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A Redox-Responsive Regulator of Photosynthesis Gene Expression in the Cyanobacterium *Synechocystis* sp. Strain PCC 6803

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We have identified genes in the unicellular cyanobacterium *Synechocystis* sp. strain PCC 6803 that are involved with redox control of photosynthesis and pigment-related genes. The genes, *rppA* (sll0797) and *rppB* (sll0798), represent a two-component regulatory system that controls the synthesis of photosystem II (PSII) and PSI genes, in addition to photopigment-related genes. *rppA* (regulator of photosynthesis- and photopigment-related gene expression) and *rppB* exhibit strong sequence similarity to prokaryotic response regulators and histidine kinases, respectively. In the wild type, the steady-state mRNA levels of PSII reaction center genes increased when the plastoquinone (PQ) pool was oxidized and decreased when the PQ pool was reduced, whereas transcription of the PSI reaction center genes was affected in an opposite fashion. Such results suggested that the redox poise of the PQ pool is critical for regulation of the photosystem reaction center genes. In Δ*rppA*, an insertion mutation of *rppA*, the PSII gene transcripts were highly up-regulated relative to the wild type under all redox conditions, whereas transcription of phycobilisome-related genes and PSI genes was decreased. The higher transcription of the *psbA* gene in Δ*rppA* was manifest by higher translation of the D1 protein and a concomitant increase in O_2 evolution. The results demonstrated that RppA is a regulator of photosynthesis- and photopigment-related gene expression, is involved in the establishment of the appropriate stoichiometry between the photosystems, and can sense changes in the PQ redox poise.

Bacteria are very adaptable organisms that can survive in a wide variety of environmental conditions. One way in which bacteria control their response to changing environmental conditions is through the mechanism of the two-component regulatory system, which consists of a sensor kinase and a response regulator (10, 26, 34). The sensor kinase has sensor and histidine phosphotransferase domains. The sensor domain recognizes the signal and autophosphorylates a histidine residue of the phosphotransferase domain. The phosphoryl group is subsequently transferred to the aspartate residue of the cognate response regulator, which is activated by the phosphoryl group (22). The output domain of a response regulator usually is a DNA-binding module, so that the regulator functions as a transcription factor (40). The Asp phosphorylation serves to control the ability to either bind its target DNA sequence or interact with other components of the transcription machinery (26). Some two-component systems utilize more than one histidine kinase protein or response regulator, and some single proteins encompassed both of the two-component elements (40, 41).

We have used unicellular cyanobacteria with high-frequency transformation systems as model organisms to study oxygenic photosynthesis. One such organism that has become a key model system is *Synechocystis* sp. strain PCC 6803 (54), which has been used for many mutagenesis, molecular biological, and biophysical studies. Most importantly, the entire genome of *Synechocystis* sp. strain PCC 6803 has been sequenced, and we can now utilize the rapidly expanding field of genomics to study the way in which this cyanobacterium controls photosynthesis and other metabolic processes (28). The genomic sequence reveals that *Synechocystis* sp. strain PCC 6803 has 80 genes that are potential two-component signal transducers within the total of 3,168 potential proteins. This includes 26 genes for sensory kinase proteins that contain both the transmitter and receiver domains (34).

In oxygenic photosynthetic organisms, light-induced charge separation is carried by two photosystems, I and II (PSII and PSI), which are major pigment-protein complexes in the thylakoid membrane (5). The D1 protein of PSII is a key element in oxygenic electron transport and in light acclimation processes. D1 and the related D2 protein form a heterodimer that bind all of the cofactors essential for the transfer of electrons from the water-splitting complex to the plastoquione (PQ) pool (5). In cyanobacteria, the phycobilisome (PBS) is the major light-harvesting, multiprotein complex and is attached extrinsically to the photosynthetic membrane (25).

It has been shown that genes encoding some photosynthesis proteins are under redox control in both cyanobacteria and higher plants (1, 2, 42). The redox status of the PQ pool has been implicated as a signal which regulates gene expression during long-term acclimation to light intensity. This hypothesis proposes that the signal transduction pathway is initiated via the PQ pool redox status or the excitation pressure on PSII, thereby coupling cellular regulatory pathways controlling gene expression and enzyme activation to utilize light energy (21). The redox state of the PQ pool seems to play a pivotal role in sensing cellular status and in regulating photosynthetic capacity. The signal of the PQ redox poise may be transduced through a redox-sensing protein kinase, which then activates the response regulator by phosphorylation or dephosphorylation. The activated regulator will then, either directly or indirectly, regulate the expression of the target genes (19).

We are particularly interested in the way in which photosynthesis and other metabolic processes are controlled by the redox poise of the PQ pool (15, 33). In photosynthetic bacteria, a two-component system called RegB-RegA (*Rhodobacter capsulatus*) or PrrB-PrrA (*R. sphaeroides*) had been demonstrated to be a global signal transduction system involved in the anaerobic induction of many physiological processes. These in-

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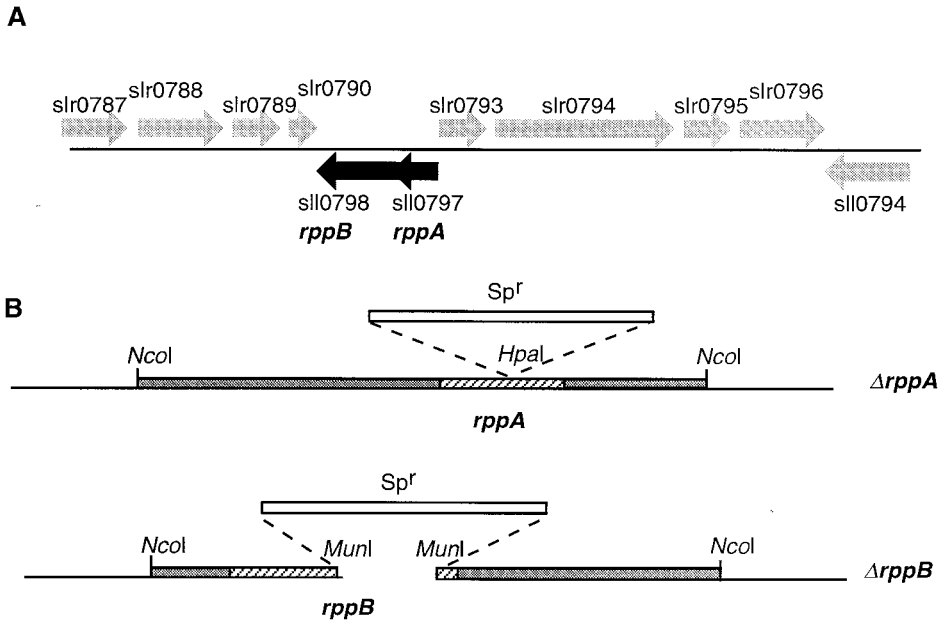

FIG. 1. Structure of the *rpp* region and mutations of *rppA* and *rppB*: genes of *Synechocystis* sp. strain PCC 6803. (A) Genomic organization of the response regulator gene *rppA* (locus sll0797) and the adjacent sensor kinase gene *rppB* (locus s110798). (B) Genetic construction of Δ*rppA* and Δ*rppB*. The *rppA* gene was interrupted by insertion of a spectinomycin resistance (Sp^r) cassette in the *Hpa*I site; the *rppB* gene was inactivated by replacement of a 789-bp *Mun*I-*Mun*I fragment with the spectinomycin resistance cassette.

clude the synthesis of the light-harvesting, reaction center, and cytochrome components of the bacterial photosystem and the assimilation of carbon dioxide and nitrogen (6, 18, 20, 38, 43, 49). The sensor RegB (PrrB) is believed to detect changes in oxygen levels, by responding to change in either the flow of reductant or a redox carrier, and then activates the response regulator RegA (PrrA) (39). Using sequence comparisons, we have identified genes in *Synechocystis* sp. strain PCC 6803 that are similar to the photosynthesis response regulator and kinase genes, *regA* (*prrA*) and *regB* (*prrB*). These genes were cloned, and knockout mutants were constructed by either insertion or deletion; all of the mutants grew under photoautotrophic conditions. These mutants were analyzed under a variety of environmental conditions that lead to changes in the PQ redox poise, including photoautotrophic (light-grown cells) and photomixotrophic (light plus 5 mM glucose) conditions. We have also used photosynthetic inhibitors, such as 3-(3,4-dichlorophenyl)-1,1-dimethyl-urea (DCMU) and 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone (DBMIB), to alter the PQ redox poise. Through this analysis, we found one of the two-component systems, *rppA* (regulator of photosynthesis- and photopigment-related genes) and *rppB*, to be most interesting. Here we report the effects of the *rppA* mutation on cellular growth, photosynthetic activity, the transcriptional regulation of photosynthesis- and photopigment-related gene expression and the accumulation of PSII reaction center proteins.

MATERIALS AND METHODS

Strains, culture conditions, and pigment analysis. *Synechocystis* sp. strain PCC 6803 wild type and mutants were cultured at 30°C, 40 microeinsteins (μE) m^{-2} s^{-1}, in a modified BG-11 medium with 5 mM $NaNO_3$ as a basal medium. This growth condition is referred to photoautotrophic or control. For photomixotrophic growth, 5 mM glucose was added to the basal medium. For different redox conditions, DCMU and DBMIB were added to the photoautotrophic growth culture at 10 μM (final concentration), and cells were treated for 6 h before harvesting. For different illumination experiments, cells were grown in low light (LL; 40 μE m^{-2} s^{-1}) until mid- to late log phase (5×10^7 to 10×10^7 cells ml^{-1}) and then transferred to the dark for 6 h, to medium light (ML; 200 μE m^{-2} s^{-1}) for 2 days, or to high light (HL; 1000 μE m^{-2} s^{-1}) for 3 h. When needed, the protein synthesis inhibitor chloramphenicol (50 μg ml^{-1}) was added to the media.

The cell density of the cultures was determined using a Coulter Counter (Coulter Electronics Inc., Hialeah, Fla.). Chlorophyll (Chl) and phycocyanin (PC) concentrations were quantified by spectrophotometry as previously described (16, 33).

Construction of *rppA* and *rppB* mutants. A 4.0-kb *Nco*I DNA fragment was cloned into plasmid pUC19 from cosmid CS1377 (a kind gift from Nakamura Yasukazu, Kazusa DNA Research Institute). The Δ*rppA* mutation was constructed by inserting a 2.0-kb spectinomycin resistance cassette (from plasmid pRL453) in the *Hpa*I site. *rppB* was inactivated by replacing a 789-bp *Mun*I fragment with the 2.0-kb spectinomycin resistance cassette (Fig. 1B). Wild-type *Synechocystis* sp. strain PCC 6803 was transformed with these plasmid constructs, and transformants were selected on plates containing the antibiotic spectinomycin (40 μg ml^{-1}). Segregation was confirmed by Southern blotting and PCR. Characterization of the mutant was repeated at least three times for each of the parameters measured.

Determination of oxygen evolution rate. Photosynthesis activity was determined by measuring O_2 production and consumption using a Clark electrode (model 5331; Yellow Springs Instruments, Yellow Springs, Ohio), with an amplifier in a cuvette thermostatted at 30°C. Actinic light was provided by a fiber-optic illuminator filtered through a CS 2-63 red filter to minimize photoinhibition. The cell suspension was supplemented with 10 mM $NaHCO_3$ (final concentration) as the terminal electron acceptors before measurement. The rate of O_2 production was obtained by adding the rates of O_2 evolution in the light and O_2 consumption in the dark due to photorespiration.

RNA isolation and Northern analysis. Total RNA was extracted and purified using phenol-chloroform extraction and $CsCl_2$ gradient purification as previously described (16, 44); 10 μg of total RNA was fractionated by electrophoresis on a 1.0% agarose gel with 0.6 M formaldehyde. RNA was transferred to a nylon membrane as previously described (46) and fixed by baking at 80°C for 2 h in a vacuum oven. The blots were hybridized with α-^{32}P-labeled DNA probes prepared by random primer labeling using a Ready-To-Go kit (Pharmacia Biotech, Piscataway, N.J.). Hybridization was performed at 41 to 42°C with 50% formamide. The equal loading of total RNA was standardized by hybridization with a *Synechocystis* sp. strain PCC 6803 rRNA probe after stripping the previously hybridized probe. The Northern blot experiments were repeated at least twice for each gene with mRNA isolated from two separate experiments.

DNA probes and primers. The following homologous probes from *Synechocystis* sp. strain PCC 6803 were used: 0.73-kb *Eco*RI fragment of *psbB* (plasmid pSL158), 0.44-kb *Bst*EII fragment of *psbD* (plasmid pKW1344), 0.70-kb *Eco*RI fragment of *psaC* (plasmid TOPO-*psaC*), 0.75-kb *Eco*RI fragment of *psaD* (plasmid TOPO-*psaD*), 0.68-kb *Apa*I-*Hin*dIII fragment of *psaLI* (plasmid TOPO-*psaLI*), 0.98-kb *Hin*dIII fragment of *apcABC* (plasmid TOPO-*apcABC*), and

0.44-kb *Hinc*II fragment of *nblA* (plasmid TOPO-*nblA*). The three heterologous probes used were a 0.60-kb *Bst*EII fragment of *psbA* from *Synechococcus* sp. strain PCC 7942 (plasmid pSG200), a 2.8-kb *Eco*RI-*Bgl*II fragment of *psaAB* from *Synechococcus* sp. strain PCC 7002 (plasmid pAQPR80), and a 1.2-kb *Sma*I-*Xho*I fragment of *cpcBA* from *Synechococcus* sp. strain PCC 7002 (plasmid pAQPR1).

The following primers were used for cloning genes by PCR from *Synechocystis* sp. strain PCC 6803: *psaC* forward (5′-GCCTAGCTTTGGTCGAAAATCG-3′) and reverse (5′-CGCCGCCAGTCTAACTTTTG-3′), *psaD* forward (5′-CACA GAAGTCCCCATAATCTCCTTG-3′) and reverse (5′-CCAACATTGAAAGA GCGAACTGTC-3′), *psaLI* forward (5′-CGTGCGTAAAATGGGGACTAAA G-3′) and reverse (5′-CGAATCGGTTCAGTCATCTTGC-3′), *apcABC* forward (5′-TTACGGGGGCAGTGTAATCAGG-3′) and reverse (5′-TGGAGCAAAA CGGTTGGACG-3′), and *nblA* forward (5′-CCCAGAGCAACAACAAGAGT TACTG-3′) and reverse (5′-CAGGTAAGATCAAGTTTGCGGC-3′). All primers were synthesized by Integrated DNA Technologies, Inc. PCR products were cloned into pCR2.1-TOPO vector (Invitrogen).

Thylakoid membrane isolation and Western blot analysis. Cells were grown under photomixotrophic conditions and harvested at mid- to late log phase. Thylakoid membranes were isolated as previously described (55, 56), with some modifications. Briefly, cell pellets were resuspended in 1 ml of inhibitor buffer (50 mM morpholine ethanesulfonic acid-NaOH [pH 6.5], 50 mM $CaCl_2$, 0.3 M sucrose, 2 mM ε-aminocaproic acid, 2 mM phenylmethylsulfonyl fluoride). About 0.5 ml of glass beads (0.1-mm diameter) was added after transfer of the cyanobacterial cells to a 2-ml microcentrifuge tube. Cells were broken in a Braun homogenizer by four bursts of 30-s duration at high speed and centrifuged at 1,600 × *g* for 10 min to remove cell debris. The supernatant was centrifuged at 13,000 × *g* for 20 min to pellet the thylakoid membrane. The pellet was resuspended in inhibitor buffer, and 5 μg of Chl of each sample was solubilized on ice for 10 min, loaded into a lithium dodecyl sulfate (LDS)–10 to 20% polyacrylamide gradient gel, and separated by polyacrylamide gel electrophoresis (PAGE) at 1.5 W at 4°C for 16 to 18 h.

Protein pulse-chase experiment with [^{35}S]methionine. Pulse-labeling was performed to detect the synthesis of D1 protein in wild-type and Δ*rppA* cells. Cells were grown under LL and photomixotrophic conditions and harvested at the mid- to late logarithmic growth stage by centrifugation at 1,200 × *g* for 10 min at room temperature. The cell pellet was resuspended in BG-11 medium without sulfate. [^{35}S]Met was added to the medium to a final concentration of 1 μCi ml^{-1}, and the culture was incubated under LL for 30 min. An aliquot of cells was rapidly harvested by centrifugation, and the remainder of the culture was used for the pulse-chase experiment.

Cells were spun down at room temperature and resuspended in BG-11 medium without SO_4^{2-}. Unlabeled Met was added to the cells (final concentration of 1.0 mM) and transferred to HL. Cells were chilled on ice and harvested at 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0 h after HL exposure. The isolation of thylakoid membranes and PAGE were the same as described above. The gel was dried at 80°C for 3 h before X-ray autoradiography.

Quantification. Quantification of Northern blots, Western blots, and pulse-chase gels was performed with IP Lab Gel (Signal Analytics, Vienna, Va.) after scanning the information into an Apple Macintosh 9500 computer. In all cases, a relatively short exposure autoradiogram or a lightly stained gel was scanned into the computer to ensure linearity. The figures were darkened for publication to permit visualization of the lighter bands. The protein sequences were analyzed by MacVector 5.0 (Genetics Computer Group, Madison, Wis.).

RESULTS

The organization of the *Synechocystis* sp. strain PCC 6803 genome in the vicinity of *rppA* (sll0797) and *rppB* (sll0798) is shown in Fig. 1A. The gene map is from the Cyanobase web site (http://www.kazusa.or.jp/cyano/cyano.html), which contains the complete nucleotide sequence of *Synechocystis* sp. strain PCC 6803. The relative positions of 11 open reading frames near *rppA* and *rppB* are displayed. RppA is the response regulator (234 amino acids), and RppB is the cognate histidine kinase (454 amino acids). The *rppA* and *rppB* genes were inactivated by insertion of and replacement with a spectinomycin resistance cassette, respectively, as shown in Fig. 1B. The constructed plasmids were used to transform *Synechocystis* wild-type cells. Knockout mutants were demonstrated by Southern blots and PCR (data not shown). The growth characteristics, photosynthetic activity, and gene expression under different redox conditions had been examined in wild-type, Δ*rppA*, and Δ*rppB* cells. Since most of the detected features of the Δ*rppB* mutant were similar to those of the wild type, we present only results for the wild type and Δ*rppA* mutant. We also constructed a knockout mutant of sll0789 which had a primary sequence similar to that of RppA. The properties of this mutant was also similar to those of the wild type, thus providing a useful control.

Sequence analysis of RppA. Amino acid alignment among RppA and several response regulators was performed by the ClustW program (Fig. 2). These response regulators have been identified as transcriptional regulators for genes involved in various metabolic responses under different stress conditions. RegA and PrrA activate the transcription of *puf* and *puh* genes in anaerobic conditions in photosynthetic bacteria (20, 49). NblR activates transcription of the *nblA* gene, which codes for a small polypeptide that triggers the complete degradation of PBSs in cells grown under nitrogen and sulfur deficiency (14, 48).

RppA demonstrated ~35% similarity and ~20% identity to PrrA/RegA. More importantly, the highly conserved residues Asp8, Asp53, Thr81, and Lys103 in RppA corresponded to Asp13, Asp57, Thr87, and Lys109 in CheY, as well as to Asp20, Asp63, Thr91, and Lys113 in PrrA/RegA. CheY is the response regulator of bacterial chemotaxis; a detailed three-dimensional structure and site-directed mutagenesis studies of this protein have shown that Asp13 is essential for phosphorylation and dephosphorylation (51) and that Asp57 is the phosphorylation site (47). Thr87 and Lys109 are important for the phosphorylation-induced conformational change (3, 31). This suggests that RppA has the characteristics appropriate for a bacterial response regulator.

A recent analysis has demonstrated a remarkable similarity among six RegA homologues from four photosynthetic bacteria and two nitrogen-fixing bacteria (32). The proteins from the nitrogen fixers are regulator proteins thought to be involved in sensing low pH or in the control of nitrogen fixation-associated genes. These comparisons established some key attributes of these proteins. In addition to the conserved amino acids mentioned above (Fig. 2), there are two important structural features: (i) a series of four prolines (in positions 133 to 136 in RegA), two of which are retained in the ActR from *Rhizobium meliloti* (52); and (ii) an extremely well conserved C-terminal region that contains a helix-turn-helix motif. The RppA protein from *Synechocystis* sp. strain PCC 6803 retains two of the conserved prolines, but they are separated by a 23-amino-acid segment with a third Pro in the center. Secondary structure analysis indicated that this region in both RppA and RegA models as a random coil plus some extended strand. Thus, this region in RppA may function similarly to that of RegA, despite the modified sequence. Last, our analysis using MacVector confirmed the helix-turn-helix motif in RegA as well as in NblR and indicated the possibility of a similar motif in RppA.

Physiological characterization of Δ*rppA*. *Synechocystis* sp. strain PCC 6803 wild type and Δ*rppA* cells grow differently under photoautotrophic and photomixotrophic (with 5 mM glucose) conditions (Table 1). Under photoautotrophic conditions, liquid cultures of Δ*rppA* grew at almost the same growth rate (half-life [$t_{1/2}$] ~20 h) and had less green color than wild-type cells, a phenomenon that could also be detected directly on plates (data not shown). When glucose was present in the media, the Δ*rppA* strain grew faster than the wild type ($t_{1/2}$ ~9.5 versus 12 h), and cell numbers increased at least 1.5-fold over wild-type levels by the late logarithmic growth phase. Interestingly, light microscopic observations indicated that more than 90% of the wild-type cells were doublets, whereas most of the Δ*rppA* cells were seen as single cells. Without taking these doublets into account, Δ*rppA* appeared to have threefold more cells than the wild type. The measurement of cellular biomass showed that photomixotrophic Δ*rppA* cultures, in the mid- to late log growth phase, had approximately 25 to 70% greater

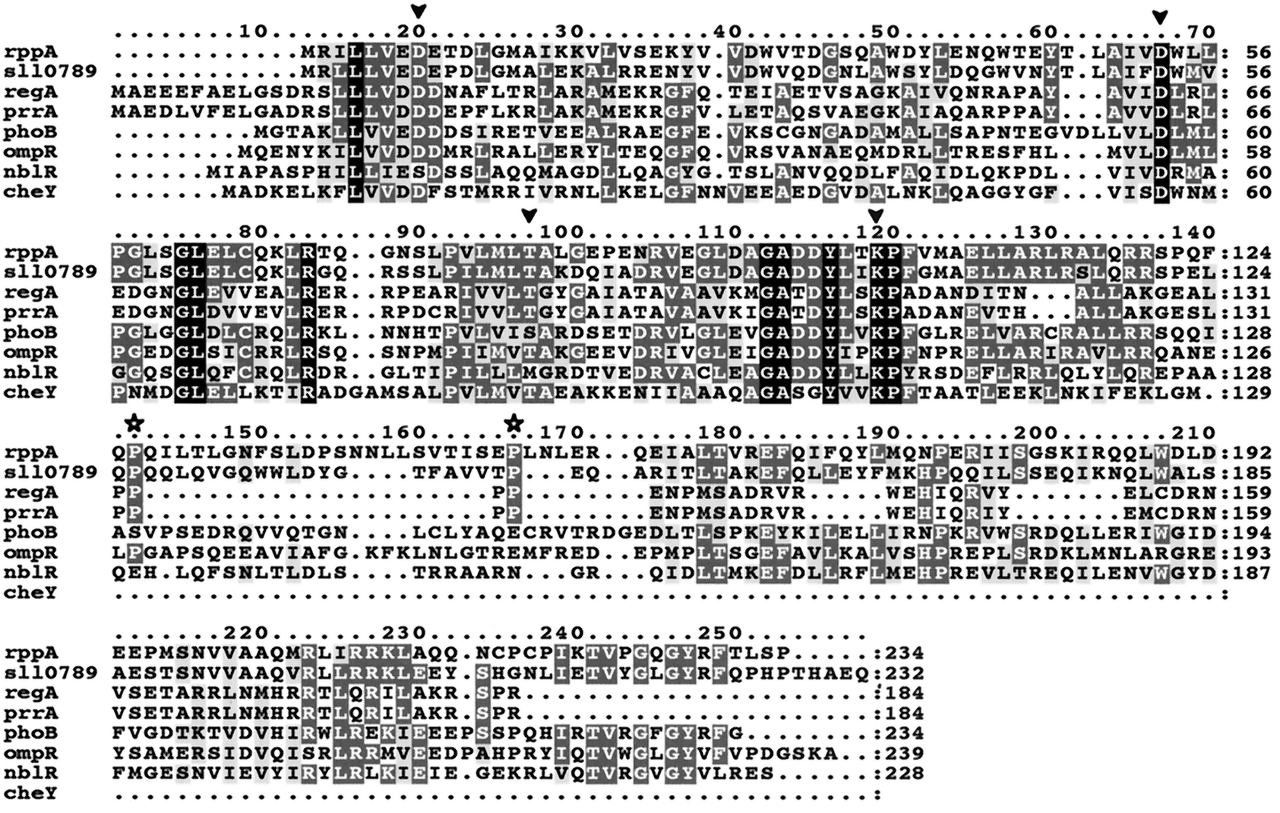

FIG. 2. Alignment of RppA with other response regulators. The sequences are as follows: RppA and sll0789, *Synechocystis* sp. strain PCC 6803; RegA, *Rhodobacter capsulatus* (GenBank accession no. M64976); PrrA, *R. sphaeroides* (L25895); PhoB, *Synechococcus* sp. WH7803 (U38917); OmpR, *Escherichia coli* (J01656); NblR, *Synechococcus* sp. strain PCC 7942 (AF049128); CheY, *E. coli* (M13463). Identical residues are shaded in black, conserved residues are shaded in dark gray, and similar residues are shaded in light gray. Arrowheads denote the highly conserved aspartic acid (Asp8 and Asp53), threonine (Thr81), and lysine (Lys103) residues in RppA, which correspond to Asp13, Asp57, Thr87, and Lys109 in CheY, as well as to Asp20, Asp63, Thr91, and Lys113 in PrrA/RegA. Stars denote the two conserved prolines in RppA, s110789, RegA, and PrrA.

wet weight than did wild-type cultures (data not shown). The whole-cell spectra showed somewhat lower Chl and PC concentrations per cell in the Δ*rppA* mutant. However, the PC/Chl ratio of the Δ*rppA* mutant is higher than that of the wild type under both growth conditions, which suggests that the mutant has a higher PSII/PSI ratio or less Chl per photosystem. The oxygen evolution activity in Δ*rppA* cells was slightly higher than the wild-type level under photoautotrophic growth conditions. However, when cells were cultured in LL with glucose, the photosynthesis activity of Δ*rppA* was about 50% higher than the wild-type level (Table 1). This difference cannot be accounted for merely on the basis of the difference in Chl concentration per cell (4.7 versus 3.9 μg of Chl/10^{-8} cells) and must represent an actual increase in the specific activity for PSII of approximately one-third.

Transcriptional regulation of PSII genes. The impact of Δ*rppA* on transcription and on redox regulation of transcription was tested using various DNA probes against wild-type and Δ*rppA* total mRNAs. The electron transfer inhibitors DCMU and DBMIB were used to generate oxidizing and reducing states of the PQ pool. The cells were grown for 6 h in the presence of these compounds so that we could analyze long-term effects on cellular growth and physiology relative to transcriptional patterns. Northern blots of PSII genes against wild-type mRNA demonstrated that *psbA* was obviously regulated by the PQ redox conditions: up-regulated when the PQ pool was oxidized by DCMU (Fig. 3A, *psbA*, lanes 3 versus 1) and down-regulated when the PQ pool was reduced by DBMIB or glucose (lanes 5 and 7 versus 1). The transcription of *psbA* was dramatically increased in Δ*rppA* cells compared to the wild

TABLE 1. Characteristics of *Synechocystis* sp. strain PCC 6803 wild-type and Δ*rppA* cells[a]

Strain	Growth conditions	Growth rate ($t_{1/2}$, h)	μg of Chl/10^8 cells	μg of PC/10^8 cells	PC/Chl	O_2 evolution [μmol of O_2 (mg of Chl)$^{-1}$ h^{-1}]
Wild type	Photoautotrophic	19	3.8	29.8	7.2	209
	Photomixotrophic	12	4.7	37.8	8.1	265
Δ*rppA*	Photoautotrophic	21	3.1	26.3	7.9	229
	Photomixotrophic	9.5	3.9	33.6	8.5	398

[a] Cells were grown under LL conditions until mid- to late log phase. Values are averages from four separate experiments.

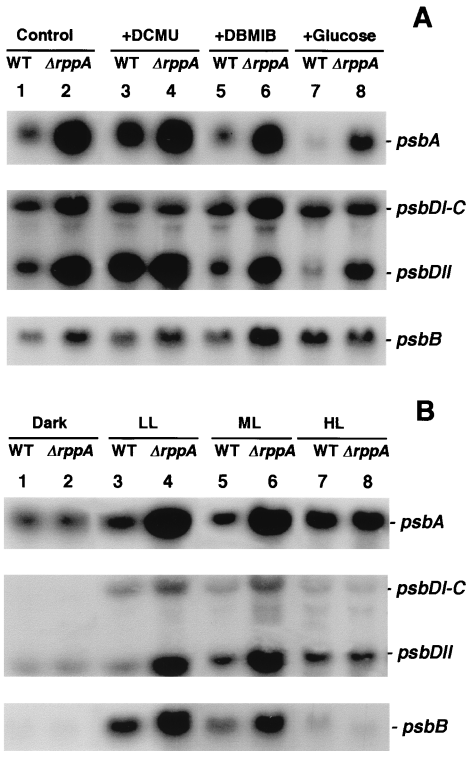

FIG. 3. Northern blot analysis of PSII gene expression in *Synechocystis* sp. strain PCC 6803 wild type (WT) and Δ*rppA* cells. RNA was isolated from both wild-type (lanes 1, 3, 5, and 7) and Δ*rppA* (lanes 2, 4, 6, and 8) cells after treatment with different redox (A) and illumination (B) conditions as described in Materials and Methods. The sizes of *psbA*, *psbDI-C*, *psbDII*, and *psbB* are 1.2, 2.5, 1.2, and 2.0 kb, respectively.

type. In Δ*rppA* cells, the *psbA* mRNA level was about twofold higher than the wild-type level when the PQ pool was oxidized (lanes 4 versus 3) and 5- to 10-fold higher when the PQ pool was reduced (lanes 6 versus 5 and 8 versus 7). It should be noted that the *psbA* family in *Synechocystis* sp. strain PCC 6803 consists of three genes, *psbA-1*, *psbA-2*, and *psbA-3*, although *psbA-1* is not expressed (27). The nucleotide sequences of *psbA-2* and *psbA-3* are more than 99% identical, the transcription sizes are also identical, and *psbA-2* accounts for the majority (>90%) of the *psbA* transcripts under normal growth conditions (37). In this study, the *psbA* signal in Fig. 3A represents the mRNA of both *psbA-2* and *psbA-3*.

We also analyzed the transcriptional activity of the *psbD* gene, which codes for the other PSII reaction center protein, D2. In *Synechocystis* sp. strain PCC 6803, there are two copies of the *psbD* gene, *psbDI* and *psbDII*. The *psbDI* is cotranscribed with *psbC*, which encodes the CP43 polypeptide of the PSII complex (35). Two different-sized bands were seen on the Northern blots: the upper band (~2.5 kb) represents the transcripts of *psbDI-psbC*, whereas the lower band (~1.2 kb) is the *psbDII* transcript. The transcriptional patterns of *psbDI-psbC* and *psbDII* under different redox conditions were not the same. In the wild type, the steady-state mRNA level of *psbDI-psbC* did not change much under various PQ redox conditions and thus more closely followed the pattern of *psbB*, the other antenna protein (see below). *psbDII* responded like the *psbA* gene and was up-regulated when the PQ pool was oxidized (Fig. 3A, *psbDII*, lanes 3 versus 1) and down-regulated when the PQ pool was reduced (lanes 5 and 7 versus 1). In Δ*rppA* cells, *psbDI-psbC* transcripts were higher than in the wild type only under photoautotrophic (control) growth conditions and in DBMIB-treated cells (Fig. 3A, *psbDI-psbC*, lanes 2 versus 1 and 6 versus 5). The presence of DCMU and glucose did not change the *psbDI-psbC* transcriptional pattern. The *psbDII* gene is highly expressed in Δ*rppA* under all examined conditions (Fig. 3A, *psbDII*, lanes 2, 4, 6, and 8). Since the *psbA* and *psbD* genes encode the two PSII reaction center proteins, D1 and D2, it is reasonable to speculate that they are under similar transcriptional control.

The transcriptional pattern of *psbB*, which encodes the CP47 polypeptide of the PSII complex, was different from that of *psbA* and *psbDII*. In the wild type, the steady-state mRNA level of *psbB* was slightly increased in both oxidized and reduced conditions (Fig. 3A, *psbB*, lanes 3, 5, and 7 versus 1). When glucose was present in the growth media, the *psbB* mRNA level was about threefold higher than the control level (lanes 7 versus 1), whereas *psbA* and *psbDII* levels were very low under this condition. In the Δ*rppA* strain, the accumulation of *psbB* transcripts was higher than in the wild type, especially when DBMIB was added (lanes 6 versus 5). At the same time, growth in the presence of glucose led to no difference in *psbB* transcription between wild-type and Δ*rppA* cells (lanes 8 versus 7).

We also used different illumination conditions in wild type and Δ*rppA* strains and demonstrated that the excessive up-regulation of PSII genes in Δ*rppA* cells was restricted to LL and ML conditions (Fig. 3B). When cells were transferred to the dark for 6 h or exposed to high light intensity for 3 h, the steady-state mRNA levels of *psbA* and *psbDII* were the same in wild type and Δ*rppA* cells (Fig. 3B, *psbA* and *psbDII*, lanes 2 versus 1 and 8 versus 7). Compared to the other PSII genes, *psbA* and *psbDII* transcripts are more stable in the dark and HL, which is consistent with previous studies (35, 36). The higher levels of *psbA* and *psbDII* transcripts under HL illumination were required to meet an accelerated turnover of the D1 and D2 proteins. The differences in transcriptional control of *psbDI* and *psbDII* were reflected in their different responses to illumination. The steady-state mRNA level of *psbDI-psbC* is much lower than that of *psbDII* in wild-type and Δ*rppA* cells. The expression pattern of *psbDI-psbC* under different illumination conditions was the same as for the *psbB* gene: the transcripts are almost undetectable in the dark, and there were very low levels under HL, suggesting that the mRNA has a short half-life under these extreme light conditions.

Transcriptional regulation of PSI genes. The PSI reaction center proteins, PsaA and PsaB, are encoded in an operon by the *psaA* and *psaB* genes. Northern blot analysis (Fig. 4) showed two distinct transcripts, a 5.5-kb transcript, which represented the entire gene cluster, and a 2.5-kb transcript, which corresponds to *psaA* and *psaB* (50). In contrast to the PSII reaction center genes, *psaAB* transcription in wild-type cells was repressed under oxidizing conditions (Fig. 4A, *psaAB*, lanes 3 versus 1). Interestingly, the *psaA-psaB* transcripts became more prevalent under reducing conditions and were induced relative to the control (Fig. 4A, *psaA* + *psaB*, lanes 5 and 7 versus 1). In the wild type, *psaAB* mRNA levels increased when glucose was present (Fig. 4A, *psaAB*, lanes 7 versus 1) but not in the presence of DBMIB. In Δ*rppA* cells, the mRNA levels of *psaAB* and *psaA-psaB* were very similar to the wild-type level in control and DBMIB-treated cells (Fig. 4A, *psaAB* and *psaA* + *psaB*, lanes 2 versus 1 and 6 versus 5) but decreased when the cells were treated with DCMU or when glucose was present in the media (lanes 4 versus 3 and 8 versus 7). The genes encoding proteins that are attached to the PSI reaction center (*psaC*, *psaD*, and *psaLI*) (11, 24) showed very similar transcriptional patterns under different redox con-

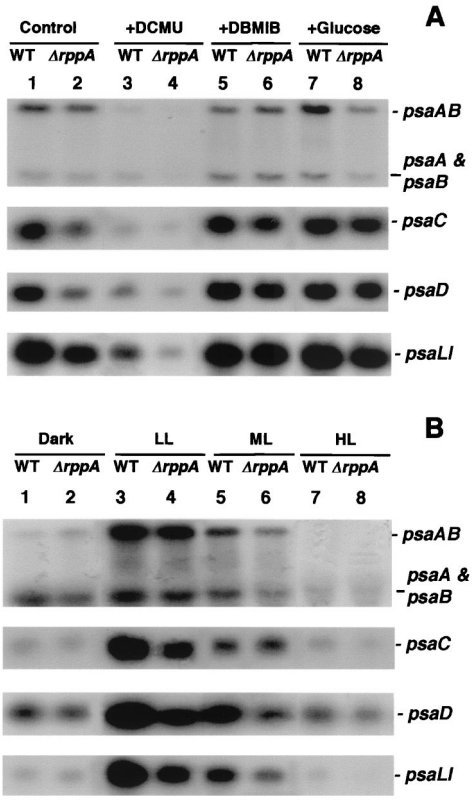

FIG. 4. Northern blot analysis of PSI gene expression in *Synechocystis* sp. strain PCC 6803 wild-type (WT) and Δ*rppA* cells. RNA was isolated from both wild-type (lanes 1, 3, 5, and 7) and Δ*rppA* (lanes 2, 4, 6, and 8) cells after treatment with different redox (A) and illumination (B) conditions as described in Materials and Methods. The sizes of *psaAB*, *psaA* and *psaB*, *psaC*, *psaD*, and *psaLI* are 5.5, 2.5, 0.6, 0.6, and 1.0 kb, respectively.

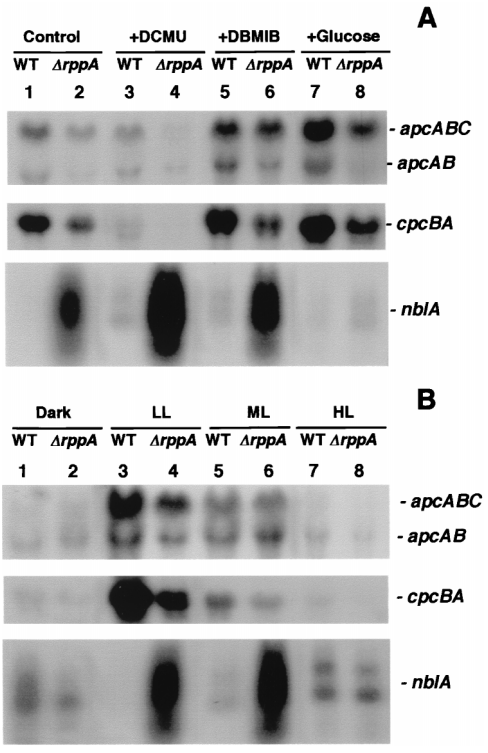

FIG. 5. Northern blot analysis of PBS-related gene expression in *Synechocystis* sp. strain PCC 6803 wild-type (WT) and Δ*rppA* cells. RNA was isolated from both wild-type (lanes 1, 3, 5, and 7) and Δ*rppA* (lanes 2, 4, 6, and 8) cells after treatment with different redox (A) and illumination (B) conditions as described in Materials and Methods. The sizes of *apcABC*, *apcAB*, *cpcBA*, and *nblA* are 1.8, 1.4, 1.5, and 0.25 to 1.0 kb, respectively.

ditions: they were all down-regulated under oxidized conditions (Fig. 4A, *psaC*, *psaD*, and *psaLI*, lanes 3 versus 1) but were at the same levels when the PQ pool was reduced by either DBMIB or glucose (lanes 5 and 7 versus 1). In Δ*rppA* cells, the *psaC*, *psaD*, and *psaLI* transcripts were under the same control: they were all decreased two- to fivefold compared to the wild type under photoautotrophic (control) and PQ-oxidized conditions (lanes 2 versus 1 and 4 versus 3) but only slightly reduced or unchanged when PQ was reduced (lanes 6 versus 5 and 8 versus 7).

When cells were kept in the dark for 6 h, the abundance of full-length *psaAB* transcripts was dramatically decreased, but the *psaA-psaB* transcripts continued to accumulate to high levels in both wild-type and Δ*rppA* cells (Fig. 4B, *psaAB* and *psaA* + *psaB*, lanes 1 and 2). This result suggested that the full-length *psaAB* transcripts were less stable in the dark and produced transcripts that encode only *psaA* and *psaB* after processing in the intergenic region. The steady-state mRNA levels of *psaAB* and *psaA-psaB* decreased as the light intensity increased. When cells were exposed to high light for 3 h, *psaAB* transcripts were undetectable in both wild-type and Δ*rppA* cells (Fig. 4B, *psaAB*, lanes 7 and 8). *psaC*, *psaD*, and *psaLI* showed the same transcriptional patterns under all illumination conditions: highly expressed under LL and ML conditions but depressed under both dark and HL conditions. This decline can be caused either by a lower transcriptional rate or faster mRNA degradation. In the Δ*rppA* strain, PSI gene expression was very similar to that in the wild type under both dark and HL conditions (Fig. 4B, lanes 2 versus 1 and 8 versus 7).

Transcriptional regulation of PBS-related genes. In *Synechocystis* sp. strain PCC 6803, the genes encoding the PBS core subunits, allophycocyanin α and β, and a small core linker protein form an operon, *apcABC*. Northern blots revealed two transcripts of 1.8 and 1.5 kb (Fig. 5). The larger band contains the *apcA*, *apcB*, and *apcC* transcripts, whereas the smaller contains the *apcA* and *apcB* transcripts. Transcription of the *apc* operon in the wild type was repressed under PQ-oxidizing conditions (Fig. 5A, *apcABC*, lanes 3 versus 1) and induced under PQ-reducing conditions (lanes 5 and 7 versus 1). In Δ*rppA* cells, the *apcABC* transcripts were down-regulated under all detected conditions compared to the wild type (lanes 2 versus 1, 4 versus 3, 6 versus 5, and 8 versus 7). Interestingly, in Δ*rppA* cells, the larger transcript (*apcABC*) decreased mainly under oxidizing conditions (lanes 4 versus 3), whereas the smaller transcript (*apcAB*) is strikingly decreased under reducing conditions (Fig. 5A, *apcAB*, lanes 6 versus 5 and 8 versus 7), especially when cells were grown in the presence of glucose. The transcript of the *cpcBA* operon, which encode α and β subunits of PC, showed the same expression pattern as the *apc* operon (Fig. 5A).

NblA is a small polypeptide, first identified in *Synechococcus* sp. strain PCC 7942 (14), that is involved with the complete degradation of light-harvesting phycobiliproteins in nutrient-deprived cyanobacteria. Two copies of the *nblA* gene (ssl0452 and ssl0453) were identified in the *Synechocystis* sp. strain PCC 6803 genomic sequence. Since we are interested in RppA regulation of photosynthesis- and photopigment-related gene ex-

pression, we examined the *nblA* transcript levels under different redox conditions. Northern analysis indicated that the *nblA* gene was extremely highly expressed in Δ*rppA* under photoautotrophic and both PQ-oxidized and PQ-reduced conditions (Fig. 5A, *nblA*, lanes 2 versus 1, 4 versus 3, and 6 versus 5). Unexpectedly, growth in the presence of glucose almost completely repressed the high expression of *nblA* in Δ*rppA* cells (lanes 8 versus 7). Such results suggested a correlation between the transcriptional regulation activity of RppA and high rates of respiration which lead to reduction of the PQ pool.

In wild-type and Δ*rppA* cells, the *apc* and *cpc* transcripts are dramatically decreased under dark and HL exposure (Fig. 5B, *apcABC*, *apcAB*, and *cpcBA*, lanes 1, 2, 7, and 8). In Δ*rppA* cells, the *apc* transcript was down-regulated under LL (Fig. 5B, *apcABC* and *apcAB*, lanes 4 versus 3), and *cpc* transcription was repressed under both LL and ML compared to the wild type (Fig. 5B, *cpcBA*, lanes 4 versus 3 and 6 versus 5). These results agreed with the absorbance spectral data showing that under LL and photoautotrophic growth conditions, the Δ*rppA* strain contained less PC per cell than the wild type. The *nblA* transcripts were differently expressed from the *apc* and *cpc* operons under different illumination conditions. In the wild type, *nblA* transcription was higher under dark, ML, and HL conditions than under LL conditions (Fig. 5B, *nblA*, lanes 1, 5, and 7 versus 3), consistent with the phycobiliprotein degradation process under these nonoptimal light conditions. In Δ*rppA* cells, the steady-state mRNA levels of *nblA* were lower in dark and HL than in LL and ML conditions (Fig. 5B, *nblA*, lanes 2 and 8 versus 4 and 6).

Photosynthesis activity and half-life of D1 protein in wild-type and Δ*rppA* cells. Under photoautotrophic, LL growth conditions, the O_2 evolution rate of Δ*rppA* cells is very close to that of the wild type. After 2 h of HL exposure, the photosynthesis activity of wild-type and Δ*rppA* cells increased about 40% ± 4% ($n = 3$) (Fig. 6A). When cells were cultured in LL conditions with glucose, the photosynthesis activity of the Δ*rppA* mutant was much higher than the wild-type level (Fig. 6B, $t = 0$ h), as shown in Table 1 for LL conditions. After 2 h of HL treatment, the photosynthesis activity of wild-type and Δ*rppA* cells also increased about 40% ± 4% ($n = 3$).

The half-life of the D1 protein under HL was examined by adding the protein synthesis inhibitor chloramphenicol to the culture. Results showed that under photoautotrophic, HL conditions, the D1 half-lives of wild-type and Δ*rppA* cells were quite similar (~60 min) (Fig. 6A). In photomixotrophic, HL conditions, Δ*rppA* showed a slightly higher D1 degradation rate than the wild type (45 versus 60 min) (Fig. 6B). These results suggested that one factor for the higher O_2 evolution activity of Δ*rppA* could be the somewhat faster de novo turnover of the D1 protein. The next step was to determine how much of this turnover was due to de novo synthesis.

Translational regulation of the D1 protein. To clarify why the oxygen evolution activity in Δ*rppA* cells is higher than in the wild type under photomixotrophic growth conditions, we analyzed protein accumulation by immunoblotting (Fig. 7A) and protein synthesis with pulse-chase experiments (Fig. 7B). For the immunoblot experiment, cells were grown in LL until mid- to late log phase, chloramphenicol was added, and the cells were exposed to HL for different time intervals. The thylakoid membranes were isolated, and membranes containing 5 μg of Chl were loaded in each lane. As shown in Fig. 7A ($t = 0$ h), the steady-state D1 protein level in Δ*rppA* cells was about 1.5-fold higher than in the wild type. When cells were exposed to HL, degradation of the D1 protein in the Δ*rppA* strain was faster than in the wild type. Notably, we observed a band above the mature D1 protein band that was previously designated the

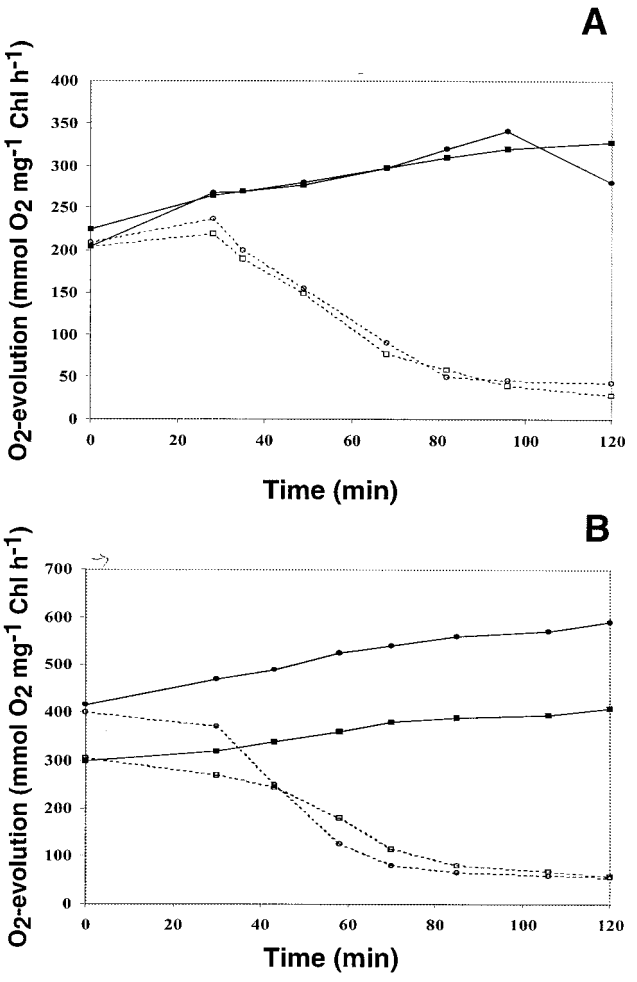

FIG. 6. Oxygen evolution activities of *Synechocystis* sp. strain PCC 6803 wild-type and Δ*rppA* cells under high light intensity (1,000 μE m^{-2} s^{-1}). Cells were grown in LL without (A) or with (B) glucose until mid- to late log phase and then transferred to HL. Samples were taken at different time points after exposure to HL. The protein synthesis inhibitor chloramphenicol was added at 0 h to a final concentration of 50 μg ml^{-1}. The oxygen evolution activity was measured as described in Materials and Methods. Samples: wild type without (■) and with (□) chloramphenicol; Δ*rppA* without (●) and with (○) chloramphenicol.

D1 precursor (55, 56). The abundance of this band was higher in Δ*rppA* than in wild-type cells, especially within the first hour of chloramphenicol treatment. This result indicated that the rate of D1 synthesis in the Δ*rppA* strain increased to keep up with the high photosynthesis activity and to compensate for the rapid D1 degradation. However, the processing rate may not have been altered, and the new precursor would accumulate to higher levels in Δ*rppA* than in wild-type cells.

Pulse-labeling experiments showed that the D1 protein synthesis in Δ*rppA* was faster than in the wild type. Quantification of the labeled band showed a twofold increase in the amount of D1 in Δ*rppA* over wild-type cells after 30 min of labeling (Fig. 7B, lane P). During the chase under HL conditions, D1 turned over more rapidly in Δ*rppA* than in wild-type cells. Again, a protein band with a slightly slower mobility than the mature D1 protein was observed at 0 h in Δ*rppA* cells, which indicated faster D1 protein synthesis in the mutant. From both Western blot and pulse-chase experiments, we conclude that the PSII reaction center protein D1 was synthesized more

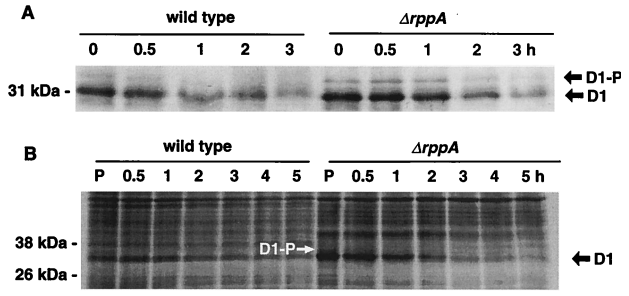

FIG. 7. (A) Immunoblot analysis of D1 protein of *Synechocystis* sp. strain PCC 6803 wild-type and Δ*rppA* cells. Thylakoid proteins were isolated from cells which had been grown under LL with glucose until mid- to late log phase, chloramphenicol was added, and the cells were exposed to high light intensity for different time intervals. Thylakoids (5 μg of Chl per lane) were loaded, separated by LDS-PAGE, blotted onto a nitrocellulose membrane, and incubated with D1 antisera. The D1 precursor (D1-P) that migrates more slowly than the mature D1 protein was observed in both wild-type and Δ*rppA* cells. (B) Pulse-chase labeling of thylakoid proteins from *Synechocystis* sp. strain PCC 6803 wild-type and Δ*rppA* cells. Cells were labeled with [^{35}S]Met in vivo under LL with glucose for 30 min (P). The radioactivity was subsequently chased for 0.5, 1, 2, 3, 4, and 5 h under HL (see Materials and Methods). The D1 precursor (D1-P; white arrow) that migrates more slowly than the mature D1 protein was observed in Δ*rppA* cells. Labeled proteins were detected by autoradiography.

rapidly and accumulated at a higher steady-state level in photomixotrophically grown Δ*rppA* cells than in the wild type. The rapid degradation of the D1 protein is balanced by an enhancement of gene expression that compensated for the loss of these proteins and maintained active PSII complexes. Thus, the high level of PSII reaction center gene transcription is physiologically relevant and is seen in a higher level of de novo synthesis of D1.

DISCUSSION

Overall characterization of photosynthesis gene transcription in Δ*rppA*. Figures 3 to 5 demonstrate that the transcription of photosynthesis genes was profoundly affected in Δ*rppA*. In general, transcription of genes coding for PSII proteins was enhanced in Δ*rppA* cells compared to the wild type, as seen for *psbA* (Fig. 3A). Conversely, transcription of genes encoding for PSI proteins and phycobiliproteins was somewhat decreased, as seen for *psaC* (Fig. 4A) or *apcABC* (Fig. 5A). These changes were not seen when cells were grown in the dark or in HL, as demonstrated for *psbA* (Fig. 3B). A major exception to the above generality was transcription of *nblA*, which was vastly increased (~100-fold) in Δ*rppA* compared to wild-type cells (Fig. 5A), whereas the PBS structural genes were down-regulated. From these results, we tentatively concluded that the RppA protein is involved in regulating the stoichiometry of the photosystems by depressing transcription of PSII and increasing the transcription of PSI. At the same time, it increases transcription of PBS genes and inactivates a system for destruction of PBS. A more detailed analysis of the RppA regulation follows, including the effects of redox poise and light on transcription.

Redox state of the PQ pool controls photosynthesis and PBS-related gene expression. Changes in the redox state of components of the electron transport chain have been implicated in controlling the transcriptional activators of photosynthetic gene expression in cyanobacteria (1, 2). To test if RppA is a regulator that responds to redox poise, we have treated *Synechocystis* sp. strain PCC6803 wild-type and Δ*rppA* cultures under different light conditions and with two specific inhibitors of electron transport, DCMU and DBMIB. DCMU blocks electron transfer from the PSII primary acceptor Q_A to the PQ pool, and DBMIB prevents the electron transfer from PQ pool to cytochrome b_6/f. Our results indicated that in wild-type cells, the steady-state mRNA levels of PSII genes increased when the PQ pool was oxidized by DCMU, decreased when the PQ pool was reduced by DBMIB, and were extremely depressed when glucose was present (Fig. 3A). In contrast, transcription of the PSI reaction center operon was depressed when the PQ pool was oxidized, and net accumulation of the *psaA-psaB* transcripts increased when the PQ pool was reduced (Fig. 4A). The inverse effects of DCMU and DBMIB strongly suggest that the balance between the reduced and oxidized forms of the PQ pool is involved in the signal transduction of PS gene expression. Compared to the wild type, expression of photosynthesis genes in Δ*rppA*, especially the PSII reaction center genes, was less sensitive to PQ redox variation (Fig. 3A). We conclude that the *rppA* gene is normally involved in the establishment of the appropriate stoichiometry between the photosystems and can sense changes in the PQ redox poise.

Our results relate to a number of important areas of photosynthesis research, including redox control of gene expression and the effect of photoinhibition on PSII gene expression. Similar results in regard to redox control of chloroplast gene expression were obtained recently by Pfannschmidt et al. (42). Their paper supported and expanded on the model of Allen (2) that relates the transcriptional activity of PSI and PSII genes to the redox poise of the PQ pool. Overall, their results indicated that when either photosystem becomes rate limiting for photosynthesis, transcription of genes for its specific reaction center proteins becomes induced. Their results for chloroplasts from mustard seedlings and ours for cyanobacteria are virtually identical in this regard. Another detailed study of redox control of *psbA* expression in *Synechocystis* (1) also concluded that such transcription is under redox control. These authors specifically emphasized that accumulation of Q_A^- activates *psbA* transcription. We have explicitly not tried to differentiate between Q_A^- and PQ pool redox state or the thiol redox state at this early stage of our studies, since it is difficult to determine which would be the actual signal. It is possible that Q_A^- could be involved directly with the mechanism to indicate that PSII centers need to be replaced. However, it is less certain if this is the direct signal for the transcriptional regulation of PSI genes or those involved with PBSs. For simplicity, we have referred just to the redox poise of the PQ pool as we begin the process of sorting out the control mechanism in the Δ*rppA*, as well as in other mutants.

Another very fruitful line of investigation has been initiated by Grossman and colleagues (14, 17, 48), who isolated mutants of *Synechococcus* sp. strain PCC 7942 that were defective in the degradation of PBSs during sulfur- or nitrogen-limited growth. They identified NblA, a small polypeptide that is critical for PBS degradation during this nutrient deprivation (14). In *Synechococcus* sp. strain PCC 7942, *nblA* transcripts were very low in nutrient-replete cells, and their abundance increased about 50-fold during sulfur or nitrogen deprivation (14). The present study showed that in *Synechocystis* sp. strain PCC 6803, RppA strongly depressed *nblA* transcription. The steady-state mRNA level of *nblA* was dramatically increased when RppA was absent. This may be one reason why Δ*rppA* cells contained less PC than the wild type, since over expression of *nblA* could trigger the PBS-degradative process. Interestingly, *nblA* transcription is also up-regulated in the RppB mutant, but to a lesser degree than in Δ*rppA* cells (data not shown). These results suggest that phosphorylation is essential for RppA activation, and RppA could be phosphorylated by other kinases in addition to RppB. In Δ*rppA* cells, the accumulation of *nblA*

transcripts increased greatly under both PQ oxidizing and reducing conditions (Fig. 5A). The regulation of RppA on *nblA* transcription was eliminated when glucose was present and when cells were transferred to dark or to HL. These data implied that *nblA* transcription is under different controls under diverse environmental stress conditions. NblR was identified to be an essential inducer of *nblA* expression in *Synechococcus* sp. strain PCC 7942 under nitrogen and sulfur deprivation conditions (48). NblR had all of the characteristics of a response regulator that is controlled by the intracellular redox state. Based on these data, we currently conclude that RppA and NblR work differently toward controlling *nblA* expression, although they may have overlapping functions. We will soon be able to study their related functions by constructing double mutants of *Synechocystis* sp. strain PCC 6803 that are deficient in both RppA and NblR.

In cyanobacteria, the light-harvesting antenna consists of the PBSs and Chl. The majority of Chl molecules are associated with PSI, whereas the PBSs are generally the major light-harvesting antenna for PSII. The state transitions are associated with the movement of the PBSs from PSII to PSI when PSII has been provided too much excitation energy (45). Our results are very similar to those of Alfonso et al. (1) in that PSI- and PBS-related genes were not under the same level of redox control as the PSII reaction center genes. Our results indicated that in the wild type, transcription of PSI- and PBS-related genes decreased in the presence of DCMU and increased in the presence of DBMIB and glucose. It is of interest that regulation of the PBS-related genes is closer to that of PSI than to those of PSII. This suggests that the main function of the redox-regulated signal is to allow for the degradation and resynthesis of PSII. Under these circumstances, new transcription of PBS-related genes and PBS synthesis could complicate the repair mechanism and would be more appropriate at a later time. In Δ*rppA* cells, transcription of the PBS structural genes (*apcABC* and *cpcBA*) was significantly reduced under both oxidizing and reducing conditions, especially the presence of glucose. Once again, the high rate of PSII synthesis may require that the transcription of the light-harvesting proteins be reduced significantly.

Light conditions affect transcription and translation of the PSII reaction center components. Exposure of oxygenic photosynthetic organisms to high light intensity causes photoinhibition of photosynthesis. Photoinhibition is associated with an inactivation of PSII electron transport and subsequent degradation of the PSII reaction center proteins (4, 29). In *Synechococcus* sp. strain PCC 7942, there are two forms of D1 protein, D1:1 and D1:2. It has been demonstrated that PSII reaction centers containing D1:2 have a higher intrinsic resistance to photoinhibition and are more photochemically efficient than PSII centers with D1:1 (9, 12, 13, 30). In *Synechocystis* sp. strain PCC 6803, only one form of D1 has been identified. The rapid degradation of D1, and possibly D2, is balanced by an induction of gene expression at the high light intensity that compensates for the loss of these proteins and maintains a functional PSII. In *Synechocystis* sp. strain PCC 6803, the accumulation of *psbA* and *psbDII* transcripts was enhanced by a shift to HL conditions (Fig. 3B). Like in *Synechococcus*, the primary function of the monocistronic *psbDII* locus in *Synechocystis* may be to produce extra D2 protein to maintain a functional PSII at high light intensity without increasing synthesis of the more stable *psbC* gene product (8). The oxygen evolution of both *Synechocystis* sp. strain PCC 6803 wild type and Δ*rppA* mutant increased 35 to 45% in photoautotrophic and photomixotrophic growth conditions, respectively, under HL for 2 h (Fig. 6). The protein synthesis inhibitor chloramphenicol caused the O_2 evolution activity to be lost completely within 2 h under high light irradiation, indicating that rapid de novo protein synthesis is required to maintain PSII activity. Fast D1 degradation and synthesis were confirmed by the pulse-chase and immunoblot experiments. Both experiments indicated that D1 synthesis was faster and the steady-state level was higher in Δ*rppA* cells than in the wild type. This phenomenon was more obvious when cells were grown in the presence of glucose. It is important to note that although D1 and D2 synthesis was enhanced, the synthesis of CP43 and CP47 was not, especially in Δ*rppA* cells. This suggests that there can be more PSII centers but with less antenna Chl on average. Thus, the O_2 evolution per milligram of Chl in Δ*rppA* cells would appear higher than the wild type (Table 1 and Fig. 6).

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