PsbQ (Sll1638) in *Synechocystis* sp. PCC 6803 Is Required for Photosystem II Activity in Specific Mutants and in Nutrient-Limiting Conditions†

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ABSTRACT: A PsbQ homologue has been found associated with photosystem II complexes in *Synechocystis* sp. PCC 6803 where it is involved in optimal photoautotrophic growth and water splitting under $CaCl_2$-depleted conditions [Thornton, L. E., Ohkawa, H., Roose, J. L., Kashino, Y., Keren, N., and Pakrasi, H. B. (2004) *Plant Cell 16*, 2164−2175]. By inactivating *psbQ* in strains carrying photosystem II-specific mutations, we have identified stringent requirements for PsbQ in vivo. Whereas under nutrient-replete conditions the ΔPsbQ mutant was similar to wild type, a strain lacking PsbQ and PsbV was not photoautotrophic, exhibiting decreased oxygen evolution and decreased photosystem II assembly compared to the ΔPsbV mutant. Combining the removal of PsbU and PsbQ introduced an altered requirement for Ca^{2+} and Cl^-, and photoautotrophic growth of the ΔPsbQ strain was prevented in nutrient-limiting media depleted in Ca^{2+}, Cl^-, and iron. Unlike other photosystem II extrinsic proteins PsbQ did not participate in the acquisition of thermotolerance; however, photoautotrophic growth at elevated temperatures was impaired in this mutant. Growth of the ΔPsbV:ΔPsbQ mutant was restored at pH 10.0: in contrast, an additional deletion between Arg-384 and Val-392 in the CP47 protein of photosystem II prevented recovery at alkaline pH. When conditions prevented photoautotrophy in strains lacking PsbQ, photoheterotrophic growth was indistinguishable to wild type, indicating that photosystem II had been inactivated. These data substantiate a role for PsbQ in optimizing photosystem II activity in *Synechocystis* sp. PCC 6803 and establish an absolute requirement for the subunit under specific biochemical and physiological conditions.

Cyanobacteria perform oxygenic photosynthesis, and several species serve as model organisms for the study of water splitting by photosystem II (PSII)[1] (*1*, *2*). Extensive manipulation of genes encoding PSII proteins in *Synechocystis* sp. PCC 6803 has been undertaken to examine structure/function relationships (*3*, *4*), and structural data for the photosystem are available from *Thermosynechococcus elongatus* and *Thermosynechococcus vulcanus* at 3.5 and 3.7 Å, respectively (*5*−*7*). Water oxidation occurs at the oxygen-evolving complex (OEC) found on the lumenal face of the photosystem incorporating a manganese cluster containing Ca^{2+} and Cl^- cofactors (*7*, *8*). Protein domains are contributed by the reaction center polypeptides D1 (PsbA) and D2 (PsbD) and include hydrophilic loops connecting membrane-spanning regions of the chlorophyll *a*-binding core antenna proteins CP47 (PsbB) and CP43 (PsbC). Three polypeptides encoded by *psbO*, *psbU*, and *psbV*, corresponding to proteins of ∼27, 10, and 15 kDa in *Synechocystis* sp. PCC 6803, are also present (*6*, *7*, *9*). Two additional proteins, PsbQ and PsbP, have been found to be associated with PSII particles from *Synechocystis* sp. PCC 6803 (*10*, *11*). However, these two proteins are absent from the crystal structures from *T. elongatus* and *T. vulcanus* (*5*−*7*).

The PsbO protein is in all oxygenic photoautotrophs (*12*, *13*). However, PsbU and PsbV (or cytochrome c_{550}) are restricted to cyanobacteria, red algae (*12*, *14*, *15*), and chlorophyll *a*/*c*-containing organisms (e.g., the diatom *Thalassiosira pseudonana*; www.jgi.doe.gov/genomes/index.html). In cyanobacteria the removal of either PsbO or PsbV resulted in fewer assembled PSII centers, a susceptibility of oxygen evolution to photoinactivation, and an altered dependence on Ca^{2+} and Cl^- for photoautotrophic growth (*16*−*21*). Additionally, the absence of PsbO in *Synechocystis* sp. PCC 6803 slowed the kinetics of oxygen release and resulted in an increase in the relative quantum yield for photoactivation of the OEC (*22*−*24*), while the mutant lacking both PsbO and PsbV has been found to be an obligate photoheterotroph (*25*). Furthermore, the absence of PsbU in vivo resulted in slowed growth under conditions where Ca^{2+} or Cl^- were limiting and oxygen evolution was reduced by up to 20% when compared to wild type (*26*−*28*). However, the requirement for PsbQ is less stringent with ΔPsbQ cells

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[1] Abbreviations: bp, base pairs; Cam^R, chloramphenicol-resistance cassette; CAPS, 3-(cyclohexylamino)-1-propanesulfonic acid; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DMBQ, 2,5-dimethyl-*p*-benzoquinone; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; kb, kilobases; nt, nucleotides; OD, optical density; OEC, oxygen-evolving complex; ORF, open reading frame; PCC, Pasteur Culture Collection; PCR, polymerase chain reaction; PSI, photosystem I; PSII, photosystem II; SDS, sodium dodecyl sulfate; $Spec^R$, spectinomycin-resistance cassette; SSC, standard saline citrate; TES, 2-[tris(hydroxymethyl)methyl]amino-1-ethanesulfonic acid; Tris, tris(hydroxymethyl)-aminomethane.

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exhibiting a slight decrease in growth compared to wild type in media lacking either Ca^{2+} or Cl^- (*11*). In addition, oxygen evolution showed an increased dependence on $CaCl_2$ concentration both in vivo and in vitro in the absence of PsbQ compared to wild type, and a similar phenotype was observed in the absence of PsbP (*11*).

The PsbP and PsbQ polypeptides were identified in higher plants and green algae in association with the OEC where they participate in Ca^{2+} and Cl^- binding (*14*, *29*−*31*), and it has been postulated that these subunits have replaced the cyanobacterial PsbU and PsbV proteins in optimizing oxygen evolution (*32*, *33*). The red alga *Cyanidium caldarium* has four extrinsic proteins associated with the OEC: PsbO, PsbU, PsbV, and a 20 kDa subunit that has been shown to have sequence similarity with PsbQ and is designated PsbQ′ (*34*, *35*). In release-reconstitution experiments the binding of PsbQ′ did not enhance oxygen evolution but is thought to facilitate binding of PsbU and PsbV (*34*). Furthermore, PsbQ′ has been shown to bind to cyanobacterial and plant PSII, although plant PsbP and PsbQ did not bind to red algal or cyanobacterial PSII-enriched membranes (*12*). The inclusion of PsbQ as an extrinsic PSII protein in *Synechocystis* sp. PCC 6803 is therefore consistent with the protein composition of the OEC in red algae, and it has been suggested that PsbQ is a regulator required for the biogenesis of optimally active PSII centers, particularly in $CaCl_2$-depleted media (*11*).

To examine the role for PsbQ in water splitting and to investigate the potential for functional redundancy between the extrinsic proteins in *Synechocystis* sp. PCC 6803, the sll1638 gene has been inactivated in cells carrying PSII-specific mutations or gene knockouts for one or more extrinsic subunits. Using this approach we have identified nutrient-limiting conditions where PsbQ is essential for photoautotrophic growth. Additionally, PsbQ is required for optimal oxygen evolution by PSII in specific strains in nutrient-replete media, and it is required for photoautotrophic growth in the absence of PsbV but not in the absence of PsbO or PsbU.

MATERIALS AND METHODS

Growth of Synechocystis sp. PCC 6803 Strains. Cultures were maintained on BG-11 plates containing 5 mM glucose and 20 μM atrazine and appropriate antibiotics. In both solid and liquid media chloramphenicol was present at a concentration of 15 μg/mL and erythromycin, kanamycin, and spectinomycin were present at 25 μg/mL. The BG-11 solid media were supplemented with 10 mM TES−NaOH (pH 8.2) and 0.3% sodium thiosulfate (*36*). Liquid cultures were grown mixotrophically in unbuffered BG-11 containing 5 mM glucose and appropriate antibiotics unless otherwise noted and photoheterotrophically grown cultures also contained 20 μM 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU). To obtain Ca^{2+}-limiting media, 0.24 mM $CaCl_2$ was replaced by 0.48 mM NaCl. For Cl^--limiting media $MnSO_4$ replaced $MnCl_2$ and $Ca(NO_3)_2$ replaced $CaCl_2$. This reduced the Cl^- concentration in the BG-11 medium from 0.48 mM to a level that could not be detected by titration with sodium borohydride (*37*). For iron-limiting conditions ferric ammonium citrate was substituted with ammonium citrate. The *Synechocystis* sp. PCC 6803 strain used in this study is the glucose-tolerant strain from Williams (*3*), and this is referred to throughout as wild type.

Cultures, except those used in heat-inactivation experiments, were maintained at 30 °C under constant illumination at 25 μE m^{-2} s^{-1} (*20*). For growth at temperatures greater than 30 °C cultures were maintained in a MLR-350 growth cabinet (Sanyo Electric Biomedical Co. Ltd.) under constant illumination at 115 μE m^{-2} s^{-1}. For oxygen evolution and herbicide-binding measurements at either pH 7.5 or pH 10.0, strains were grown in BG-11 media containing either 25 mM HEPES (pH 7.5) or 25 mM CAPS (pH 10.0). Starter cultures for pH-dependent growth experiments were grown in unbuffered BG-11 and then transferred into buffered media at the start of the growth measurement (*36*).

Construction of Strains Lacking PsbQ. The *Synechocystis* sp. PCC 6803 open-reading frame sll1638, encoding the PsbQ protein, was obtained by PCR using the forward primer 5′-TAATGTCCTGCAAACGGGTG-3′ and the reverse primer 5′-GGGCATTGGGAGATTCGTAA-3′ corresponding to 665 bp upstream of the initiation codon and 651 bp downstream from the termination codon. The PCR product was cloned into pGEM-T Easy (Promega) and interrupted at a unique intragenic *Bst*EII site by either a chloramphenicol-resistance cassette (Cam^R) derived from pBR325 or a spectinomycin-resistance cassette ($Spec^R$) derived from pHP45ω (*38*−*40*). Cyanobacterial strains were transformed according to established protocols as previously described (*3*, *36*).

RNA Extraction and Northern Hybridization. Synechocystis sp. PCC 6803 was grown from a starting OD_{730nm} of 0.05 for 60 h either in unbuffered or buffered BG-11 and total RNA extracted from exponentially growing 300 mL cultures as described previously (*41*). The RNA was fractionated on a 1% agarose gel containing 2% formaldehyde and blotted onto a nylon membrane (Dupont). The membrane was hybridized with 25 ng of a [^{32}P]dCTP-labeled *psbQ* intragenic probe obtained as a PCR-generated fragment using the forward primer 5′-ATGTCTCGTTTACGTTCGTT-3′ and the reverse primer 5′-CAAACACTTCTTTAGCCAGG-3′ and a [^{32}P]dCTP-labeled *psbB* intragenic probe that was obtained as a PCR-generated fragment using the forward primer 5′-ACCGGTGCTATGAAACAGTGG-3′ and the reverse primer 5′-CTTCTTTCCGGGTGGAAAGG-3′. The membrane was hybridized overnight at 42 °C in 50% formamide, 1% sodium dodecyl sulfate (SDS), 5× standard saline citrate (SSC), and 5× Denhardt's solution. The membrane was then washed twice in 2× SSC at room temperature for 5 min, followed by two washes in 2× SSC containing 0.2% SDS at 60 °C for 30 min and finally by two washes in 0.1× SSC at room temperature for 10 min.

Oxygen Evolution Assays. A Clark-type electrode (Hansatech) was used to measure oxygen evolution at 30 °C. Measurements were made in buffered BG-11 at 10 μg/mL chlorophyll *a* (hereafter referred to as chlorophyll). Saturating actinic light was provided by an FLS1 light source (Hansatech) (2.0 mE m^{-2} s^{-1}) passed through a Melles Griot OG 590 sharp cutoff red glass filter. The electron acceptors were 1.0 mM $K_3Fe(CN)_6$ and 0.2 mM 2,5-dimethyl-*p*-benzoquinone (DMBQ) for PSII-specific electron transport and 15 mM $NaHCO_3$ for whole-chain measurements. Chlorophyll determinations were performed according to MacKinney (*42*).

Measurement of the Relative Level of Assembled PSII Centers. The relative level of assembled PSII centers was estimated on a chlorophyll basis by herbicide-binding assays

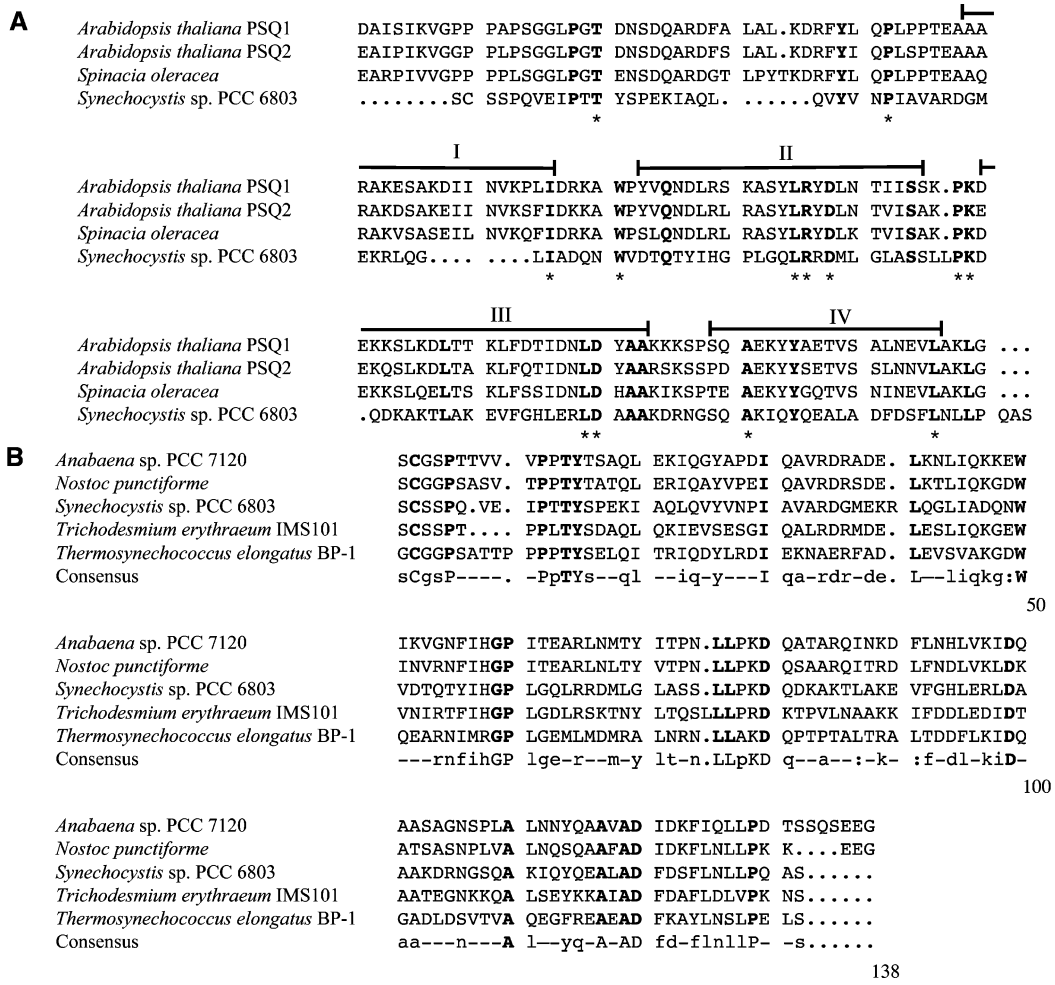

FIGURE 1: Comparison of PsbQ-like protein sequences. The alignments were performed using ClustalX default parameters (*65*). (A) Alignment of amino acid sequences of PsbQ proteins from *A. thaliana* PSQ1 (accession number Q9XFTE), PSQ2 (accession number Q41932), *S. oleracea* (accession number P12301), and the *Synechocystis* sp. PCC 6803 PsbQ protein (Sll1638). The solid black lines indicate the positions of α-helices in the three-dimensional structure of PsbQ from *S. oleracea* (*47*). Amino acids conserved in the aligned sequences are indicated in bold, and residues also conserved in the higher plant sequences aligned in ref *48* are marked by an asterisk. Of these 13 residues Thr-20, Pro-42 and Pro-97 are not conserved in green algae. (B) Alignment of predicted amino acid sequences of PsbQ proteins from *Anabaena* sp. PCC 7120 (All1355), *N. punctiforme* (accession number ZP00112081), *Synechocystis* sp. PCC 6803 (Sll1638), *T. erythraeum* IMS101 (accession number ZP00073276), and *T. elongatus* BP-1 (Tll2057). Amino acids conserved in the aligned sequences are indicated in bold. In the consensus sequence conserved residues are indicated in upper case, and those residues also conserved in higher plant sequences are shown in bold. Amino acids marked in lower case are present in at least three of the sequences, conservative substitutions are indicated by a colon, and positions with variable amino acids are indicated by a dash.

employing [^{14}C]atrazine (*20*). The specific activity of the [^{14}C]atrazine was 17.1 mCi/mmol, and the chlorophyll concentration was 50 μg/mL; however, when the relative amount of assembled PSII centers was below 50% of the wild-type level, the chlorophyll concentration was increased to 100 μg/mL.

RESULTS

Comparison of Sll1638 with PsbQ and PsbQ-like Sequences. The *Synechocystis* sp. PCC 6803 PsbQ protein (i.e., Sll1638) has 32% identity to PsbQ from *Arabidopsis thaliana* (*10*): this refers to the PSQ1 protein which has the highest identity to Sll1638 of the four predicted proteins encoded by *psbQ*-like ORFs in the *Arabidopsis* genome. There is evidence that all four ORFs are expressed, and the PSQ1 and PSQ2 proteins have been observed in lumenal extracts (*43*−*46*). These two PsbQ protein sequences are compared to the sequences from spinach and *Synechocystis* sp. PCC 6803 in Figure 1A. Also shown are the helical domains that form a four-helix bundle in the X-ray crystal structure of PsbQ from spinach (*47*). The greatest variation was observed between the N-terminal sequences of the *Synechocystis* sp. PCC 6803 and plant PsbQ proteins; however, 22 residues are conserved in the four proteins, and a number of conservative substitutions are present. Thirteen of these conserved amino acids were retained in the 10 PsbQ sequences of green plants aligned in ref *48*, and these are indicated in Figure 1A. This compares with a total of 63 conserved amino acids in the PsbQ sequences from green plants, and 24 of these residues were conserved between plants and green algae (*48*). However, only 10 of the 24 conserved residues found in plants and green algae were also found in *Synechocystis* sp. PCC 6803.

A BLAST search (*49*) using Sll1638 led to the identification of *psbQ*-like ORFs within the genomes of a number of cyanobacterial strains. An alignment of predicted PsbQ-like proteins from five cyanobacteria in Figure 1B revealed 19 conserved residues (shown in the consensus sequence)

together with several conservative substitutions. Four residues were conserved between the cyanobacterial and plant sequences, and only 3 of the 10 amino acids found in plants, green algae, and *Synechocystis* sp. PCC 6803 were found in all five cyanobacterial sequences. Given the degree of identity demonstrated in Figure 1, it is difficult to predict if Sll1638 would play a similar role to that observed for PsbQ or PsbQ-like proteins in other oxygenic photoautotrophic organisms. To examine this, we investigated the removal of Sll1638 in wild type or in mutants lacking extrinsic proteins associated with the OEC. In particular, conditions where the extrinsic proteins have been shown to contribute in optimizing oxygen evolution were selected. Additionally, to probe the specificity of removing the PsbQ protein encoded by sll1638, strains were characterized in which this *psbQ* gene had been inactivated in combination with other PSII-specific mutations in CP47.

Expression of psbQ. The expression of *psbQ* was investigated under each of the growth conditions utilized in this study: normal BG-11; Ca^{2+}-limiting media; Cl^--limiting media; iron-limiting media, and in normal BG-11 at either pH 7.5 or pH 10.0. The RNA isolated from these cells was hybridized with an intragenic probe for either *psbQ* or the constitutively expressed *psbB* that encodes CP47. An ~800 nt transcript corresponding to *psbQ* was detected in RNA isolated from wild-type cells grown under all conditions investigated (Figure 2A), and although the level of expression of *psbQ* appeared slightly elevated under Cl^--limiting conditions, this corresponded to variation observed between experiments rather than a significant increase. Additionally, the level of the *psbB* transcript (~2000 nt) was similar in each lane.

Effect of Removing PsbQ in Wild Type or in Strains Lacking PsbO, PsbU, and PsbV. To conduct this study, nine new mutants were constructed in which *psbQ* was inactivated by the insertion of an antibiotic-resistance cassette. Since *Synechocystis* sp. PCC 6803 has multiple copies of its genome, full segregation for the inactivated *psbQ* gene was confirmed by PCR and is shown in Figure 2B. It has been suggested that the extrinsic proteins PsbU and PsbV in cyanobacteria have equivalent functions to PsbP and PsbQ in plants and green algae (*32*, *33*). To examine the possibility that PsbQ is required when PsbU or PsbV is absent, *psbQ* was inactivated in strains lacking these proteins as well as in the wild type and ΔPsbO mutant. Photoautotrophic growth of the ΔPsbQ, ΔPsbO:ΔPsbQ, ΔPsbU:ΔPsbQ, and ΔPsbV:ΔPsbQ mutants was compared to wild type, and the photoautotrophic doubling times, following the removal of PsbQ, for the wild-type, ΔPsbO, ΔPsbU, and ΔPsbV strains are presented in Table 1. The removal of PsbQ resulted in little change in the photoautotrophic doubling time of wild type (Figure 3A) but extended the doubling time of the ΔPsbO:ΔPsbQ and ΔPsbU:ΔPsbQ mutants (Table 1). Growth was eliminated in the ΔPsbV:ΔPsbQ strain (Figure 3A); however, photoheterotrophic growth in the presence of DCMU and glucose resembled that of wild type, demonstrating that the absence of photoautotrophic growth was a PSII-specific phenotype (data not shown).

The removal of PsbQ had little effect on PSII-specific oxygen evolution supported by $K_3Fe(CN)_6$ and DMBQ in wild type, and a similar result was obtained when *psbQ* had been inactivated in the ΔPsbO and ΔPsbU strains (data not shown). Under these conditions the ΔPsbV mutant evolved oxygen at 58% of the wild-type rate; however, the additional removal of PsbQ lowered the rate further to 32% (Figure 3B). Oxygen evolution for the ΔPsbV:ΔPsbQ mutant was also readily photoinactivated and was zero when the actinic light was increased to 6.5 mE m^{-2} s^{-1} (data not shown). A

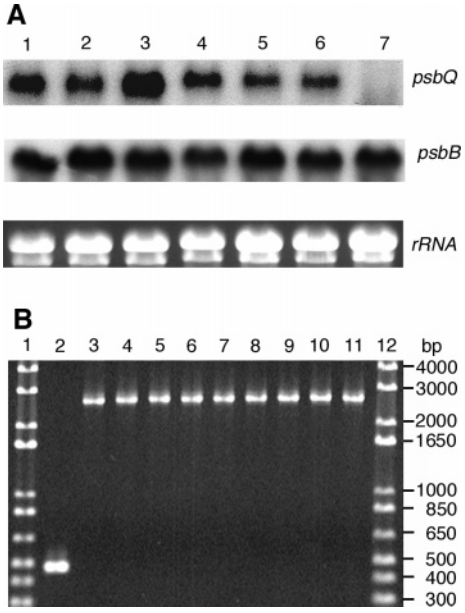

FIGURE 2: Expression of *psbQ* in wild-type *Synechocystis* sp. PCC 6803 and PCR verification of segregation of interrupted sll1638 in the different strains. (A) Northern blot showing expression of *psbQ* in wild type under different growth conditions: lane 1, unbuffered BG-11; lane 2, Ca^{2+}-limiting medium; lane 3, Cl^--limiting medium; lane 4, iron-limiting medium; lane 5, BG-11 at pH 7.5; lane 6, BG-11 at pH 10.0; and lane 7, RNA from the ΔPsbQ mutant in unbuffered BG-11. (B) PCR confirmation of the segregation of interrupted sll1638 in the different strains. The sll1638 gene was interrupted in the ΔPsbO, ΔPsbU, and ΔPsbV mutants, previously described in ref *28*, and the CP47 mutants E364Q, E364Q:ΔPsbV, Δ(R384−V392), and Δ(R384−V392):ΔPsbV strains described in ref *20*. In addition, the triple mutant ΔPsbO:ΔPsbU:ΔPsbQ was constructed. The wild-type and mutant strains were transformed with a plasmid in which sll1638 had been interrupted by either a 2.0 kb spectinomycin-resistance cassette ($Spec^R$) or a 2.0 kb chloramphenicol-resistance cassette (Cam^R) as described in the Materials and Methods. Lanes: 1, 1 kb ladder supplied by Invitrogen; 2, wild type; 3, ΔPsbQ (Cam^R); 4, ΔPsbO:ΔPsbQ (Cam^R); 5, ΔPsbU:ΔPsbQ (Cam^R); 6, ΔPsbV:ΔPsbQ (Cam^R); 7, ΔPsbO:ΔPsbU:ΔPsbQ ($Spec^R$); 8, CP47:E364Q:ΔPsbQ (Cam^R); 9, CP47:E364Q:ΔPsbV:ΔPsbQ (Cam^R); 10, CP47:Δ(R384−V392):ΔPsbQ (Cam^R); 11, CP47:Δ(R384−V392):ΔPsbV:ΔPsbQ ($Spec^R$); 12, 1 kb ladder supplied by Invitrogen.

Table 1: Photoautotrophic Doubling Time of *Synechocystis* sp. PCC 6803 Strains Lacking PsbQ and (in Parentheses) in the Presence of PsbQ

	media			
strain	BG-11	BG-11 calcium limiting	BG-11 chloride limiting	BG-11 iron limiting
ΔPsbQ	14 (13)	15 (13)	17 (15)	17 (16)
ΔPsbO:ΔPsbQ	26 (21)	ng[b] (ng)	32[a] (22)	25[a] (17)[a]
ΔPsbU:ΔPsbQ	17 (13)	50[a] (20)	18[a] (18)	18[a] (18)
ΔPsbV:ΔPsbQ	ng (17)	ng (ng)	ng (ng)	ng (20)

[a] The estimated doubling time used for this calculation was based on initial growth (see Figure 4). [b] ng = no growth.

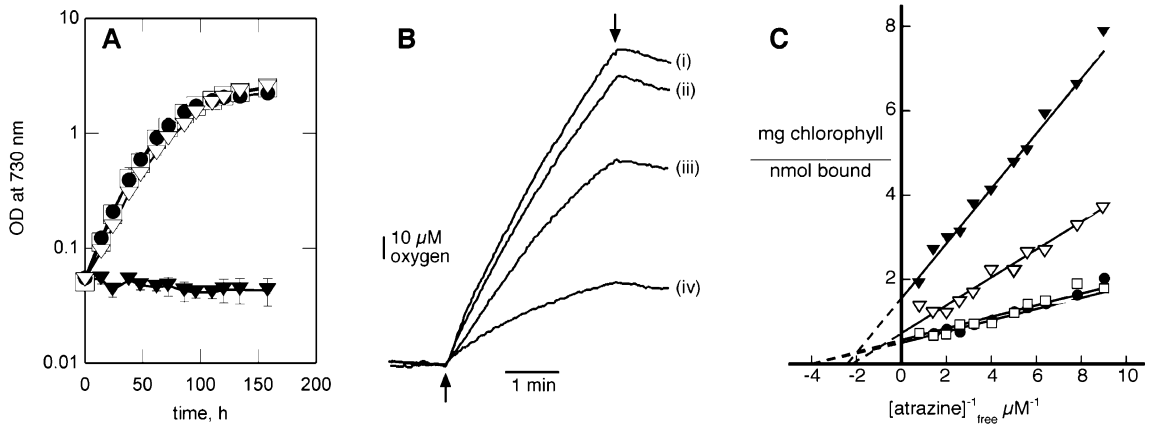

FIGURE 3: Characterization of *Synechocystis* sp. PCC 6803 strains lacking PsbQ. (A) Photoautotrophic growth in BG-11 medium as measured by the optical density at 730 nm. The strains shown are wild type (closed circles), ΔPsbV (open inverted triangles), ΔPsbQ (open squares), and ΔPsbV:ΔPsbQ (closed inverted triangles). The data are the average ± standard error of three to five independent experiments. Error bars not visible are smaller than the symbols. (B) Traces of oxygen evolution determined polargraphically in the presence of $K_3Fe(CN)_6$ and DMBQ. The reaction chamber held cell suspensions containing 10 μg of chlorophyll/mL. Key: wild type (i), ΔPsbQ (ii), ΔPsbV (iii), and ΔPsbV:ΔPsbQ (iv). The corresponding rates of oxygen evolution for the different strains in μmol of O_2 (mg of chlorophyll)$^{-1}$ h^{-1} were as follows: wild type, 283; ΔPsbQ, 249; ΔPsbV, 163; and ΔPsbV:ΔPsbQ, 90. The arrows indicate when the light was turned on and off. (C) Atrazine-binding assays for the strains shown in (B). Key: wild type (closed circles), ΔPsbQ (open squares), ΔPsbV (inverted open triangles), and ΔPsbV:ΔPsbQ (inverted closed triangles). The relative number of chlorophylls per PSII were as follows: wild type, 560; ΔPsbQ, 640; ΔPsbV, 830; and ΔPsbV:ΔPsbQ, 1730. The measurements shown for the strains presented in (B) and (C) were reproduced in two additional independent experiments with similar results.

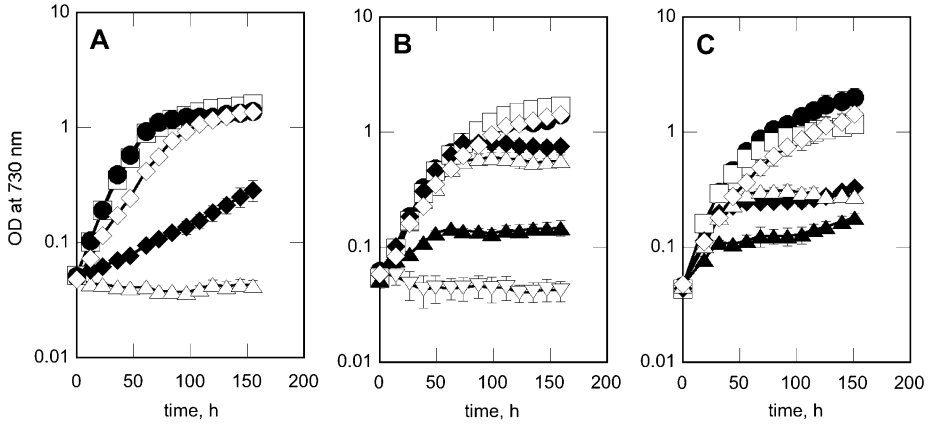

FIGURE 4: Photoautotrophic growth of *Synechocystis* sp. PCC 6803 strains in nutrient-limiting BG-11 media as measured by the optical density at 730 nm. (A) Ca^{2+}-limiting BG-11 medium: wild type (closed circles), ΔPsbO (open triangles), ΔPsbU (open diamonds), ΔPsbQ (open squares), and ΔPsbU:ΔPsbQ (closed diamonds). (B) Cl^--limiting BG-11 medium: wild type (closed circles), ΔPsbO (open triangles), ΔPsbU (open diamonds), ΔPsbV (open inverted triangles), ΔPsbQ (open squares), ΔPsbO:ΔPsbQ (closed triangles), and ΔPsbU:ΔPsbQ (closed diamonds). (C) Iron-limiting BG-11 medium: wild type (closed circles), ΔPsbO (open triangles), ΔPsbU (open diamonds), ΔPsbQ (open squares), ΔPsbO:ΔPsbQ (closed triangles), and ΔPsbU:ΔPsbQ (closed diamonds). In (A)−(C) the data are the average ± the standard error of three to five independent experiments. Error bars not visible are smaller than the symbols.

similar trend was observed when electron transport was measured in the presence of HCO_3^-. Oxygen evolution ranged between 88% and 91% of the wild-type rate in the ΔPsbQ, ΔPsbO:ΔPsbQ, and ΔPsbU:ΔPsbQ mutants; however, while the rate for the ΔPsbV mutant was 95% of the wild type, oxygen evolution for the ΔPsbV:ΔPsbQ strain was reduced to 47% (data not shown).

To assess PSII assembly in the absence of PsbQ, DCMU-replaceable [^{14}C]atrazine binding was performed in whole cells. These data, shown in Figure 3C, indicated that PSII assembled in the ΔPsbQ strain at a similar level as wild type; however, the ΔPsbV:ΔPsbQ strain was found to have assembled centers at ∼32% of wild type whereas the level was 67% of wild type in the ΔPsbV strain. These data were in agreement with the PSII-specific oxygen evolution observed for each of the strains (Figure 3B). However, as previously noted (*50*), oxygen evolution in the presence of HCO_3^- did not correlate with the relative levels of PSII. Additionally the ΔPsbO:ΔPsbQ and ΔPsbU:ΔPsbQ strains exhibited a decrease in assembled PSII centers at ∼46% and 61% of wild type, compared to levels of ∼67% and 100% for the ΔPsbO and ΔPsbU strains, respectively (data not shown).

Effect of Removing PsbQ in Nutrient-Limiting Conditions. In both plant and cyanobacterial PSII it has been reported that absence of the extrinsic proteins alters the requirement for both Ca^{2+} and Cl^-. Therefore, photoautotrophic growth of strains lacking PsbQ was examined in Ca^{2+}-limiting and Cl^--limiting media. The ΔPsbO and ΔPsbV mutants are not photoautotrophic in Ca^{2+}-limiting media, and Ca^{2+}-limiting conditions were verified in Figure 4A by the inclusion of the ΔPsbO strain (*17*, *20*, *21*, *51*). For wild type and the ΔPsbQ strain these conditions resulted in both photoautotrophic growth curves entering a stationary phase at an

OD_{730nm} of ~1.1 at ~80 h. Additionally, the removal of PsbQ in the ΔPsbU strain extended the photoautotrophic doubling time from 20 to 50 h (Table 1; cf. ref *26*).

Photoautotrophic growth in Cl^--limiting conditions resulted in similar phenotypes for the wild type and the ΔPsbQ strain where these exhibited little difference in doubling time (Table 1). Removal of PsbQ had a large effect in the case of the ΔPsbO:ΔPsbQ mutant. In Figure 4B photoautotrophic growth of the ΔPsbO:ΔPsbQ strain reached a plateau at an OD_{730nm} of ~0.14 after ~60 h of growth, whereas the ΔPsbO strain grew to an OD_{730nm} of ~0.6. The initial photoautotrophic doubling time of the ΔPsbO strain was also extended by 10 h on removal of PsbQ (Table 1). The observation of photoautotrophic growth in the ΔPsbO mutant in Cl^--limiting media is in agreement with the data in ref *17*; however, in other studies Cl^--limiting media did not support photoautotrophic growth in this strain (*20*). Moreover, in Cl^--limiting media, Engels et al. (*51*) observed photoautotrophic growth in their ΔPsbO mutant from *Synechococcus* sp. PCC 7942 but not in their ΔPsbO mutant from *Synechocystis* sp. PCC 6803. These observations suggest that the photoautotrophic growth of ΔPsbO cells in Cl^--limiting media is dependent on as yet unspecified growth conditions. The Cl^--limiting media produced a slight slowing of photoautotrophic growth in the ΔPsbU strain from ~13 h in Cl^--sufficient media to ~18 h in Figure 4B (see also ref *26*). Under Cl^--limiting conditions a similar doubling time was observed for the ΔPsbU and ΔPsbU:ΔPsbQ mutants as shown in Table 1 although the ΔPsbU strain reached an OD_{730nm} of ~1.4 while the ΔPsbU:ΔPsbQ strain reached a plateau at an the OD_{730nm} of ~0.8 at ~70 h. Strains lacking cytochrome c_{550} are not photoautotrophic in Cl^--limiting media (*20*, *21*), and the conditions in Figure 4B prevented the ΔPsbV strain from undergoing photoautotrophic growth.

In cyanobacteria iron deficiency results in a number of morphological and physiological changes (*52*). Microarray data have also indicated that the expression of the genes encoding PsbO, PsbU, and PsbV is downregulated in iron-deficient conditions in *Synechocystis* sp. PCC 6803 (*53*). We examined the photoautotrophic growth of the ΔPsbQ strain and the ΔPsbO:ΔPsbQ and ΔPsbU:ΔPsbQ double mutants in iron-limiting media to test the possibility that PsbQ may be important under stress conditions where the extrinsic proteins might be depleted. Under the iron-limiting conditions used in Figure 4C removal of PsbQ from the wild type and the ΔPsbU strain had little effect on the initial doubling times. However, the doubling time of the ΔPsbO:ΔPsbQ mutant was 8 h longer than observed for the ΔPsbO strain (Table 1). Furthermore, the photoautotrophic growth of the ΔPsbO:ΔPsbQ mutant reached a plateau at an OD_{730nm} of <0.2 compared to ~0.3 for the ΔPsbO strain, and the ΔPsbU:ΔPsbQ strain reached an OD_{730nm} of ~0.3, whereas the ΔPsbU strain in Figure 4C reached an OD_{730nm} of >1.0.

Natural populations of cyanobacteria occupy environments that may be simultaneously limiting for more than one nutrient. The effect of combining more than one nutrient-limiting condition on photoautotrophic growth was therefore investigated. When grown under conditions where two or more nutrients were limiting, the wild-type cultures were still able to reach an OD_{730nm} of ~1.0. In BG-11 medium without Ca^{2+} and Cl^-, growth was slower for the ΔPsbQ strain than for wild type, where the doubling times were ~34 and ~26 h, respectively, and there was no growth of the ΔPsbU mutant. Where the medium was limiting for Ca^{2+} and iron, the ΔPsbQ strain and wild type had similar doubling times of ~36 h, whereas the growth of the ΔPsbU strain decreased, reaching an OD_{730nm} of ~0.3, and ΔPsbU:ΔPsbQ cells were not photoautotrophic. When Cl^--limiting and iron-limiting conditions were combined, the ΔPsbQ strain had a photoautotrophic doubling time that was increased by ~12 h compared to wild type and reached an OD_{730nm} of ~0.5. Under these conditions the ΔPsbU and ΔPsbU:ΔPsbQ strains grew to an OD_{730nm} of ~0.14. In addition, Figure 5 shows the effect of simultaneously limiting Ca^{2+}, Cl^-, and iron: under this condition there was no photoautotrophic growth of the ΔPsbQ strain. However, the addition of DCMU and glucose to ΔPsbQ cells resulted in photoheterotrophic growth rates similar to that observed for the wild type in this media, thereby showing that the lack of photoautotrophy resulted from impaired PSII activity.

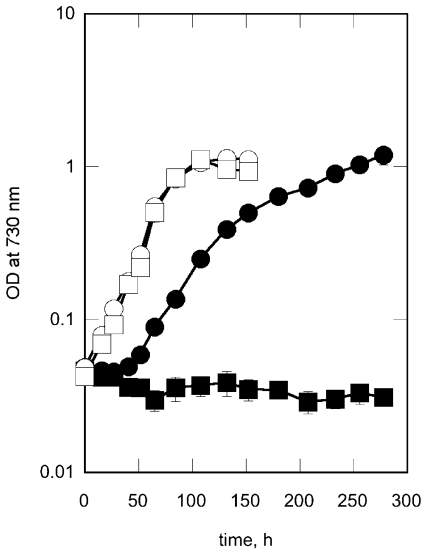

FIGURE 5: Growth of *Synechocystis* sp. PCC 6803 strains as measured by the optical density at 730 nm in BG-11 medium that was limiting for Ca^{2+}, Cl^-, and iron. Photoautotrophic growth (closed symbols) and photoheterotrophic growth in the presence 20 μM DCMU and 5 mM glucose (open symbols): wild type (circles) and ΔPsbQ (squares). The data are the average ± standard error of three to five independent experiments. Error bars not visible are smaller than the symbols.

Effect of Removing PsbQ on Growth at Elevated Temperature. The PsbO, PsbU, and PsbV proteins have been shown to be required for thermal stability and the acquisition of thermotolerance in *Synechococcus* sp. PCC 7002, and similar results have been obtained in *Synechocystis* sp. PCC 6803 (*28*, *54*−*56*). Therefore, we examined whether the PsbQ protein had a role in thermal stability and acclimation. At 40 °C the wild type and the ΔPsbU, ΔPsbQ, and ΔPsbU:ΔPsbQ strains remained photoautotrophic (Figure 6A) although the ΔPsbQ strain reached an OD_{730nm} of ~2.3 compared to the wild type which grew to an OD_{730nm} of ~2.8. However, as described in ref *28*, no photoautotrophic growth was observed for the ΔPsbO and ΔPsbV strains. At 41 °C (Figure 6B) cells lacking PsbQ grew to an OD_{730nm} of ~1.2 whereas wild-type cells grew to an OD_{730nm} of ~2.1. Additionally, the ΔPsbU strain reached an OD_{730nm} of ~0.4 and the ΔPsbU:ΔPsbQ strain exhibited little growth, reaching an OD_{730nm} of ~0.1, and rapidly bleached. Hence the thermal

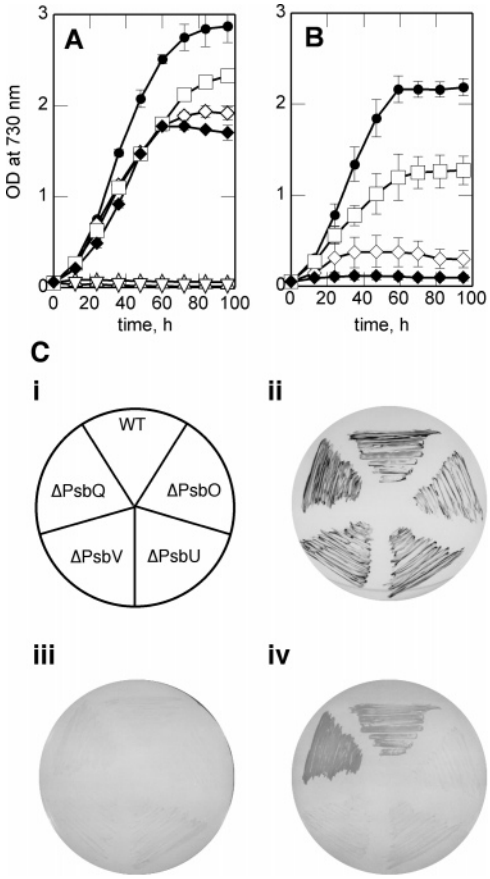

FIGURE 6: Effect of temperature on photoautotrophic growth of *Synechocystis* sp. PCC 6803 strains. (A) Photoautotrophic growth for different strains as measured by the optical density at 730 nm in BG-11 medium at 40 °C: wild type (closed circles), ΔPsbO (open triangles), ΔPsbU (open diamonds), ΔPsbV (open inverted triangles), ΔPsbQ (open squares), and ΔPsbU:ΔPsbQ (closed diamonds). (B) Photoautotrophic growth at 41 °C. Strains as in (A) but the ΔPsbO and ΔPsbV mutants have been omitted as these were not photoautotrophic at either 40 or 41 °C. (C) Heat treatment of the wild type, ΔPsbO, ΔPsbU, ΔPsbV, and ΔPsbQ strains: (i) position of strains on BG-11 plate; (ii) growth of strains at 30 °C after 7 days; (iii) growth following transfer to 43 °C for 2 days, and (iv) growth following transfer to 38 °C for 2 days and then 43 °C for 2 days. In (A) and (B) the data are the average ± standard error of three to five independent experiments. Error bars not visible are smaller than the symbols. In (C) four independent experiments were performed with similar results.

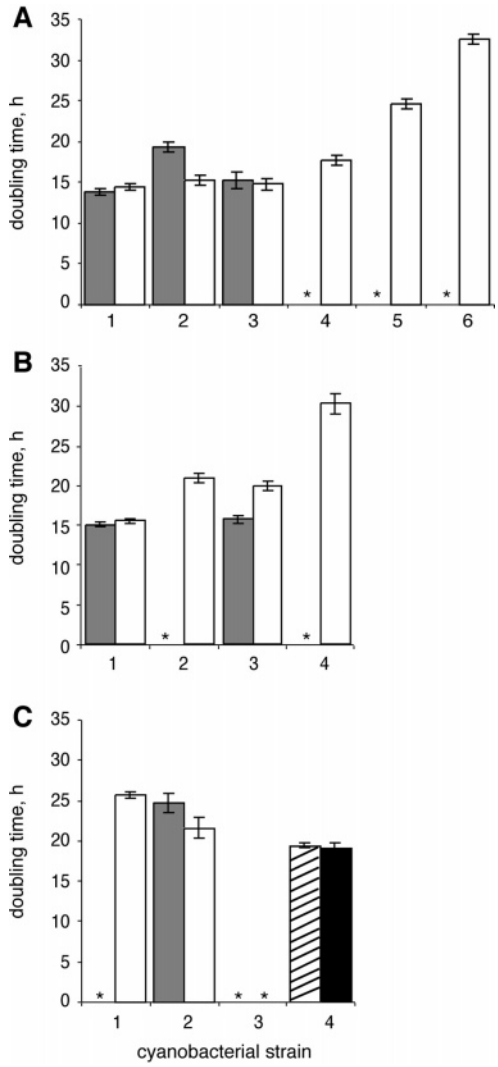

FIGURE 7: Histograms showing doubling time of *Synechocystis* sp. PCC 6803 strains in BG-11 medium. Gray bars indicate growth in BG-11 medium at pH 7.5; open bars indicate growth in BG-11 medium at pH 10.0. (A) Photoautotrophic growth of strains: 1, wild type; 2, ΔPsbV; 3, ΔPsbQ; 4, ΔPsbV:ΔPsbQ; 5, ΔPsbO:ΔPsbU; and 6, ΔPsbO:ΔPsbU:ΔPsbQ. (B) Photoautotrophic growth of strains: 1, control; 2, E364Q:ΔPsbV; 3, E364Q:ΔPsbQ; and 4, E364Q:ΔPsbV:ΔPsbQ. (C) Photoautotrophic growth of strains: 1, Δ(R384−V392):ΔPsbV; 2, Δ(R384−V392):ΔPsbQ; 3, Δ(R384−V392):ΔPsbV:ΔPsbQ; and photoheterotrophic growth in unbuffered BG-11 medium of wild type (hatched bar) and Δ(R384−V392):ΔPsbV:ΔPsbQ (black bar). No doubling times were estimated for strains with an OD_{730nm} of less than 0.1; these strains are indicated by an asterisk. All strains for which a doubling time was calculated had reached a minimum OD_{730nm} of 0.9 by 150 h. The control strain was described in ref *66* and has a phenotype that is indistinguishable from wild type. The data are the average of three to five independent experiments, and standard error bars are shown.

stability in the ΔPsbQ strain, and to a lesser extent the ΔPsbU:ΔPsbQ strain, was reduced by the removal of PsbQ.

Acquisition of thermotolerance does not occur in the ΔPsbO and ΔPsbV mutants and is diminished in the ΔPsbU strain of *Synechocystis* sp. PCC 6803 (*28*). Since at elevated temperature the ΔPsbQ cells grew less well than wild type, we tested the effect of the removal of PsbQ on cellular thermotolerance. Wild type and the ΔPsbO, ΔPsbU, ΔPsbV, and ΔPsbQ strains were grown photoautotrophically at 30 °C on BG-11 plates and then transferred to 43 °C. This resulted in all strains bleaching within 2 days as shown in Figure 6Ciii. Incubation at the intermediate temperature of 38 °C for 2 days prior to the 43 °C treatment resulted in death of the ΔPsbO and ΔPsbV strains and little or no growth of the ΔPsbU cells as reported (*28*). However, the wild type and the ΔPsbQ mutant were able to survive at 43 °C following acclimation at 38 °C (Figure 6Civ), and therefore, these data indicate that PsbQ is not required for acquisition of cellular thermotolerance.

Effect of PsbQ in BG-11 at pH 7.5 or 10.0. Photoautotrophic growth has been found to be pH dependent in certain PSII mutants which are not photoautotrophic in unbuffered BG-11 or in BG-11 at pH 7.5; however, photoautotrophic growth was restored when grown at pH 10.0 (*50*). To investigate if removal of PsbQ was associated with this PSII-specific effect, we examined photoautotrophic growth in the ΔPsbV:ΔPsbQ strain at these pH values. In Figure 7 the

Table 2: Relative Rates of Oxygen Evolution and PSII Assembly at pH 7.5 and 10.0 in *Synechocystis* sp. PCC 6803 Strains Lacking PsbQ[a]

	pH 7.5			pH 10.0		
	rate of oxygen evolution[b]		relative	rate of oxygen evolution[b]		relative
	HCO_3^- [d]	$K_3Fe(CN)_6$ [e]	level of PSII[c]	HCO_3^- [d]	$K_3Fe(CN)_6$ [e]	level of PSII[c]
wild type	1.00	1.00	1.00	1.00	1.00	1.00
ΔPsbV	0.87	0.33	0.44	0.77	0.66	0.56
ΔPsbQ	0.71	0.60	0.80	0.78	0.94	0.91
ΔPsbV:ΔPsbQ	0.58	0.26	0.24	0.63	0.45	0.37
ΔPsbO:ΔPsbU	0.59	0.34	0.52	0.73	0.38	0.99
ΔPsbO:ΔPsbU:ΔPsbQ	0.54	0.36	0.40	0.68	0.39	0.64

[a] These data represent the average of at least three independent measurements and were reproducible to within 15% of the average. [b] The rate of oxygen evolution was stable for at least 2 min of illumination. [c] The values for PSII abundance are normalized to a chlorophyll/PSII ratio of 460 at pH 7.5 and 480 at pH 10.0 and have been determined by [^{14}C]atrazine binding. [d] Oxygen evolution supported by HCO_3^- was normalized to wild-type rates of 387 μmol of O_2 (mg of chlorophyll)$^{-1}$ h^{-1} at pH 7.5 and 371 μmol of O_2 (mg of chlorophyll)$^{-1}$ h^{-1} at pH 10.0. [e] Oxygen evolution supported by $K_3Fe(CN)_6$ and DMBQ was normalized to wild-type rates of 394 μmol of O_2 (mg of chlorophyll)$^{-1}$ h^{-1} at pH 7.5 and 407 μmol of O_2 (mg of chlorophyll)$^{-1}$ h^{-1} at pH 10.0.

doubling time has been shown for strains grown at pH 7.5 and 10.0. At pH 7.5 photoautotrophic growth was observed for the wild-type, ΔPsbV, and ΔPsbQ strains but not the ΔPsbV:ΔPsbQ mutant (Figure 7A), whereas at pH 10.0 photoautotrophic growth of the ΔPsbV:ΔPsbQ strain was restored and exhibited a doubling time of ~18 h, while that of the wild type was ~14 h at this pH. The effect of removing PsbQ from the pH-sensitive mutant lacking PsbO and PsbU was also investigated since a ΔPsbO:ΔPsbU strain had previously been reported to have limited growth at pH 7.5, reaching an OD_{730nm} of <0.1, while stable photoautotrophic growth was restored at pH 10.0 (*50*). The ΔPsbO:ΔPsbU:ΔPsbQ mutant showed no growth at pH 7.5, and although it grew at pH 10.0, the observed photoautotrophic doubling time was extended by ~8 h when compared to the ΔPsbO:ΔPsbU strain (Figure 7A).

To obtain additional information on the effect of removing PsbQ on PSII activity and assembly at pH 7.5 and 10.0, oxygen evolution and PSII abundance were measured (Table 2). At both pH 7.5 and pH 10.0 the oxygen evolution rates observed for the ΔPsbV:ΔPsbQ mutant were lower than for either of the ΔPsbV and ΔPsbQ strains, thus indicating a specific effect on PSII activity following the removal of PsbQ when PsbV is already absent. Also, at both pH values and in both PSII-specific and whole-chain assays, the observed rates of oxygen evolution for the ΔPsbV:ΔPsbQ and the ΔPsbO:ΔPsbU strains were similar despite ~2-fold fewer centers being detected for the ΔPsbV:ΔPsbQ mutant at pH 7.5. In addition, the recovery of photoautotrophic growth at pH 10.0 in these strains was accompanied by an increase in assembled PSII centers. Furthermore, a PSII-specific effect was apparent when PsbQ was absent from the ΔPsbO:ΔPsbU strain. The effect at pH 7.5 was less than observed when PsbQ was removed from the strain lacking PsbV; however, at pH 10.0 there were a decreased number of centers detected by the herbicide-binding assay in the triple-deletion mutant.

The PSII-specific effects arising from the inactivation of *psbQ* were further investigated in strains where *psbV* had been deleted and which carry mutations in loop E of CP47. Each of these strains required alkaline pH for photoautotrophic growth (*50*). The photoautotrophic doubling times presented in Figure 7B for the E364Q:ΔPsbQ and E364Q:ΔPsbV:ΔPsbQ strains demonstrate that the pH-recoverable phenotype of E364Q:ΔPsbV cells remained after the removal of PsbQ. In contrast, Figure 7C shows that the pH-recoverable phenotype of the Δ(R384−V392):ΔPsbV strain, which carries a deletion between Arg-384 and Val-392 in loop E of CP47, has been abolished by removing PsbQ.

The effect on oxygen evolution and the relative levels of PSII following the removal of PsbQ in strains where mutations in CP47 are combined with the removal of PsbV has been summarized in Table 3. The removal of PsbQ in the E364Q:ΔPsbV mutant resulted in reduced rates of oxygen evolution, particularly at pH 7.5, despite only having a moderate effect on the number of PSII centers detected. In contrast, the strain E364Q:ΔPsbQ had a similar number of PSII centers to the corresponding control strain at both pH 7.5 and 10.0 and similar oxygen evolution rates. In the case of the Δ(R384−V392):ΔPsbV strain the removal of PsbQ resulted in an almost complete loss of oxygen evolution; however, considerable rates of oxygen evolution were detected in the ΔPsbV:ΔPsbQ mutant at pH 7.5 which was determined to have a comparable number of assembled PSII centers in Table 2. The low rate of oxygen evolution observed in the Δ(R384−V392):ΔPsbV:ΔPsbQ mutant again indicates a direct effect on PSII activity upon inactivation of *psbQ*. Moreover, this conclusion is strengthened by the similarity of the photoheterotrophic doubling times for wild type and the Δ(R384−V392):ΔPsbV:ΔPsbQ mutant (Figure 7C).

DISCUSSION

Structural analysis of PsbQ from spinach has shown the core of the protein to be a four-helix bundle while the N-terminal domain, which is required for binding to the PSII complex, was disordered (*47*, *48*, *57*). Variation between the amino acid sequence of *Synechocystis* sp. PCC 6803 and plant PsbQ sequences was most evident at the N-termini of these proteins, and green algal and plant PsbQ sequences also exhibited variation in this domain (*48*). This may reflect different binding properties, since the association of PsbQ with PSII differed between higher plants and green algae (*57*, *58*). In spinach both PsbO and PsbP were required for binding PsbQ, although PsbO has been partially extracted, leaving PsbP and PsbQ in situ (*59*, *60*). In contrast, PsbP was not required for the reconstitution of PsbQ in green algae (*58*). In the case of cyanobacteria the binding requirement of the PsbQ protein would be expected to differ to plants or green algae due to the presence of PsbU and PsbV. The red alga *C. caldarium* contains PsbU and PsbV in addition to a

Table 3: Relative Rates of Oxygen Evolution and PSII Assembly at pH 7.5 and 10.0 in *Synechocystis* sp. PCC 6803 Strains Lacking PsbQ and Carrying Mutations in Loop E of CP47[a]

	pH 7.5			pH 10.0		
	rate of oxygen evolution[b]		relative	rate of oxygen evolution[b]		relative
	HCO_3^- [d]	$K_3Fe(CN)_6$[e]	level of PSII[c]	HCO_3^- [d]	$K_3Fe(CN)_6$[e]	level of PSII[c]
control	1.00	1.00	1.00	1.00	1.00	1.00
E364Q:ΔPsbV	0.62	0.33	0.35[f]	0.79	0.45	0.58[f]
E364Q:ΔPsbQ	0.97	1.02	1.00	0.94	1.06	0.89
E364Q:ΔPsbV:ΔPsbQ	0.32	0.13	0.26	0.70	0.33	0.46
Δ(R384−V392):ΔPsbV	0.29	0.17	0.37[f]	0.51	0.19	0.37[f]
Δ(R384−V392):ΔPsbQ	0.50	0.40	0.50	0.81	0.37	0.60
Δ(R384−V392):ΔPsbV:ΔPsbQ	0.09	0.04	0.22	0.17	0.04	0.26

[a] These data represent the average of at least three independent measurements and were reproducible to within 15% of the average. [b] The rate of oxygen evolution was stable for at least 2 min of illumination. [c] The values for PSII abundance are normalized to a chlorophyll/PSII ratio of 460 at pH 7.5 and 450 at pH 10.0 and have been determined by [^{14}C]atrazine binding. [d] Oxygen evolution supported by HCO_3^- was normalized to control rates of 393 μmol of O_2 (mg of chlorophyll)$^{-1}$ h^{-1} at pH 7.5 and 343 μmol of O_2 (mg of chlorophyll)$^{-1}$ h^{-1} at pH 10.0. [e] Oxygen evolution supported by $K_3Fe(CN)_6$ and DMBQ was normalized to control rates of 476 μmol of O_2 (mg of chlorophyll)$^{-1}$ h^{-1} at pH 7.5 and 439 μmol of O_2 (mg of chlorophyll)$^{-1}$ h^{-1} at pH 10.0. [f] Data from ref *50*; independent confirmation of these values was obtained in this experiment (data not shown).

PsbQ-like protein (PsbQ′). Association of PsbQ′ with PSII also required the presence of PsbO, and PsbQ′ may facilitate the binding of PsbU and PsbV rather than directly contributing to oxygen evolution (*34*). However, the N-terminal sequences of PsbQ′ and Sll1638 exhibit little sequence similarity, and their overall identity is low (*35*); therefore, an identical role for these proteins cannot be assumed despite the presence of PsbU and PsbV in both taxonomic groups.

In Figure 1B PsbQ from the thermophilic cyanobacterium *T. elongatus* exhibited the most sequence variation from PsbQ of *Synechocystis* sp. PCC 6803 and was not detected in either PSII crystal structure from this organism (*5*, *7*). However, the PsbQ protein was present at a level of one per PSII complex in *Synechocystis* sp. PCC 6803, and it has been reported that oxygen evolution rates decreased in $CaCl_2$-depleted conditions in a ΔPsbQ strain (*10*, *11*). One possibility for the absence of PsbQ in the crystal structure is that it is only associated with PSII under specific environmental conditions and that it is not required under the optimized conditions for crystallization. To examine this, we also considered the role of PsbQ in Ca^{2+}- and Cl^--limiting media since PsbQ has been implicated in optimizing the requirement for these PSII cofactors in green algae and plants (*14*). Iron-limiting conditions were also considered.

The approach taken for these experiments utilized the construction of double (or triple) gene deletions in a single cyanobacterial strain. Since it has been proposed that PsbU and PsbV play a role similar to that of PsbP and PsbQ in green algae and plants, evaluating the effect of removing PsbQ in the ΔPsbU and ΔPsbV strains targeted the ability of this polypeptide to substitute for either of these subunits. Importantly, photoautotrophic growth was prevented in the ΔPsbV:ΔPsbQ mutant. The dependence of the ΔPsbU:ΔPsbQ and ΔPsbO:ΔPsbQ mutants on Ca^{2+}, Cl^-, and iron, as documented in Figure 4, prompted investigation of photoautotrophic growth of the ΔPsbQ strain in media where these nutrients were all limiting. This revealed that the ΔPsbQ mutant was unable to grow under these conditions whereas wild type remained photoautotrophic (Figure 5). This effect was shown to be specific to PSII by the addition of glucose, and the PSII-specific inhibitor DCMU, as these conditions resulted in photoheterotrophic growth of the ΔPsbQ mutant that was indistinguishable from that of wild type. A PSII-specific role was also evident when we tested the effect of removing PsbQ at elevated growth temperatures. Although thermal acclimation was unaffected, our data were consistent with decreased thermal stability of PSII.

The phenotype of the ΔPsbV:ΔPsbQ strain was also consistent with PsbQ being important for photosystem stability. In unbuffered BG-11 media the ΔPsbV:ΔPsbQ strain assembled PSII centers at ∼30% of the level seen in wild type. Oxygen evolution from these cells was sensitive to photoinactivation and was fully inactivated under saturating light. This phenotype was similar to that of a previously constructed mutant lacking PsbO and PsbU (*50*). This mutant and the ΔPsbO:ΔPsbU strain constructed for the present study were unable to support photoautotrophic growth in unbuffered BG-11 or in media at pH 7.5; however, photoautotrophic growth was restored to both at pH 10.0. In Figure 7A the ΔPsbV:ΔPsbQ strain was also photoautotrophic at pH 10.0. The molecular mechanism for this pH dependence has yet to be identified; however, in the E364Q and Δ(R384−V392) mutants studied here, a pH-dependent phenotype was also observed when PsbV was removed (*50*, *61*, *62*). As additional verification of a functional role for PsbQ in oxygen evolution, we investigated the impact of the removal of PsbQ in pH-dependent strains. In particular, oxygen evolution was essentially eliminated in Δ(R384−V392):ΔPsbV:ΔPsbQ cells, and photoautotrophic growth was not recovered at pH 10.0. However, the Δ(R384−V392):ΔPsbV:ΔPsbQ mutant was found to grow with a photoheterotrophic doubling time similar to that observed for wild type. The deletion between Arg-384 and Val-392 has been shown to alter binding of PsbO and lower the quantum yield of photoactivation of the OEC (*63*, *64*). Therefore, these data indicate that the removal of PsbQ has further altered the stability of PSII in this strain.

The genome of *Synechocystis* sp. PCC 6803 also contains a gene (sll1418) predicted to encode a PsbP-like protein. We inactivated this gene and constructed double gene-interruption mutants similar to the ΔPsbQ strains reported here. In contrast to the mutants lacking PsbQ, the ΔPsbP strains retained a phenotype similar to that observed in the presence of PsbP under the experimental conditions investigated in this study. Furthermore, a lower level of expression of *psbP* compared to *psbQ* was detected by northern hybridization

(T. Summerfield and J. Eaton-Rye, unpublished results). These data suggest a different role for the PsbP protein to that of the PSII-specific requirement for the PsbQ protein in *Synechocystis* sp. PCC 6803 and are consistent with the estimate that PsbP was associated with only 3% of PSII centers (*11*).

In conclusion, the inability of the ΔPsbV:ΔPsbQ strain to grow photoautotrophically and its susceptibility to photoinactivation indicated that PsbQ was associated with PSII in *Synechocystis* sp. PCC 6803. Furthermore, photoautotrophic growth of this mutant could be recovered when grown at alkaline pH. These results were obtained in nutrient-replete media, suggesting that PsbQ is important for PSII stability and is not solely associated with a $CaCl_2$ requirement. This conclusion is supported by the impaired growth of the ΔPsbQ strain at 41 °C and the inability of the Δ(R384−V392):ΔPsbV:ΔPsbQ mutant to exhibit restored photoautotrophic growth at pH 10.0 or for PSII assembly to be enhanced in this strain at this pH. Additionally, the results obtained in nutrient-limiting media revealed that the PsbQ protein is absolutely required for photoautotrophic growth in conditions that will be encountered in natural environments. This requirement was also found to be specific for PSII since photoheterotrophic growth under these conditions was indistinguishable from that of wild type.

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