activity. A small irreversible change did occur in the heated protein's far-UV circular dichroism and FTIR spectra indicating that some structural changes had occurred which persisted after cooling. It is possible that these changes are related to the observed loss of GTPase activity. If this is the case, PS II membranes or dimeric LHCII-PS II supercomplexes reconstituted with heat-treated PsbO should exhibit no GTPase activity and, consequently, subsequent treatment of the reconstituted PS II complex with high light regimes should not lead to the release of the PsbO protein if GTPase activity is required for this process. A second question arises with respect to the association of the GTPase activity with various PS II preparations. While the purified PsbO protein exhibited low activity, PS II membranes and dimeric LHCII-PS II supercomplexes exhibited high activity. On the other hand, monomeric PS II core preparations exhibited low activity. These results would seem to indicate that the formation of PS II monomers containing the PsbO protein *per se* modulates the observed GTPase activity and not *vice versa*.

Finally, the actual GTP-binding site has not been rigorously identified. While the authors hypothesize that a number of residues are involved in GTP binding/GTPase activity, this must be tested experimentally, particularly since the identified residues apparently form a non-canonical GTP-binding domain. Site-directed mutagenesis followed by heterologous expression and *in vitro* refolding coupled with GTPase assays should be quite useful in elucidating the residues involved in the putative GTP-binding domain. Additionally, since the covalent labeling of PsbO with [α-^{32}P]8-N_3GTP was reported to be quite efficient (one PsbO labeled/PS II dimer [24]), mapping of the labeling site by proteolytic digestion followed by mass spectrometry should be relatively straightforward and provide critical data with respect to the location of the putative GTP-binding site.

Clearly, one problem in evaluating the role of the PsbO protein with respect to these studies is that in the spinach model system the PsbO component is hypothesized to function both in support of normal oxygen evolution and in control of PS II turnover (via GTP binding and GTPase activity). A biological system, however, may exist in which these two functions are separated. In *Arabidopsis thaliana*, two genes which encode the PsbO component (*psb*O-1, At5g66570 and *psb*O-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 [9]. It has been suggested that the primary function of the PsbO-1 protein is to support normal oxygen evolution by PS II and that the PsbO-2 protein principally acts to regulate the phosphorylation state and turnover of the D1 protein (Fig. 1.) [58]. This suggestion was based principally 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 [61]. If this proposed dichotomy of function is correct several predictions can be made. First, mutants lacking the PsbO-1 protein should exhibit very poor PS II functionality. Second, mutants lacking the PsbO-2 protein should exhibit defective PS II turnover characteristics, particularly under conditions where PS II damage is accelerated (i.e. photoinactivating light conditions). Under these conditions, the mutants lacking PsbO-2 should be unable to bind and hydrolyze GTP efficiently, which should markedly affect dephosphorylation of the D1 protein and induce defects in the ability to replace PS II reaction centers damaged by high light treatment. Given this hypothesized functional differentiation between the PsbO-1 and PsbO-2 isoforms, one would expect substantial PS II defects in mutants lacking either protein, particularly in plants grown for extended periods under high light regimes.

While the former has been well documented, the latter is the subject of some controversy. Certainly, mutants which lack the PsbO-1 protein exhibit defective PS II function. It was demonstrated that PsbO-1 is the major isoform in the wild type under normal growth conditions, and that in the absence of PsbO-1, the PsbO-2 protein is up-regulated in a semi-compensatory manner [9]. Mutants containing only the PsbO-2 isoform exhibit retarded growth [9], a lower quantum yield for energy trapping by PS II (F_V/F_M), lower rates of steady state oxygen evolution [68,69], and exhibit an extremely low relative ETR (as determined by fluorescence) under saturating light conditions and lower qP [58] as compared to wild type. Analysis of fluorescence decay kinetics and flash oxygen yield indicated that the mutant also exhibited a pronounced stabilization of the S_2-state [70]. This was due principally to a defect in the ability to use utilize calcium in support of oxygen evolution [71]. Under photoinactivating conditions, these mutants exhibit enhanced rates of D1 dephosphorylation and concomitant D1 degradation [58].

Mutants which lack the PsbO-2 protein and contain only the PsbO-1 isoform exhibit retarded growth characteristics when grown hydroponically [58]; this phenotype was exacerbated under photoinhibitory growth conditions. However, in later studies performed on plants grown in soil no growth differences were observed ([69], Yi, Frankel and Bricker, unpublished observations). Mutants containing only the PsbO-1 protein exhibited essentially no functional defects in PS II. Fluorescence decay after a single saturating flash in either the presence or absence of DCMU was indistinguish-

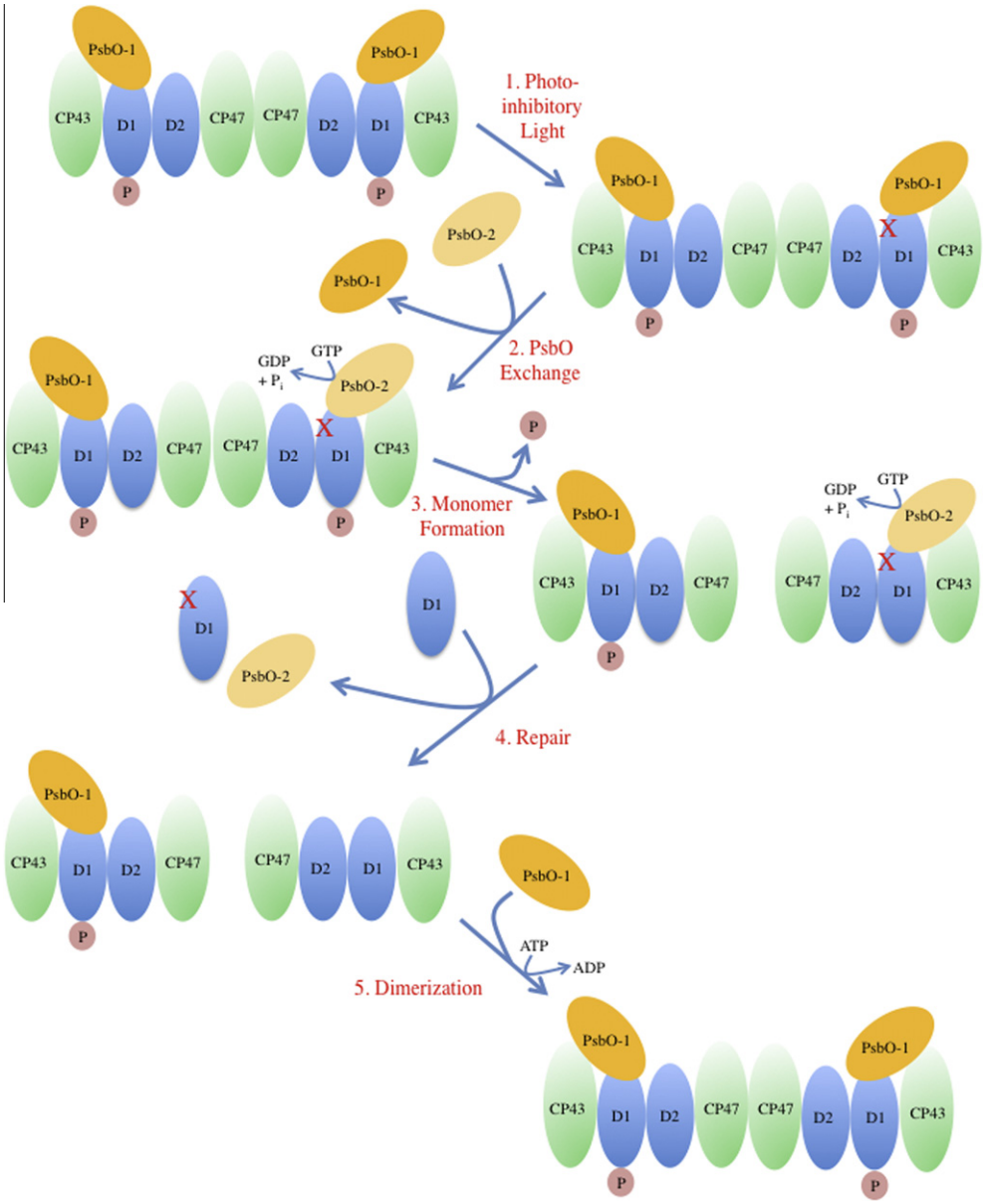

Fig. 1. Hypothesized Roles for the PsbO-2 Protein of Arabidopsis in the Photosystem II Repair Cycle. For simplicity, many of the details of the repair cycle have been omitted. Photoinhibitory light (Step 1) leads to damage of the D1 Protein (X), which then undergoes a conformational change favoring the binding of the PsbO-2 protein over its PsbO-1 counterpart (Step 2). GTP hydrolysis facilitated by the PsbO-2 protein leads to dephosphorylation of the D1 protein and monomer formation (Step 3). A second hypothesized GTP hydrolysis step leads to dissociation of CP43, PsbO-2 release, replacement of the damaged D1 protein with a new D1 protein and re-association of CP43 with the monomer (Step 4). PsbO-1 then binds to the repaired monomer, dimerization occurs and the new D1 protein is phosphorylated.

able from wild type. The quantum yield for energy trapping (F_V/F_M) and the F_O under either growth light condition were not significantly different. The oxygen evolution rate, e^-/P_{700} and the PS I/PS II ratio were also indistinguishable. The formation of the S_2-multiline and signal Q_A^-–Fe^{2+} were the same in both strains. Additionally, the PS II/PS I ratio was the same as observed in control plants (1:1). A small difference was observed in the location of the B-band in a thermoluminescence experiment. This band, which originates from the $Q_B^-S_2$ charge recombination was downshifted 3 °C in the mutant strain in comparison to wild type. These results indicate that plants containing only the PsbO-1 isoform have completely normal PS II complexes as measured by a variety of functional assays.

The loss of the PsbO-2 protein was reported to lead to defects in D1 protein turnover under short-term photoinactivation conditions (2–3 h photoinhibitory light) [58]. The D1 protein is not degraded even though photoinhibitory damage, as evidenced by a decrease in the oxygen evolution rate, occurs in these short-term experiments. Surprisingly, the control, PsbO-1- and PsbO-2-deficient plants all lost oxygen evolution capability at similar rates in these short-term experiments. The quantum yield for energy trapping by PS II (F_V/F_M) did not decrease during the time course of photoinactivation in the PsbO-2-deficient mutant, whereas decreases were observed in the control and PsbO-1-deficient strains.

Under long-term (weeks) high light growth conditions relatively few functional differences between wild type and mutants lacking the PsbO-2 protein have been documented [69]. In particular, mutants lacking the PsbO-2 protein exhibited an F_V/F_M and, perhaps more interestingly, an F_O, which were essentially identical to that observed in the control strains. If the PsbO-2 protein is required for efficient PS II turnover, one would expect an accumulation of damaged reaction centers under these conditions and a significant alteration in these and other PS II parameters. Within this context it is interesting to note that under these growth conditions this mutant appears to assemble substantially fewer

LHC-II supercomplexes and PS II dimers when compared to control strains when monitored by two-dimensional BN-SDS–PAGE [61]. These results would seem to indicate that the F_V/F_M is not strongly coupled to the organizational state of the PS II complexes in these mutants. Clearly, additional experiments examining a broader spectrum of PS II functional parameters under long-term high light growth conditions would be most welcomed.

Given the hypothesized differential roles for PsbO-1 and PsbO-2, one would predict that photodamaged reaction centers would undergo the replacement of PsbO-1 with PsbO-2 as a prelude to the initiation of photosystem repair. This would require that PsbO-1 exhibit a lower affinity for photodamaged PS II than PsbO-2, and that undamaged PS II should exhibit a higher affinity for PsbO-1 than PsbO-2. Murakami et al. [68] performed binding experiments using Arabidopsis PsbO-1 and PsbO-2 proteins expressed in *E. coli*. When reconstituted with equimolar amounts of PsbO-1 and PsbO-2, equivalent amounts of both proteins bound to the urea-washed PS II membranes. Since the majority of reaction centers in these membranes were in all likelihood not photodamaged, this experiment would seem to indicate that there is no selectivity of undamaged reaction centers for the binding of PsbO-1. However, the interpretation of this experiment is greatly complicated by the fact that the two Arabidopsis proteins were reconstituted onto urea-washed PS II membranes isolated from spinach. It is possible that a difference in binding would have been observed if urea-washed Arabidopsis PS II membranes were used in the experiment. In this context, it should be noted that the reconstitution of spinach PsbO onto spinach PS II membranes was much more efficient than the reconstitution of Arabidopsis PsbO-1 onto spinach membranes [72]. Clearly, additional experiments along these lines are warranted.

While the hypothesis that the Arabidopsis PsbO-1 and PsbO-2 proteins perform markedly different functions within Arabidopsis is certainly attractive, another hypothesis exists. It is possible that the PsbO-2 protein actually is preferred under some environmental conditions. *A. thaliana* Col-0 was derived from an accession which, in its native habitat, is frequently exposed to low temperature conditions. One might expect that these plants would have evolved a variety of mechanisms for surviving extended episodes of cold environmental stress [73]. In short-term experiments (2 h at 4 °C), no difference in either transcript or protein abundance for PsbO-1 and PsbO-2 was observed [61]. In long-term experiments, however, differences were apparent. In a Serial Analysis of Gene Expression (SAGE) experiment the PsbO-1 transcript was found to be down-regulated about 3.5-fold in plants which were incubated 7 days at 5 °C [74]. In a proteomics study examining the expression of chloroplast proteins after acclimatization to low temperature (40 days at 5 °C) using two-dimensional differential in gel electrophoresis (DIGE), the PsbO-1 protein was found to be down-regulated by a factor of 2.6 and the PsbO-2 component was up-regulated by a factor of 2.2 [75]. Given an initial ratio of PsbO-1 to PsbO-2 of about 9–1 [68], this would indicate that after cold acclimatization nearly 40% of the PsbO complement was the PsbO-2 isoform. The authors speculated that replacement of the photosynthetically competent PsbO-1 with the significantly less competent PsbO-2 protein under these conditions was a mechanism for rebalancing the photosynthetic energy flux required by the decrease in proton transport through the ATP Synthase, a known consequence of exposure to low temperature, and the consequent decrease in the lumenal pH [76]. Replacement of PsbO-1 with PsbO-2 would lower PS II activity and would reduce the rate of lumenal proton deposition. In this regard, as noted above, fluorescence decay and flash oxygen yield analysis indicated that mutants lacking the PsbO-1 protein and containing only the PsbO-2 component exhibited a marked stabilization of the S_2-state [70]. A similar stabilization of the S_2-state was observed by fluorescence decay in cold-acclimatized Arabidopsis [77]. Additional experiments examining the consequences of this apparent differential regulation of the PsbO-1 and PsbO-2 protein after cold acclimatization would be interesting and appropriate.

4. The extrinsic proteins as assembly/stability factors

In cyanobacteria, neither the PsbO protein [78,79] nor the cyanobacterial PsbP or PsbQ homologues are required for PS II accumulation or photoautotrophic growth [80], although all of these are required for maximal rates of oxygen-evolving activity. Deletion of any of these genes does not affect the accumulation of the others and the effects on reaction center assembly are minor, with the singular exception that deletion of the PsbO component results in the accumulation of PS II monomers [81] (Table 1). This is significantly different from the situation found in green algae and higher plants. Initial studies, performed in *Chlamydomonas* [82] demonstrated that the FUD44 mutant lacked the PsbO protein. This mutant exhibited a complete loss of oxygen evolution capability and could not grow photoautotrophically. While the mutant accumulated relatively normal amounts of the PsbP and PsbQ components, it was not formally determined if these were associated with the PS II reaction center or were unassembled in the thylakoid lumen. Later studies demonstrated that these components were not assembled to PS II [83]. Immunoblot analysis of the steady

Table 1
Effects of the loss of the PsbO, PsbP and PsbQ proteins on the assembly/stability of PS II in various model systems.

Organism	Absent protein	Effects on PS II assembly
Synechocystis 6803	PsbO	Loss of PS II dimers [81]
	cyanoPsbP	No effect on cyanoPsbQ accumulation [80]
	cyanoPsbQ	No effect on cyanoPsbP accumulation [80]
Chlamydomonas reinhardtii	PsbO	PsbP and PsbQ accumulate to near normal levels [82] but are not assembled into the photosystem [83]. CP47, CP43, D1 and D2 are strongly depleted [83] apparently due to instability of the assembled photosystem [84].
	PsbP	PsbO and PsbQ accumulate to near normal levels but are not assembled into the photosystem [83]. CP47, CP43 D1, and D2 depleted to 30–65% wild-type levels [83]
	PsbQ	No mutants available
Arabidopsis/ Nicotiana	PsbO	Loss of PsbQ , and to a lesser extent, PsbP [11]; CP43 and D1 strongly depleted, CP47 only modestly affected [11]
	PsbP	Loss of PsbQ [12,14], PsbO slightly affected [12]; Upon moderate suppression (5–10% wild-type levels), CP47, CP43 D1 and D2 accumulate to near normal amounts [12,14]; LHCII-PS II supercomplexes do not form, LHC and core proteins not phosphorylated [31]; Upon strong suppression (<1% wild-type levels), CP47 and D2 do not accumulate [14]; Defective thylakoid assembly under both moderate and strong suppression [30,31]
	PsbQ	No effects under normal growth light regimes [12,13]; Under low light growth regimes, CP47, CP43 and D2 strongly depleted, D1 only modestly affected [13]

state levels of the intrinsic, reaction center components CP47, CP43, D1 and D2 indicated that all of these components were strongly depleted in the FUD44 mutant while the LHC II protein, associated with the antennae of the photosystem, was not affected. This loss of the reaction center proteins was not due to a loss of transcription, as "Northern" blot analysis indicated that the mRNA populations for the CP47 and CP43 components were similar in wild type and the mutant strains, while significantly more mRNA for the D1 and D2 proteins were observed in the mutant. Pulse-chase experiments demonstrated that while these mRNAs were translated and apparently processed efficiently in the mutant, the proteins were quite unstable and were degraded rapidly. These results clearly indicated that the PsbO protein is required for the stability and accumulation of the reaction center proteins. It is unclear, however, if the instability observed in the absence of PsbO is a function of an inability of the newly synthesized reaction center components to assemble *per se* or that the reaction centers assemble, but are not stable in the absence of PsbO. These two alternatives are very difficult to differentiate. Recent studies, however, indicate that the latter may be the case. *Chlamydomonas* can grow heterotrophically on acetate in the dark. In marked contrast to the studies discussed above, in which the FUD44 mutant was grown under mixotrophic conditions (light + acetate), cultures of the FUD44 mutant under heterotrophic conditions led to a substantial accumulation of both the D1 and D2 proteins [84]. While incapable of oxygen evolution, the dark-grown mutant did exhibit a significant amount of variable fluorescence, indicative of at least partial assembly of the PS II reaction center.

A second *Chlamydomonas* mutant (BF25) was examined which lacked the PsbP component [85]. This mutant exhibited drastically reduced oxygen evolution capacity (5% of wild-type levels) but could grow very slowly photoautotrophically. While initial studies indicated that, in marked contrast to the FUD44 mutant, all of the reaction center subunits examined (CP47, CP43, D1 and D2) accumulated to near normal levels, later studies [83] indicated that BF25 and a second mutant lacking PsbP, FUD39, accumulated less of these reaction center components (about 65% and 30% of wild type, respectively). These results indicated that PsbP also contributes to the stability of the PS II reaction center components in *Chlamydomonas*. It should be noted that in the absence of the PsbP protein both the PsbO and PsbQ proteins accumulated to normal levels and were present as unassembled components in the thylakoid lumen. Interestingly, unassembled PsbO and PsbP both appeared to be present in the appressed regions of the thylakoids in mutants which lacked the D1 and D2 proteins, while the PsbP protein appeared to be present only in the unappressed regions in a mutant lacking CP43. These authors also noted that PsbP protected CP43 from proteolytic degradation and hypothesized that CP43 contains a binding domain for PsbP. Unfortunately, no mutants lacking the PsbQ protein have been reported in this organism.

Until recently, studies with higher plants have lagged significantly in this area due to the lack of appropriate mutants. In Arabidopsis, T-DNA mutant lines are now available for all of the extrinsic components of PS II. As noted above, two different genes for the PsbO protein (*psb*O-1, At5g66570 and *psb*O-2, At3g50820) are present in this organism. Two genes are also present which encode the PsbP (*psb*P-1, At1g06680 and *psb*P-2, At2g30790) and PsbQ (*psb*Q-1, At4g21280 and *psb*Q-2, At4g05180) components. Additionally, RNAi techniques have been used to suppress the expression of these proteins in both tobacco [12] and Arabidopsis [11,13,14]. Proper design of the RNAi vectors can allow either the specific suppression of individual members of a gene family [12] or the simultaneous suppression of all members of a gene family [11,13,14]. In these experiments, phenotypic series of mutants are typically generated which contain variable amounts of the targeted protein(s); these may exhibit variable phenotypes which are dependent on protein dosage.

In Arabidopsis, simultaneous RNAi suppression of PsbO-1 and PsbO-2 leads to loss of photoautotrophy and a variety of PS II functional defects which strongly correlate to both the amount of PsbO protein accumulation and the amount of functional PS II reaction centers [11]. Thylakoids isolated from these mutants exhibited loss of the D1 and CP43 proteins which paralleled the decreases observed for PsbO. Interestingly, the accumulation of CP47 was only modestly affected even under conditions where accumulation of PsbO was strongly suppressed (5–10% of wild-type levels). This situation differs somewhat from that observed in *Chlamydomonas* [82], where loss of PsbO leads to the near complete loss of all of the reaction center components (CP47, CP43, D1 and D2). Additionally, in Arabidopsis the loss of PsbO is correlated with a loss of PsbQ and, to a lesser extent, loss of PsbP. This is significantly different from what is observed in the *Chlamydomonas* system.

RNAi suppression has also been used to examine the effects of the loss of PsbP in tobacco [12] and Arabidopsis [14]. In large measure, the results obtained in these two experimental systems are highly congruent. Moderately strong suppression of PsbP (5–10% wild-type levels) leads to slow photoautotrophic growth and a variety of PS II functional defects including markedly decreased oxygen evolution rates, lower quantum yield for PS II charge separation (F_V/F_M), an increased susceptibility to photoinhibitory light, slower Q_A^- to Q_B electron transport in the absence of DCMU and a slower charge recombination with the S_2-state of the oxygen-evolving complex in the presence of DCMU [12,14], probably resulting from a more stable Q_A^-/S_2 couple [31]. In tobacco, measurements of the thermoluminescence B-band indicated that the manganese cluster was dark labile, being lost with a $T_{1/2}$ of about 20 min. The manganese cluster could be reassembled by illumination at low light intensities and exhibited a $T_{1/2}$ of about 1 min. It is unclear if these latter effects are due to loss of PsbP or to the concomitant loss of the PsbQ protein (see below). Interestingly, 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 centers and LHC proteins are not phosphorylated [31]. Additionally, significant defects in thylakoid architecture are apparent in these plants (see below, [30,31]). 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 further compromised, the mutant's ability to accumulate the PS II core proteins CP47 and D2 is also markedly reduced [14]. It is interesting to note that the loss of the reaction center components does not parallel the loss of PsbP (as observed for the PsbO RNAi mutants discussed above); even low levels of PsbP support the near normal accumulation of the 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 PsbO, leads to the complete loss of the PsbQ component, even in plants containing detectable levels of PsbP [12,14]. These results highlight the differences between the *Chlamydomonas* and higher plant systems. In *Chlamydomonas* the accumulation of the various extrinsic proteins appears to be uncoupled. Loss of any one component has little effect on the accumulation of the others. In the higher plant system, loss of either PsbO or PsbP leads to a near complete loss of the PsbQ protein. It is unclear at this time whether the loss of the PsbQ protein is due to decreased synthesis

or increased degradation. It should be noted, however, that large pools of unassembled mature PsbO, PsbQ and PsbP proteins normally exist in the thylakoid lumen without being degraded either in the presence [86,87] or in the absence [83,88] 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 PsbO and/or PsbP proteins and not a consequence of the reduced levels of PS II reaction centers observed when PsbO and PsbP are strongly suppressed.

The PsbQ protein has also been examined using RNAi suppression techniques in both tobacco [12] and Arabidopsis [13]. Under normal growth light conditions, the complete suppression of PsbQ expression did not affect photoautotrophic growth, PS II assembly, or a variety of PS II functional parameters (oxygen evolution rate, F_V/F_M, etc.) in either system. In Arabidopsis some modest defects were apparent with respect to S-state distribution and the stability of the oxygen-evolving complex under dark incubation conditions [13] were observed. A major alteration in phenotype was observed, however, when Arabidopsis mutants lacking the PsbQ proteins were placed under low light conditions. Plants grown under normal light conditions (80 μmoles photons m^{-2} s^{-1}) and then transferred to low light conditions (5 μmoles photons m^{-2} s^{-1}) for 3 weeks, progressively yellowed and then died. These 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. Wild-type plants remained green and survived the low light stress with only minimal alterations in their phenotype [13].

These results seem to indicate that subtle differences exist between the *Chlamydomonas* and higher plant model systems in the regulatory network which governs PS II accumulation (Table 1) The dependence of PsbQ, and to a lesser extent PsbP, on the presence of PsbO was quite unexpected. Additionally, the pattern of loss of the intrinsic components of the reaction center is also unusual. CP43 and D1 are preferentially lost concomitant to PsbO depletion while CP47 and D2 are preferentially lost upon PsbP depletion. Clearly further studies are warranted examining the basis for these differences in *Chlamydomonas* and the higher plant models. In particular, BN-SDS–PAGE analysis (or, alternatively sucrose density ultracentrifugation methods) of the assembled PS II complexes and sub-complexes in both systems would provide critical information concerning the differences in these plant models. These experiments would allow a detailed analysis of the different sub-complexes which form in the presence or absence of the various extrinsic components. Additionally, experiments designed to elucidate the mechanism by which the presence of PsbO and/or PsbP regulate the accumulation of PsbQ would be very interesting as the regulation could be at the transcriptional, translational, or post-translational (import or proteolytic) levels.

5. A possible role for the PsbP and PsbQ proteins in grana stacking

Recently, de Las Rivas and co-workers have hypothesized that the extrinsic proteins of PS II play significant roles in the regulation of PS II and the maintenance of normal thylakoid membrane architecture, particularly with respect to the integrity of the grana stacks [28,29]. Specifically, protein–protein interactions involving PsbQ and occurring across the lumenal space between opposing PS II dimers were suggested [28]. One specific prediction which would flow from this hypothesis is that loss of PsbQ should lead to a state of persistent lumenal diameter expansion, similar to the expansion observed in the dark which is due to the efflux of

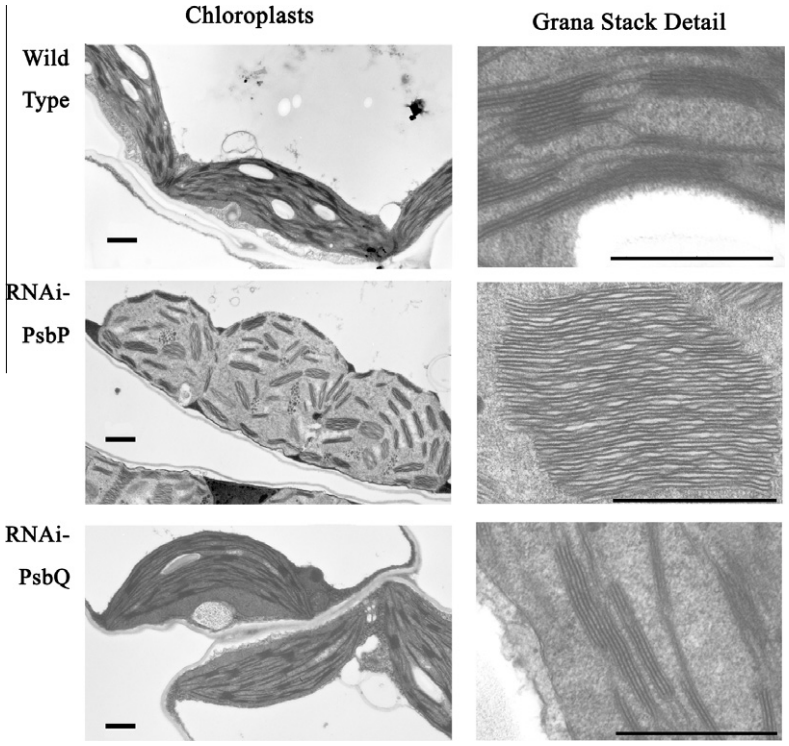

Fig. 2. Chloroplast and Thylakoid Architecture in Arabidopsis Lacking the PsbP and PsbQ Proteins. Chloroplasts and thylakoids from wild-type Arabidopsis and RNAi mutants suppressed in either PsbP expression (RNAi-PsbP) or PsbQ expression (RNAi-PsbQ) are shown. It should be noted that the RNAi-PsbP plants are deficient in both the PsbP and PsbQ proteins while the RNAi-PsbQ plants lack only the PsbQ protein. The RNAi-P plants contained 13% of the PsbP protein found in wild type, and contained no detectable PsbQ protein, while the RNAi-PsbQ plants contained <5% of wild-type levels of the PsbQ protein and wild-type levels of the PsbP protein. Both the RNAi-PsbP and RNAi-PsbQ plants contained wild-type levels of the PsbO component. Note that the RNAi-PsbQ plants exhibit no alterations in either chloroplast or thylakoid membrane morphology. For the chloroplast images the scale bar = 1 μm and for the thylakoid images the scale bar = 0.5 μm. Redrawn from [30].

protons and the influx of Mg^{2+} ions. Discussion of the origin of this hypothesis, the details of the evidence in its support and the consequences of this proposal for thylakoid architecture under various light regimes and ionic conditions are well beyond the scope of this communication. The reader is encouraged to examine the original references for these details [28,29]. It should be noted, however, that the availability of RNAi-suppressed PsbP and PsbQ mutants in tobacco [12] and Arabidopsis [13,14] should allow a direct test of this hypothesis.

As noted above, the phenotypes of the PsbP-suppressed and PsbQ-suppressed mutants are very similar in both the tobacco and Arabidopsis systems. The principal difference is that it was possible to isolate mutants which exhibited stronger suppression in Arabidopsis, where mutants could be obtained which expressed <1% of wild-type levels of the PsbP protein [30]. Transmission electron microscopy has been performed in both systems, again, with very similar results [30,31]. First, PsbQ-suppressed mutants exhibit no observable defects in chloroplast architecture under normal growth light conditions (Fig. 2). The grana stacks are indistinguishable from wild type as are a variety of other chloroplast structural parameters (chloroplast size, extent of stroma lamellae, apparent stroma density, abundance of plastoglobuli, abundance of starch grains, etc.). This observation is fully consistent with the modest functional defects in PS II observed under normal growth light conditions as discussed previously. The effects of PsbQ suppression on chloroplast architecture in plants grown under low light conditions has not been examined at this time.

The PsbP-suppressed Arabidopsis mutants, which contained about 13% of the PsbP found in wild type, however exhibit striking defects in their chloroplast architecture. With respect to grana stacking, the individual grana stacks are much larger than observed for wild type and the lumenal diameter becomes larger and highly irregular (Fig. 2). It should be noted that these plants, which accumulate a relatively large amount of PsbP (comparable to the RNAi-P11 plant examined in [14]), exhibit very few defects with respect to PS II structure or function. Additional chloroplast structural defects are also apparent. The chloroplasts appear globular with a less dense stroma, there are also many fewer stroma lamellae evident, starch grains are absent and there are many more plastoglobuli present in the chloroplast. Interestingly, these chloroplasts resemble those of the deep-shade tolerant (5 μmoles photons m^{-2} s^{-1}) tropical plant *Alocasia macrorrhiza* in almost every detail (see Fig. 1 of [89], [90]). The authors presented a hypothesis that the PsbP-suppressed mutants were "locked" in a deep shade state due to the depletion of PsbP [30].

The results with the RNAi-suppressed PsbP and PsbQ mutants support the hypothesis of De Las Rivas et al. [28,29], at least in part. Certainly, depletion of the PsbP subunit has dramatic effects on grana architecture (and chloroplast structure) even under conditions where PS II function is not markedly affected. The specific prediction that the lumenal diameter should expand in the absence of the extrinsic components, was confirmed. However, the PS II–PS II interactions hypothesized by De Las Rivas et al. [28,29], and critical for maintenance of the lumenal diameter, do not appear to be dependent on the PsbQ protein, but rather the PsbP component, at least under normal growth light conditions. It is unclear if PsbP-PsbP interactions, PsbP-X interactions, or other processes are involved in the observed structural phenotypes of these mutants. Additionally, the participation of PsbQ in thylakoid organization under low light conditions cannot be ruled out at this time.

6. PsbP as a manganese carrier participating in photoactivation

Photoactivation is a multistep low quantum yield process in which four manganese, one calcium and one or two chloride ions are assembled into a functional $Mn_4Ca_1Cl_{1-2}$ metal center functioning at the active site for water oxidation in PS II. While photoactivation can proceed in the absence of any extrinsic proteins [91], it has been proposed that the PsbP component acts as a manganese-carrying protein in support of the initial stages of this process [26]. These authors hypothesize that during the photoactivation process the PsbP protein participates in the formation of the unstable intermediate that contains two manganese(III). In their model, the first manganese(II) binds to the assembly site and becomes photooxidized to manganese(III). Subsequently, a PsbP-manganese(II) complex binds to the photosystem, undergoes a conformational change which lowers the affinity of manganese for PsbP, with manganese(II) being released to the assembly site. This manganese binds, and is then photooxidized to manganese(III). In an initial study, these authors presented evidence that the photoactivation process was enhanced by the presence of PsbP protein containing bound manganese [26]. The authors first demonstrated that the PsbP protein could bind manganese by incubating the protein with manganese(II) and observing the resultant X-band room temperature EPR signal. Under these conditions, free manganese(II) exhibits a six-line signal indicative of hexaaqua-manganese(II); protein-bound manganese, however, is usually not visible because of inhomogeneous broadening of the EPR signal. The PsbP component exhibited no EPR signal even when incubated at a manganese:protein ratio of 10:1. This indicated that PsbP could bind multiple (at least 10) manganese(II) ions. The PsbO and PsbQ proteins, however, exhibited to a lesser or greater degree, respectively, the typical six line EPR spectra, indicating that these components could not bind manganese effectively. Photoactivation experiments were then performed. NaCl-washed PS II membranes which were depleted of bound manganese by NH_2OH treatment were photoactivated for 20 min with varying amounts of $MnCl_2$ alone, or in the presence of the PsbP protein which had been pre-saturated with manganese(II) (PsbP-manganese protein). In the absence of the PsbP-manganese protein, photoactivation appeared sigmoidal with a $K_{1/2}$ for photoactivation of about 18 μM manganese. In the presence of the PsbP-manganese protein, the photoactivation appeared more hyperbolic with a $K_{1/2}$ for photoactivation of about 5 μM. The reported error in this experiment was rather large and it is unclear if these differences are significant. A time course for photoactivation, however, yielded substantially clearer results. Photoactivation in the absence of the PsbP-manganese protein exhibited a $T_{1/2}$ of about 20 min, while in the presence of PsbP-manganese protein the $T_{1/2}$ was about 1 min. The addition of the PsbP component to the reaction mixtures immediately after the photoactivation period had no effect on the $T_{1/2}$. This observation was interpreted by the authors as indicating that the role of the PsbP component was not to stabilize photoassembled reaction centers but rather to directly donate manganese during the photoactivation process.

In a second study, the binding of manganese to the PsbP protein was more fully characterized [27]. The authors determined that while the PsbP protein could bind numerous manganese [26], most of these were non-specific, weakly bound and could be removed readily by dialysis. One manganese per PsbP protein, however, was bound tightly and it was concluded that there was a stoichiometry of 1 manganese:1 PsbP at the high affinity site. The binding affinity was determined by dialysis against EDTA-containing buffers and reported to be extraordinarily strong, exhibiting a K_d of 10^{-17} M (in the original paper an apparent typographical error occurred and a K_A value of 10^{-17} M^{-1} was reported; this of course would indicate extremely weak binding). High field EPR was then used to investigate the PsbP-manganese protein. At high field, inhomogeneous broadening of the manganese(II) EPR signal is suppressed. The PsbP-manganese protein exhibited a sharp six-line signal indicative of protein-bound manganese(II) in an electronically symmetrical environment. These authors also reported the

identification of an unusual fluorescence signal associated with the PsbP-manganese complex. In the absence of bound manganese, excitation of the PsbP protein at 297 nm yielded a modest fluorescence emission peak centered at 340 nm. The excitation spectra indicated that the observed fluorescence arose from a tryptophan residue(s). The PsbP-manganese protein, however, exhibited emission peaks at 415 and 435 nm of variable intensity (depending on the PsbP-manganese protein preparation) with concomitant loss of the 340 nm emission band. The authors speculated that resonance energy transfer from tryptophan to a manganese-ligand complex could result in a metal-to-ligand charge transfer reaction accounting for the observed fluorescence signal. This would require the bound manganese(II) being photooxidized to manganese(III). No other known protein-associated metal-to-ligand charge transfer reactions involving manganese have been documented. The authors proposed two possible binding sites for the manganese, one involving ^{50}Glu, ^{51}Asp, ^{54}Asp and ^{39}Glu, which are in the vicinity of ^{34}Trp, and ^{177}Glu, ^{165}Asp, and ^{144}His, which are close to ^{168}Trp (Fig. 3A). Interestingly, X-ray crystallography of the PsbP protein from spinach at 1.98 Å resolution (PDB:2VU4, [92,93]) revealed a zinc ion which was ligated to ^{165}Asp and ^{144}His (Fig. 3B). This zinc-binding domain was conserved in the structure of the cyanobacterial homologue of PsbP (pdb:2XB3, [94]. It is unclear if these proteins natively bind zinc or, due to the crystallization conditions used, zinc displaces other bound metal ions such as manganese which are normally bound to the site, or if the zinc binds artifactually to the PsbP protein (due to the crystallization conditions used), which contains no actual metal-binding sites.

Some other lines of experimentation are at least consistent with the hypothesis that PsbP is a manganese-binding protein which is functional during photoactivation. When grown in the dark on acetate, *Chlamydomonas* assembles near normal amounts of PS II reaction centers; however, these centers lack functional manganese clusters and cannot evolve oxygen. These PS II complexes are fully competent in photoassembling functional manganese clusters [95]. The FUD39 mutant, which lacks the PsbP protein, also assembles normal amounts of PS II reaction centers which lack manganese clusters. During photoactivation of thylakoid membranes, however, the wild-type strain exhibits a $T_{1/2}$ for photoactivation of less than 1 min, while the FUD39 mutant exhibits a $T_{1/2}$ of 5–6 min. Additionally, only about 60% of the reaction centers are photoactivated vs. 100% in the wild-type thylakoids. These authors attributed the slower photoactivation of the FUD39 thylakoids to a defect in the ability to sequester chloride at the photoactivation site in the absence of the PsbP component. This was extrapolated from the observation that, with respect to oxygen evolution, wild-type thylakoids exhibited a K_d of 30 μM for chloride while the FUD35 exhibited a K_d of about 4 mM. [96]. This interpretation is certainly consistent with the large body of work examining the role of PsbP in support of the sequestration of chloride [21] and calcium [20] required for oxygen evolution and the specific requirement for chloride in photoactivation [97]. Nevertheless, it is formally possible that the PsbP protein both stabilizes chloride at the site of photoactivation and provides the manganese required for the initial assembly steps of the manganese cluster.

But several questions remain. First, it had earlier been demonstrated in cyanobacteria that photoactivation occurs at an accelerated rate in the absence of the PsbO component [91]. The PsbO protein apparently acts as a diffusion barrier for manganese access to the assembly site. In higher plants, photoactivation can also occur in the absence of the PsbO protein [98,99]. Taking these observations into account, many current models for assembly of the manganese cluster predict that the metal center assembles prior to the binding of the PsbO component [100,101]. In higher plants, a rather vast body of research indicates that the PsbO protein participates in the formation of the stable binding site for PsbP (for example see [102,103]). Indeed, in the absence of the PsbO protein, no binding of the PsbP protein to PS II is observed. The model presented by Bondarava et al. [26,27] would seem to require that a stable (or at least transiently stable) binding domain for the PsbP protein must exist prior to metal center assembly. Consequently, it would appear that the PsbO component would need to be associated with the PS II complex prior to photoactivation to provide a binding site for the PsbP-manganese complex. Such binding would seem to be necessary to facilitate the significant conformational changes required in the PsbP protein to facilitate the transfer of the extremely tightly bound PsbP-associated manganese to the site of manganese cluster assembly (see below). It could be hypothe-

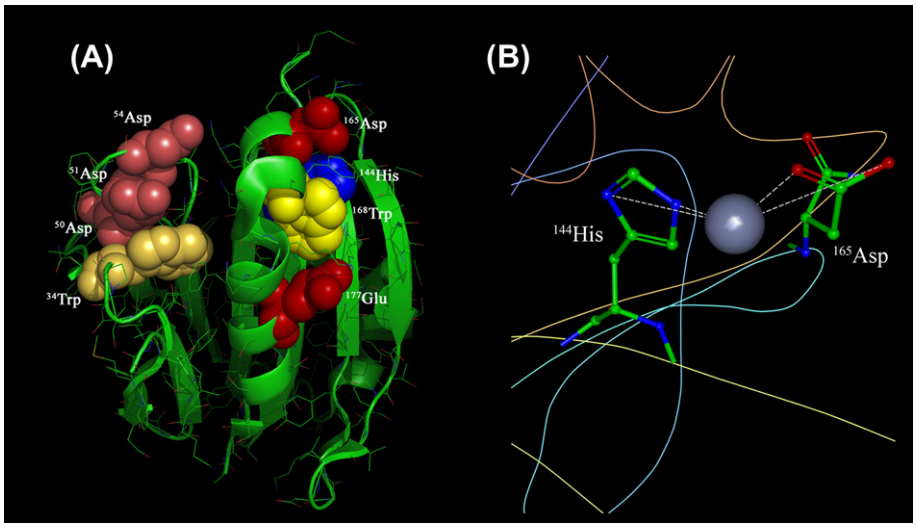

Fig. 3. Possible Metal-Binding Sites of the Spinach PsbP Protein. A. Hypothesized binding domains for manganese proposed by Bondarava et al. [27]. The N-terminal binding domain is shown to the left and the C-terminal binding domain is shown to the right. The two tryptophan residues (^{34}Trp and ^{168}Trp) involved in the observed fluorescence and hypothesized to be involved in resonance energy transfer coupled to a metal–ligand charge transfer process are shown in shades of yellow. Putative acidic ligands to the bound manganese are shown in shades of red while the basic ligand is shown in blue. Individual amino acid residues are labeled. Image produced in PYMOL (http://pymol.sourceforge.net/faq.html#CITE). B. The zinc-binding site identified in the 1.9 Å spinach PsbP crystal structure. The zinc ion is shown in grey while the nitrogen ligands from ^{144}His are shown in blue and the oxygen ligands from ^{165}Asp are shown in red. Image produced using the Ligand Explorer Tool found at the PDB database (www.rcsb.org). For both A. and B. the 1.98 Å spinach PsbP crystal structure (PDB:2VU4, [92,93]) is illustrated.

sized that other PsbP-binding assembly factors exist which would allow the binding of PsbP-manganese to the reaction center and facilitate manganese transfer during photoactivation in the absence of PsbO. However, at this time no such PsbP-binding proteins have been identified.

A second question arises as to the nature and strength of binding of manganese to the PsbP protein. The reported K_d (10^{-17} M) for manganese binding is extraordinarily low and nearly unprecedented in biological systems. Given that the K_d for manganese during photoactivation is about 8 μM [104,105], it is unclear how manganese could be transferred from a PsbP-manganese complex to the site of photoactivation. Clearly, large conformational changes in the PsbP protein occurring upon the putative PsbP-manganese complex binding to PS II could lower the affinity of PsbP for manganese and possibly facilitate manganese transfer. However, no major conformational changes in the PsbP protein appear to occur. In an FTIR study using ^{13}C-labeled PsbP, Tomita and co-workers [106] specifically probed for conformational changes in PS II arising from PsbP binding. While conformational changes occuring in the vicinity of the water-oxidizing complex were identified, no conformational changes were observed in the PsbP protein itself. However, these experiments were performed with PsbP protein which presumably did not contain bound manganese. It would be extremely interesting to perform analogous experiments in which ^{13}C-labeled PsbP-manganese complex was used in the reconstitution of PS II membrane lacking assembled manganese clusters. It is, of course, possible that the reported K_d for manganese binding by the PsbP protein is erroneous. Weaker binding in the range of 10^{-6}–10^{-9} M could facilitate manganese transfer during photoactivation, perhaps without concomitant major conformational changes. The binding constant of manganese to the PsbP protein should certainly be verified, if possible, by a variety analytical methods. Finally, site-directed mutagenesis of the residues proposed to be involved in the two putative manganese binding sites would be quite useful in determining the involvement of the PsbP protein in manganese (and zinc) binding and its possible role in the photoactivation of PS II.

7. Alteration of photosystem II reducing-side properties by removal of PsbO, PsbP and PsbQ

As noted in the introduction, the PsbO, PsbP and PsbQ proteins bind to the lumenal side of Photosystem II and function principally in support of water oxidation. These appear to function in maintaining optimal levels of calcium and chloride at the oxygen-evolving site. Removal of these components has significant consequences on the water oxidation process which, in large measure, are reversed upon the addition of millimolar concentrations of these ions. These effects have been documented by numerous investigators over the last 30 years and have been reviewed extensively ([6,19–21] Bricker and Eaton-Rye, in preparation). Additionally, either biochemical or genetic removal of the PsbP and PsbQ proteins have consequences with respect to the other enzymatic function performed by PS II, namely its role as a plastoquinone reductase. Two quinone-binding sites are located on the stromal side of the PS II complex [55]; the plastoquinones occupying these sites are termed Q_A and Q_B. Q_A is strongly bound and acts as a one electron carrier accepting an electron from reduced pheophytin and transferring it to Q_B. The plastoquinone Q_B acts as a two electron, two proton carrier and is strongly bound to PS II in the quinone and semiquinone forms but weakly bound when fully reduced as a plastoquinol. After full reduction, the plastoquinol leaves the Q_B binding site and is replaced by another plastoquinone from the quinone pool in the thylakoid membrane (for full review, see [107]).

In an early study, it was shown that removal of the PsbP and PsbQ proteins by treatment with high concentrations of NaCl led to a loss of the period-two oscillations in fluorescence which are associated with normal Q_A–Q_B electron transfer [108]. This result indicated that electron transfer between Q_A^- and Q_B had been compromised. This defect was not reversed by either reconstitution of the PsbP and PsbQ components onto the protein-depleted PS II membranes or by the addition of $CaCl_2$. Consequently, this result was interpreted by the authors as indicating that the modification of electron transfer between Q_A^- and Q_B was a direct consequence of the NaCl treatment itself, and was not directly related to removal of the PsbP and PsbQ proteins. In a later study, removal of the PsbP and PsbQ proteins by treatment of PS II membranes with high concentrations of NaCl was shown to modify the Q_B site [32]. Atrazine is a potent inhibitor of PS II electron transport, binding to the Q_B site and displacing the normally bound plastoquinone [109]. Upon removal of the PsbP and PsbQ components, the I_{50} for atrazine decreased from 10 μM in intact PS II membranes to 1 μM. This result was interpreted to indicate that the accessibility of the inhibitor to the Q_B site was increased in the absence of the PsbP and PsbQ components. It was also found that the effect on atrazine binding could be partially reversed by the addition of millimolar concentrations of NaCl or $CaCl_2$. These authors hypothesized that the loss of calcium and chloride from the oxygen-evolving site, which occurs upon the depletion of the PsbP and PsbQ proteins, modifies the conformation of the D1 protein at the lumenal side of the photosystem. Since the Q_B site is located on the stromal side of the D1 protein, these conformational changes also lead to transmembrane conformational changes near the Q_B site which alter the affinity for atrazine.

In a complementary study [110], removal of the PsbP and PsbQ proteins was shown to lead to electron transport defects on both the oxidizing and reducing sides of the photosystem. In this study, a K_d for calcium was determined to be about 50 μM and all of the defects on the reducing side of the photosystem which these authors observed were abolished with the addition of about 1 mM calcium. These authors also hypothesized that structural changes in the D1 protein following calcium depletion were transmitted to the reducing side of the photosystem by conformational changes in the vicinity of the Q_A and/or Q_B binding sites. Finally, it was recently demonstrated that after removal of the PsbP and PsbQ proteins, significant electron transport defects persist even in the presence of relatively high (10 mM $CaCl_2$) calcium concentrations [33]. Electron transfer from Q_A^- to Q_B in PS II reaction centers with an occupied Q_B site was slowed by a factor of 12, while electron transport from Q_A^- to Q_B in centers with an unoccupied Q_B site was slowed by a factor of 6. Subsequent removal of the PsbO protein by treatment with 200 mM NaCl + 2.6 M urea did not induce further reducing-side alterations. Since these results were obtained under saturating calcium conditions, it would appear that removal of the PsbP and PsbQ components, *per se*, affect the Q_A and/or Q_B binding sites. It was hypothesized that removal of the PsbP and PsbQ proteins leads to structural alterations in membrane-spanning components of the photosystem, leading to a structural alteration of the Q_A and/or Q_B binding site environment or modifying the plastoquinone-plastoquinol exchange channel [55].

While all of these studies have been performed *in vitro* with NaCl-washed PS II membranes, very similar reducing-side defects have been observed *in vivo* upon the RNAi suppression of PsbP expression [14]. The RNAi-P1 mutant, for instance, contained no detectable PsbP or PsbQ proteins, had a F_V/F_M 78% of that observed for wild type, and exhibited a significant slowing of electron transfer from Q_A^- to Q_B. The equilibrium between Q_A^- and Q_B was also shifted significantly in favor of Q_A^- in this mutant. These defects appear to be directly associated with the loss of the PsbP component since similar RNAi mutants which lacked the PsbQ protein did not exhibit alterations in either Q_A^- to Q_B electron transfer or the equi-

librium between Q_A^- and Q_B, at least under normal growth light conditions [13].

Image analysis of PS II supercomplexes of higher plants from which the PsbP and PsbQ components were removed by high salt treatment supports the hypothesis that removal of the PsbP and PsbQ proteins elicits conformational changes in the membrane-spanning components of the photosystem. The light-harvesting chlorophyll-protein CP29 was observed to move 12 Å towards the central core of the PS II complex upon removal of PsbP and PsbQ [111], which indicates that a modification in the packing of the transmembrane helixes within the complex occurs in the absence of these proteins. Conformational changes in the D1 and/or CP43 protein have been directly observed by FTIR studies of NaCl-washed PS II membranes reconstituted with ^{13}C-labeled PsbP [106]. Changes in the amide I band which are normally observed during the $S_1 \rightarrow S_2$ transition of the oxygen-evolving complex [112] were altered upon depletion of both the PsbP and PsbQ components. These features, however, were fully restored upon reconstitution with the PsbP protein, alone. No conformational changes in the PsbP protein were detected in this study. It is also important to note that these investigations were performed in the presence of 5 mM $CaCl_2$ and consequently the observed conformational changes were the direct result of the removal of the PsbP protein and not loss of calcium from the oxygen-evolving site. These conformational changes are very likely coupled to those hypothesized to give rise to the reducing-side effects observed upon chemical removal of the PsbP and PsbQ components. This latter study, coupled with the *in vivo* observations noted above [13,14], highlight the specific role of the PsbP protein in these conformational changes.

Clearly additional experiments are in order. While the FTIR studies noted above identify possible conformational changes in the vicinity of the oxygen-evolving complex, the details of how these local conformational changes are coupled to long range transmembrane effects are not understood. One can speculate that these involve changes in the environments of the Q_A and Q_B binding sites. These could easily lead to alteration of the reduction potentials of these plastoquinones. The identification of these changes, however, are severely hampered by the lack of a higher plant PS II crystal structure. One alternative approach might be the use of ^{13}C-labeled plastoquione in FTIR experiments designed to probe for alterations in the quinone-binding environment upon PsbP protein binding to the photosystem.

8. Conclusions

The proposed auxiliary roles for the extrinsic proteins of PS II which have been discussed in this review illustrate the wide range of alternative functional activities which have been attributed to these components. It is hoped that this critical evaluation of these putative functions will engender discussion, re-examination and, hopefully, additional experimentation to clarify the validity of these proposals.

9. Abbreviations

BN blue-native
DIGE differential in gel electrophoresis
DCMU 3-(3,4-dichlorophenyl)-1,1-dimethylurea
EPR electron paramagnetic resonance
ETR electron transfer rate
FTIR Fourier transform infrared spectroscopy
LHCII light-harvesting chlorophyll-protein II
P-loop phosphate-binding loop
PS photosystem
qP photochemical quenching
RNAi nterfering RNA,
SAGE serial Analysis of Gene Expression
SDS–PAGE sodium dodecyl sulfate polyacrylamide gel electrophoresis
T-DNA transposon insertion mutants
X-band 9 GHz-band.

Acknowledgements

This work was supported by a grant from the Chemical Sciences, Geosciences, and Biosciences Division, Office of Basic Energy Sciences, Office of Science, U.S. Department of Energy to T.M.B. and L.K.F.

References

[1] N. Murata, M. Miyao, T. Omata, H. Matsunami, T. Kuwabara, Stoichiometry of components in the photosynthetic oxygen evolution system of Photosystem II particles prepared with Triton X-100 from spinach chloroplast, Biochim. Biophys. Acta 765 (1984) 363–369.
[2] T.M. Bricker, Oxygen evolution in the absence of the 33 kDa manganese-stabilizing protein, Biochemistry 31 (1992) 4623–4628.
[3] R. Burnap, J.R. Shen, P.A. Jursinic, Y. Inoue, L.A. Sherman, Oxygen yield and thermoluminescence characteristics of a cyanobacterium lacking the manganese-stabilizing protein of Photosystem II, Biochemistry 31 (1992) 7404–7410.
[4] T.M. Bricker, D.F. Ghanotakis, Introduction to oxygen evolution and the oxygen-evolving complex, in: D.R. Ort, C.F. Yocum (Eds.), Oxygenic Photosynthesis: The Light Reactions, vol. 4, Kluwer Academic Publishers, Dordrecht, 1996, pp. 113–136.
[5] D.A. Berthold, G.T. Babcock, C.F. Yocum, A highly resolved oxygen-evolving Photosystem II preparation from spinach thylakoid membranes, FEBS Lett. 134 (1981) 231–234.
[6] T.M. Bricker, R.L. Burnap, The extrinsic proteins of Photosystem II, in: T. Wydrzynski, K. Satoh (Eds.), Photosystem II: The Water/Plastoquinone Oxido-Reductase of Photosynthesis, Springer, Dordrecht, 2005, pp. 95–120.
[7] S.D. Betts, J.R. Ross, E. Pichersky, C.F. Yocum, Mutation Val235Ala weakens binding of the 33-kDa manganese stabilizing protein of Photosystem II to one of two sites, Biochemistry 36 (1997) 4047–4053.
[8] H. Popelkova, M.M. Im, J. D'Auria, S.D. Betts, N. Lydakis-Simantiris, C.F. Yocum, N-terminus of the Photosystem II manganese-stabilizing protein: effects of sequence elongation and truncation, Biochemistry 41 (2002) 2702–2711.
[9] R. Murakami, K. Ifuku, A. Takabayashi, T. Shikanai, T. Endo, F. Sato, Characterization of an *Arabidopsis thaliana* mutant with impaired psbO, one of two genes encoding extrinsic 33-kDa proteins in Photosystem II, FEBS Lett. 523 (2002) 138–142.
[10] H. Popelkova, M.M. Im, C.F. Yocum, Binding of manganese-stabilizing protein to Photosystem II: identification of essential N-terminal threonine residues and domains that prevent nonspecific binding, Biochemistry 42 (2003) 6193–6200.
[11] X. Yi, M. McChargue, S.M. Laborde, L.K. Frankel, T.M. Bricker, The manganese-stabilizing protein is required for Photosystem II assembly/stability and photoautotrophy in higher plants, J. Biol. Chem. 280 (2005) 16170–16174.
[12] K. Ifuku, J. Yamamoto, T.-a. Ono, S. Ishihara, F. Sato, PsbP protein, but not PsbQ protein, is essential for the regulation and stabilization of Photosystem II in higher plants, Plant Physiol. 139 (2005) 1175–1184.
[13] X. Yi, S.R. Hargett, L.K. Frankel, T.M. Bricker, The PsbQ protein is required in Arabidopsis for Photosystem II assembly/stability and photoautotrophy under low light conditions, J. Biol. Chem. 281 (2006) 26260–26267.
[14] X. Yi, H. Liu, S.R. Hargett, L.K. Frankel, T.M. Bricker, The PsbP protein is required for Photosystem II complex assembly/stability and photoautotrophy in *Arabidopsis thaliana*, J. Biol. Chem. 34 (2007) 24833–24841.
[15] K.N. Ferreira, T.M. Iverson, K. Maghlaoui, J. Barber, S. Iwata, Architecture of the photosynthetic oxygen-evolving center, Science 303 (2004) 1831–1838.
[16] B. Loll, N. Kern, W. Saenger, A. Zouni, J. Biesiadka, Towards complete cofactor arrangement in the 3.0 Å resolution structure of Photosystem II, Nature 438 (2006) 1040–1044.
[17] K. Ifuku, T. Nakatsu, H. Kato, F. Sato, Crystal structure of the PsbP protein of Photosystem II from *Nicotiana tabacum*, EMBO Rep. 5 (2004) 362–367.
[18] V.T. Calderone, M. Trabucco, A. Vujicic, R. Battistutta, G.M. Giacometti, F. Andreucci, R. Barbato, G. Zanotti, Crystal structure of the PsbQ protein of Photosystem II from higher plants, EMBO Rep. 4 (2003) 900–905.
[19] J. Roose, K. Wegener, H. Pakrasi, The extrinsic proteins of Photosystem II, Photosynth. Res. 92 (2007) 369–387.
[20] M. Miqyass, H.J. van Gorkom, C.F. Yocum, The PS II calcium site revisited, Photosynth. Res. 92 (2007) 275–287.
[21] H. Popelkova, C.F. Yocum, Current status of the role of Cl^- ion in the oxygen-evolving complex, Photosynth. Res. 93 (2007) 111–121.
[22] Y.K. Lu, A.J. Stemler, Extrinsic Photosystem II carbonic anhydrase in maize mesophyll chloroplasts, Plant Physiol. 128 (2002) 643–649.

[23] A.V. Shitov, O.V. Pobeguts, T.N. Smolova, S.I. Allakhverdiev, V.V. Klimov, Manganese-dependent carboanhydrase activity of Photosystem II proteins, Biochemistry (Moskow) 74 (2009) 509–517.
[24] C. Spetea, T. Hundal, B. Lundin, M. Heddad, I. Adamska, B. Andersson, Multiple evidence for nucleotide metabolism in the chloroplast thylakoid lumen, Proc. Natl. Acad. Sci. USA 101 (2004) 1409–1414.
[25] B. Lundin, S. Thuswaldner, T. Shutova, S. Eshaghi, G. Samuelsson, J. Barber, B. Andersson, C. Spetea, Subsequent events to GTP binding by the plant PsbO protein: structural changes, GTP hydrolysis and dissociation from the Photosystem II complex, Biochim. Biophys. Acta 1767 (2007) 500–508.
[26] N. Bondarava, P. Beyer, A. Krieger-Liszkay, Function of the 23 kDa extrinsic protein of Photosystem II as a manganese binding protein and its role in photoactivation, Biochim. Biophys. Acta 1708 (2005) 63–70.
[27] N. Bondarava, A. Krieger-Liszkay, Manganese binding to the 23 kDa extrinsic protein of Photosystem II, Biochim. Biophys. Acta 1767 (2007) 583–588.
[28] J. de Las Rivas, P. Heredia, A. Roman, Oxygen-evolving extrinsic proteins (PsbO, P, Q, R): bioinformatic and functional analysis, Biochim. Biophys. Acta 1767 (2007) 575–582.
[29] J.M. Anderson, W.S. Chow, J. De Las Rivas, Dynamic flexibility in the structure and function of Photosystem II in higher plant thylakoid membranes: the grana enigma, Photosynth. Res. 98 (2008) 575–587.
[30] X. Yi, S. Hargett, L.K. Frankel, T.M. Bricker, The PsbP protein, but not the PsbQ protein, is required for normal thylakoid membrane architecture in *Arabidopsis thaliana*, FEBS Lett. 583 (2009) 2142–2147.
[31] K. Ido, K. Ifuku, Y. Yamamoto, S. Ishihara, A. Murakami, K. Takabe, C. Miyake, F. Sato, Knockdown of 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 (2009) 873–881.
[32] A. Rashid, R. Carpentier, The 16 and 23 kDa extrinsic polypeptides and the associated Ca^{2+} and Cl^{-} modify atrazine interaction with the Photosystem II core complex, Photosynth. Res. 24 (1990) 221–227.
[33] J.L. Roose, L.K. Frankel, T.M. Bricker, Documentation of significant electron transport defects on the reducing side of Photosystem II upon removal of the PsbP and PsbQ extrinsic proteins, Biochemistry 49 (2010) 36–41.
[34] J.J.S. Van Rensen, V.V. Klimov, Bicarbonate interactions, in: T. Wydrznski, K. Satoh (Eds.), Photosystem II: The Light-Driven Water: Plastoquinone Oxidoreductase, Springer, Dordrecht, 2005, pp. 329–345.
[35] J. Clausen, K. Baeckmann, W. Junge, J. Messinger, Evidence that bicarbonate is not the substrate in photosynthetic oxygen evolution, Plant Physiol. 139 (2005) 1444–1450.
[36] G. Ulas, G. Olack, G.W. Brudvig, Evidence against bicarbonate bound in the O_2-evolving complex of Photosystem II, Biochemistry 47 (2008) 3073–3075.
[37] C. Aoyama, H. Suzuki, M. Sigiura, T. Noguchi, Flash-induced FTIR difference spectroscopy shows no evidence for the structural coupling of bicarbonate to the oxygen-evolving Mn cluster in Photosystem II, Biochemistry 47 (2008) 2760–2765.
[38] D. Shevela, J.-H. Su, V.V. Klimov, J. Messinger, Hydrogencarbonate is not a tightly bound constituent of the water oxidizing complex in Photosystem II, Biochim. Biophys. Acta 1777 (2008) 532–539.
[39] A. Guskov, A. Gabdulkhakov, M. Broser, C. Glockner, J. Hellmich, J. Kern, J. Frank, F. Muh, W. Saenger, A. Zouni, Recent progress in the crystallographic studies of Photosystem II, Chem. Phys. Chem. 11 (2010) 1160–1171.
[40] Y.-K. Lu, S.M. Theg, A.J. Stemler, Carbonic anhydrase activity of the Photosystem II OEC33 protein from pea, Plant Cell Physiol. 46 (2005) 1944–1953.
[41] Y.-K. Lu, A.J. Stemler, Differing responses of the two forms of Photosystem II carbonic anhydrase to chloride, cations, and pH, Biochim. Biophys. Acta 1767 (2007) 633–638.
[42] N. Lydakis-Simantiris, R.S. Hutchison, S.D. Betts, B.A. Barry, C.F. Yocum, Manganese stabilizing protein of Photosystem II is a thermostable, natively unfolded pypeptide, Biochemistry 38 (1999) 404–414.
[43] T.W. Lane, M.A. Saito, G.N. George, I.J. Pikering, R.C. Prince, F.M.M. Morel, A cadmium enzyme from a marine diatom, Nature 435 (2005) 42.
[44] Y. Xu, L. Feng, P.D. Jeffrey, Y. Shi, F.M.M. Morel, Structure and metal exchange in the cadmium carbonic anhydrase of marine diatoms, Nature 452 (2008) 52–61.
[45] S. Betts, J.R. Ross, K.U. Hall, E. Pichersky, C.F. Yocum, Functional reconstitution of Photosystem II with recombinant manganese-stabilizing proteins containing mutations that remove the disulfide bridge, Biochim. Biophys. Acta 1274 (1996) 135–142.
[46] B.B. Buchanan, S. Luan, Redox regulation in the chloroplast thylakoid lumen: a new frontier in photosynthesis research, J. Exp. Bot. 56 (2005) 1439–1447.
[47] M. Hall, W.P. Schröder, T. Kieselbach, Thioredoxin interactions of the chloroplast lumen of *Arabidopsis thaliana* indicate redox regulation of the xanthophyll cycle, in: J.F. Allen, J.H. Golbeck, B. Osmond (Eds.), Photosynthesis, Energy from the Sun: 14th International Congress on Photosynthesis, Springer, 2008.
[48] E. Ströher, K.-J. Dietz, The dynamic thiol–disulphide redox proteome of the *Arabidopsis thaliana* chloroplast as revealed by differential electrophoretic mobility, Physiol. Plant 133 (2008) 566–583.
[49] N. Majeau, J.R. Coleman, Correlation of carbonic anhydrase and ribulose-1,5 bisphosphate carboxylase/oxygenase expression in pea, Plant Physiol. 104 (1994) 1393–1399.
[50] C. Merlin, M. Masters, S. McAteer, A. Coulson, Why is carbonic anhydrase essential to *Escherichia coli*?, J Bacteriol. 185 (2003) 6415–6424.
[51] M. Mitra, C.B. Mason, Y. Xiao, R.A. Ynalvez, S.M. Lato, J.V. Moroney, The carbonic anhydrase gene families of *Chlamydomonas reinhardtii*, Can. J. Bot. 83 (2005) 780–795.
[52] L.K. Frankel, T.M. Bricker, Monoclonal antibodies directed against the 33, 24, and 17 kDa extrinsic proteins of spinach Photosystem II, in: M. Batcheffsky (Ed.), Current Research in Photosynthesis, vol. I, Kluwer Academic Press, Dordrech, 1990, pp. 825–828.
[53] L.K. Frankel, J.A. Cruz, T.M. Bricker, The role of carboxylic acid residues on the manganese-stabilizing protein in its binding to Photosystem II, Biochemistry 38 (1999) 14271–14278.
[54] J.W. Murray, J. Barber, Identification of a calcium-binding site in the PsbO protein of Photosystem II, Biochemistry 45 (2006) 4128–4130.
[55] A. Guskov, J. Kern, A. Gabdulkhakov, M. Broser, A. Zouni, W. Saenger, Cyanobacterial Photosystem II at 2.9-Å resolution and the roles of quinones, lipids, channels and chloride, Nat. Struct. Mol. Biol. 16 (2009) 334–342.
[56] A. Lanir, S. Gradstajn, G. Navon, Temperature and frequency dependence of solvent proton relaxation rates in solutions of manganese(II) carbonic anhydrase, Biochemistry (1975) 242–248.
[57] I.L. McConnell, M.R. Badger, T. Wydrzynski, W. Hiller, A quantitative assessment of the carbonic anhydrase activity in Photosystem II, Biochim. Biophys. Acta 1767 (2007) 639–647.
[58] B. Lundin, M. Hansson, B. Schoefs, A.V. Vener, C. Spetea, The Arabidopsis $PsbO_2$ protein regulates dephosphorylation and turnover of the Photosystem II reaction center D1 protein, Plant J. 49 (2007) 528–539.
[59] E.-M. Aro, I. Virgin, B. Andersson, Photoinhibition of Photosystem II. Inactivation, protein damage and turnover, Biochem. Biophys. Acta 1143 (1993) 113–134.
[60] E-M. Aro, M. Suorsa, A. Rokka, Y. Allahverdiyeva, V. Paakkarinen, A. Saleem, N. Battchikova, E. Rintamaki, Dynamics of Photosystem II: a proteomic approach to thylakoid protein complexes, J. Exp. Bot. 56 (2005) 347–356.
[61] B. Lundin, M. Nurmi, M. Rojas-Stuetz, E.-M. Aro, I. Adamski, C. Spetea, Towards understanding the functional difference between the two PsbO isoforms in *Arabidopsis thaliana*—insights from phenotypic analyses of PsbO knockout mutants, Photosynth. Res. 98 (2008) 405–414.
[62] V. Bonardi, P. Pesaresi, T. Becker, E. Schleiff, R. Wagner, T. Pfannschmidt, P. Jahns, D. Leister, Photosystem II core phosphorylation and photosynthetic acclimation require two different protein kinases, Nature 437 (2005) 1179–1182.
[63] I.S. Booij-James, M. Edelman, A.K. Mattoo, Nitric oxide donor-mediated inhibition of phosphorylation shows that light-mediated degradation of Photosystem II D1 protein and phosphorylation are not tightly linked, Planta 229 (2009) 1347–1352.
[64] T. Shutova, G. Deikus, K.D. Irrgang, V.V. Klimov, G. Renger, Origin and properties of fluorescence emission from the extrinsic 33 kDa manganese stabilizing protein of higher plant water oxidizing complex, Biochim. Biophys. Acta 1504 (2001) 371–378.
[65] A.J. Wyman, H. Popelkova, C.F. Yocum, Site-directed mutagenesis of conserved C-terminal tyrosine and tryptophan residues of PsbO, the Photosystem II manganese-stabilizing protein, alters its activity and fluorescence properties, Biochemistry 47 (2008) 6490–6498.
[66] J. de Gunzberg, Proteins of the Ras pathway as novel potential anticancer therapeutic targets, Cell Biol. Toxicol. 15 (1999) 345–358.
[67] C. Sundby, S. McCaffery, J.M. Anderson, Turnover of the Photosystem II D1 protein in higher plants under photoinhibitory and nonphotoinhibitory irradiance, J. Biol. Chem. 268 (1993) 25476–25482.
[68] R. Murakami, K. Ifuku, A. Takabayashi, T. Shikanai, T. Endo, F. Sato, Functional dissection of two Arabidopsis PsbO proteins $PsbO_1$ and $PsbO_2$, FEBS J. 272 (2005) 2165–2175.
[69] Y. Allahverdiyeva, F. Mamedov, M. Holmstrom, M. Nurmi, B. Lundin, S. Styring, C. Spetea, E.-M. Aro, Comparison of the electron transport properties of the $PsbO_1$ and $PsbO_2$ mutants of *Arabidopsis thaliana*, Biochim. Biophys. Acta 1787 (2009) 1230–1237.
[70] H. Liu, L.K. Frankel, T.M. Bricker, Functional analysis of Photosystem II in a PsbO-1 deficient mutant in *Arabidopsis thaliana*, Biochemistry 46 (2007) 7607–7613.
[71] T.M. Bricker, L.K. Frankel, The Arabidopsis $PsbO_1$ mutant cannot efficiently utilize calcium in support of photosynthetic oxygen evolution, J. Biol. Chem. 283 (2008) 29022–29027.
[72] S.D. Betts, T.D. Hachigian, E. Pichersky, C.F. Yocum, Reconstitution of the spinach oxygen-evolving complex with recombinant Arabidopsis manganese-stabilizing protein, Plant Mol. Biol. 26 (1994) 117–130.
[73] I. Ensminger, F. Busch, P.A. Huner, Photostasis and cold acclimation: sensing low temperature through photosynthesis, Physiol. Plant 126 (2006) 28–44.
[74] S.J. Robinson, I.A.P. Parkin, Differential SAGE analysis in Arabidopsis uncovers increased transcriptome complexity in response to low temperature, BMC Genome 9 (2008) 434–450.
[75] E. Goulas, M. Schubert, T. Kieselbach, L.A. Kleczkowski, P. Gardestrom, W.P. Schroder, V. Hurry, The chloroplast lumen and stromal proteomes of *Arabidopsis thaliana* show differential sensitivity to short- and long-term exposure to low temperature, Plant J. 47 (2006) 720–734.
[76] A.M. Gilmore, O. Bjorkman, Temperature sensitive coupling and uncoupling of ATPase-mediated nonradiative energy dissipation: similarities between chloroplasts and leaves, Planta 197 (1995) 646–654.
[77] P.V. Sane, A.G. Ivanov, V. Hurry, N.P.A. Huner, G. Oquist, Changes in the redox potential of primary and secondary electron-accepting quinones in Photosystem II confer increased resistance to photoinhibition in

low-temperature-acclimated Arabidopsis, Plant Physiol. 132 (2003) 2144–2151.
[78] R.L. Burnap, R.K. Trench, Sites of synthesis of polypeptides of the cyanelles of *Cyanophora paradoxa*: II. In vivo labeling in the presence of translational and transcriptional inhibitors, Proc. Roy. Soc. Lond. B. 238 (1989) 73–87.
[79] J.B. Philbrick, B.A. Diner, B.A. Zilinskas, Construction and characterization of cyanobacterial mutants lacking the manganese-stabilizing protein of Photosystem II, J. Biol. Chem. 266 (1991) 13370–13376.
[80] L. Thornton, H. Ohkawa, J. Roose, Y. Kashino, N. Keren, H. Pakrasi, Homologs of plant PsbP and PsbQ proteins are necessary for regulation of Photosystem II activity in the cyanobacterium Synechocystis 6803, Plant Cell 16 (2004) 2164–2175.
[81] F.K. Bentley, J. Eaton-Rye, The effect of removing Photosystem II extrinsic proteins on dimer formation and recovery from photodamage in Synechocystis sp. PCC 6803, in: J.F. Allen, E. Gantt, J.H. Golbeck, B. Osmond (Eds.), Photosynthesis. Energy from the Sun: 14th International Congress on Photosynthesis, Dordrecht, 2008, pp. 715–717.
[82] S.P. Mayfield, P. Bennoun, J.D. Rochaix, Expression of the nuclear-encoded OEE1 protein is required for oxygen evolution and stability of Photosystem II particles in *Chlamydomonas reinhardtii*, EMBO J. 6 (1987) 313–318.
[83] C. de Vitry, J. Olive, D. Drapier, M. Recouvreur, F.A. Wollman, Post-translational events leading to the assembly of Photosystem II protein complex: a study using photosynthesis mutants from *Chlamydomonas reinhardtii*, J. Cell Biol. 109 (1989) 991–1006.
[84] A.V. Pigolev, S.K. Zharmukhamedov, V.V. Klimov, The PsbO mutant of *Chlamydomonas reinhardtii* is capable of assembling stable, photochemically active reaction center of Photosystem II, Biol. Memb. 26 (2009) 31–40.
[85] S.P. Mayfield, M. Rahire, G. Frank, H. Zuber, J.D. Rochaix, Expression of the nuclear gene encoding oxygen-evolving enhancer protein 2 is required for high levels of photosynthetic oxygen evolution in *Chlamydomonas reinhardtii*, Proc. Natl. Acad. Sci. USA 84 (1987) 749–753.
[86] W.F. Ettinger, S.M. Theg, Physiologically active chloroplasts contain pools of unassembled extrinsic proteins of the photosynthetic oxygen-evolving enzyme complex in the thylakoid lumen, J. Cell Biol. 115 (1991) 321–328.
[87] A. Hashimoto, W.F. Ettinger, Y. Yamamoto, S.M. Theg, Assembly of newly imported oxygen-evolving complex subunits in isolated chloroplasts: sites of assembly and mechanism binding, Plant Cell 9 (1997) 441–452.
[88] S.P. Mayfield, M. Schirmer-Rahire, G. Frank, H. Zuber, J.-D. Rochaix, Analysis of the genes of the OEE1 and OEE3 proteins of the Photosystem II complex from *Chlamydomonas reinhardtii*, Plant Mol. Biol. 12 (1989) 683–693.
[89] J.M. Anderson, Insights into the consequences of grana stacking of thylakoid membranes in vascular plants: a personal perspective, Aust. J. Plant Physiol. 26 (1999) 625–639.
[90] D.J. Goodchild, O. Björkman, N.A. Pyliotis, Chloroplast ultrastructure, leaf anatomy, and soluble protein in rainforest species, Carnegie Inst. Washington Year Book 71 (1972) 102–107.
[91] R.L. Burnap, M. Qian, C. Pierce, The manganese-stabilizing protein (MSP) of Photosystem II modifies the in vivo deactivation and photoactivation kinetics of the H_2O-oxidation complex in Synechocystis sp. PCC6803, Biochemistry 35 (1996) 874–882.
[92] J. Kohoutova, I.K. Smatanova, J. Brynda, M. Lapkouski, J.L. Revuelta, J.B. Arellano, R. Ettrich, Crystallization and preliminary crystallographic characterization of the extrinsic PsbP protein of Photosystem II from Spinacia oleracea, Act. Cryst. F 65 (2009) 111–115.
[93] R. Ristvejova, M. Lapkouski, J. Kopecky, V, K. Hofbauerova, Z. Sofova, S. Gonzalez-Perez, I.K. Smatanova, J.L. Revuelta, J.B. Arellano, R. Ettrich, Structure of PsbP protein from *Spinacea oleracea* at 1.98 Å, in preparation.
[94] F. Michoux, K. Takasaka, M. Boehm, P.J. Nixon, J.W. Murray, Structure of CyanoP at 2.8 Å: implications for the evolution and function of the PsbP subunit of Photosystem II, Biochemistry 49 (2010) 7411–7413.
[95] E.M. Rova, B. Mc Ewen, P.O. Fredriksson, S. Styring, Photoactivation and photoinhibition are competing in a mutant of *Chlamydomonas reinhardtii* lacking the 23 kDa extrinsic subunit of Photosystem II, J. Biol. Chem. 271 (1996) 28918–28924.
[96] M. Rova, L.-G. Franzen, P.-O. Fredriksson, S. Styring, Photosystem II in a mutant of *Chlamydomonas reinhardtii* lacking the 23 kDa psbP protein shows increased sensitivity to photoinhibition in the absence of chloride, Photosynth. Res. 39 (1994) 75–83.
[97] M. Miyao, Y. Inoue, Enhancement by chloride ions of photoactivation of oxygen evolution in manganese-depleted Photosystem II membranes, Biochemistry 30 (1991) 5379–5387.
[98] N. Tamura, G. Cheniae, Photoactivation of the water-oxidizing complex in Photosystem II membranes depleted of Mn and extrinsic proteins. I. Biochemical and kinetic characterization, Biochim. Biophys. Acta 890 (1987) 179–193.
[99] N. Tamura, Y. Inoue, G. Cheniae, Photoactivation of the water-oxidizing complex in Photosystem II membranes depleted of Mn, Ca, and extrinsic proteins. II. Studies on the function of Ca^{2+}., Biochim. Biophys. Acta 976 (1989) 173–181.
[100] J.L. Roose, H.B. Pakrasi, The Psb27 protein facilitates manganese cluster assembly in Photosystem II, J. Biol. Chem. 283 (2007) 4044–4050.
[101] J. Dasgupta, G.M. Ananyev, G.C. Dismukes, Photoassembly of the water-oxidizing complex in Photosystem II, Coord. Chem. Rev. 252 (2008) 347–360.
[102] B. Andersson, C. Larsson, C. Jansson, U. Ljungberg, H.-E. Akerlund, Immunological studies on the organization of proteins in photosynthetic oxygen evolution, Biochim. Biophys. Acta 766 (1984) 21–26.
[103] K. Kavelaki, D.F. Ghanotakis, Effects of the manganese complex on the binding of the extrinsic proteins (17, 23 and 33 kDa) of Photosystem II, Photosynth. Res. 29 (1991) 149–155.
[104] D.J. Blubaugh, G.M. Cheniae, Kinetics of photoinhibition in hydroxylamine extracted Photosystem II membranes: relevance to photoactivation and sites of electron donation, Biochemistry 29 (1990) 5109–5118.
[105] C.W. Hoganson, D.F. Ghanotakis, G.T. Babcock, C.F. Yocum, Manganese ion reduces redox activated tyrosine in manganese-depleted Photosystem II preparations, Photosynth. Res. 22 (1989) 285–294.
[106] M. Tomita, K. Ifuku, F. Satoh, T. Noguchi, FTIR evidence that the PsbP extrinsic protein induces protein conformational changes around the oxygen-evolving Mn cluster in Photosystem II, Biochemistry 48 (2009) 6318–6325.
[107] V. Petrouleas, A.R. Crofts, The iron-quinone acceptor complex, in: T. Wydrzynski, K. Satoh (Eds.), Photosystem II: The Light-Driven Water: Plastoquinone Oxidoreductase, Springer, Dordrecht, 2005, pp. 177–206.
[108] J.P. Dekker, D.F. Ghanotakis, J.J. Plijter, H.J. Van Gorkom, G.T. Babcock, Kinetics of the oxygen-evolving complex in salt-washed Photosystem II preparations, Biochim. Biophys. Acta 767 (1984) 515–523.
[109] K. Pfister, C.J. Arntzen, The mode of action of Photosystem II-specific inhibitors in herbicide-resistant weed biotypes, Z. Naturforsch. 34C (1979) 996–1009.
[110] L.-E. Andreasson, I. Vass, S. Styring, Ca^{2+} depletion modifies the electron transfer on both donor and acceptor sides in Photosystem II from spinach, Biochim. Biophys. Acta 1230 (1995) 155–164.
[111] E.J. Boekema, J.F.L. van Breeman, H. van Roon, J.P. Dekker, Conformational changes in Photosystem II supercomplexes upon removal of extrinsic subunits, Biochemistry 39 (2000) 12907–12915.
[112] T. Noguchi, T.-a. Ono, Y. Inoue, Direct detection of a carboxylate bridge between Mn and Ca^{2+} in the photosynthetic oxygen-evolving center by means of Fourier transform infrared spectroscopy, Biochim. Biophys. Acta 1228 (1995) 189–200.