Biogenesis of Phycobiliproteins

I. cpcS-I AND cpcU MUTANTS OF THE CYANOBACTERIUM SYNECHOCOCUS sp. PCC 7002 DEFINE A HETERO DIMERIC PHYCOCYANIN LYASE SPECIFIC FOR β-PH YOCYANIN AND ALLOPHYCOCYANIN SUBUNITS

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Phycobilin lyases covalently attach phycobilin chromophores to apo-phycobiliproteins (PBPs). Genome analyses of the unicellular, marine cyanobacterium Synechococcus sp. PCC 7002 identified three genes, denoted cpcS-I, cpcU, and cpcV, that were possible candidates to encode phycocyanobilin (PCB) lyases. Single and double mutant strains for cpcS-I and cpcU exhibited slower growth rates, reduced PBP levels, and impaired assembly of phycobilisomes, but a cpcV mutant had no discernable phenotype. A cpcS-I cpcU cpcT triple mutant was nearly devoid of PBP. SDS-PAGE and mass spectrometry demonstrated that the cpcS-I and cpcU mutants produced an altered form of the phycocyanin (PC) β subunit, which had a mass ∼588 Da smaller than the wild-type protein. Some free PCB (mass = 588 Da) was tentatively detected in the phycobilisome fraction purified from the mutants. The modified PC from the cpcS-I, cpcU, and cpcS-I cpcU mutant strains was purified, and biochemical analyses showed that Cys-153 of CpcB carried a PCB chromophore but Cys-82 did not. These results show that both CpcS-I and CpcU are required for covalent attachment of PCB to Cys-82 of the PC subunit. SDS-PAGE and mass spectrometry demonstrated that the products of the cpcS-I and cpcU genes form heterodimers (1–3). Toroid-shaped trimers (αβ)3 and hexamers (αβ)6 form the fundamental assembly units of PCB. The precise supramolecular architecture of PBS, as well as the specific locations of the PBPs within them, is mediated by so-called linker proteins (1–3). PCB structures exhibit high similarity in the overall structure, pigment binding environment, and subunit interactions (12–16).

PBPs owe their brilliant colors and light absorption properties to the presence of linear tetrapyrrole prosthetic groups, phycobilins, which are covalently attached to PBPs through thioether linkages to highly conserved cysteine residues (1–3). Each AP subunit type carries a single phycocyanobilin (PCB) chromophore at Cys-82 (12, 13), but in PC one PCB is attached to Cys-84 of the α subunit (α-PC, CpcA) and two PCB chromophores are attached to Cys-82 and Cys-153 of the β subunit (β-PC, CpcB) (14–16). Genetic and biochemical studies have shown that the products of the cpeE and cpeF genes form a heterodimeric lyase that specifically attaches PCB to Cys-82 of α-PC (17–21). Intergenic suppressors of cpeE and cpeF mutant strains of Synechococcus sp. PCC 7002 were characterized that allowed α-PC to be chromophorylated by another unidentified lyase(s) present in the cells (17, 18).

In the cyanobacterium Fremyella diplosiphon, a cpeCDESTR operon was sequenced and some of the genes were shown to function in the biogenesis of phycoerythrin (PE) (22). The cpeCDE operon encodes three PE-associated linker proteins that are responsible for the proper assembly of PE into PBS. The cpeR gene product was proposed to encode an activator for control of expression of the cpeBA operon (22), but the roles of the cpeS and cpeT products were not identified. Analysis of the genome sequences of Synechococcus sp. PCC 7002 and other...
strains that do not synthesize PE revealed paralogs, whose products displayed very high sequence similarity to CpeS and CpeT (23). Paralogs of cpeS and cpeT were present in all PBP-containing cyanobacteria, were absent in organisms that do not synthesize PBP, and were encoded within operons with other PBP-related genes. Because these observations suggested that these gene products were involved in PBP synthesis, it seemed plausible that the CpeS and CpeT paralogs might be PBP lyases for PC and/or AP (23, 24). Because Synechococcus sp. PCC 7002 does not synthesize PE, the three proteins from Synechococcus sp. PCC 7002 with sequence similarity to CpeS were named CpcS-I, CpcU, and CpcV, and the single protein with similarity to CpeT was named CpcT (23, 24). Mutational analyses and in vitro studies showed that CpcT is the lyase that attaches PCB to Cys-153 of β-PC (24). Recently, Zhao et al. (25, 26) reported that a CpeS-like protein from Anabaena sp. PCC 7120 can attach PCB to β-PC, the β subunit of phyceroerythrocyanin, and the ApcA, ApcB, ApcD, and ApcE subunits.

In this report we describe the characterization of single, double, and triple mutants of the cpcS-I, cpcU, cpcV, and cpcT genes of Synechococcus sp. PCC 7002. Our results show that, in this cyanobacterium, a heterodimer of CpcS-I and CpcU functions together as the lyase for the attachment of the PCB chromophore at Cys-82 to Cys-81/82 Phycobiliprotein Lyase.

**EXPERIMENTAL PROCEDURES**

**Cultures and Growth Conditions**—The wild-type and mutant strains of Synechococcus sp. PCC 7002 were grown in medium A supplemented with 1 mg NaNO₃ ml⁻¹ (denoted medium A⁺) as previously described (24, 28). Growth of the wild-type and mutant strains was monitored turbidometrically at 730 nm. The mutant strains were adapted to and grown in A⁺ medium with 10 mM glycerol as a supplemental carbon source to increase the growth rate. Additionally, the medium was supplemented with the appropriate antibiotic(s) at concentrations of 50 µg of gentamicin ml⁻¹, 100 µg of kanamycin ml⁻¹, and 50 µg of spectinomycin ml⁻¹.

**DNA and Protein Sequence Analysis**—The DNA sequences of the cpcS-I, cpcU, and cpcV genes from Synechococcus sp. PCC 7002 were deduced from the complete genome sequence of this organism. The nucleotide sequences of these three genes have been deposited in GenBank® as accessions EU145731, EU145732, and EU145733. The amino acid sequence data of homologs of CpcS-I and CpeS proteins was obtained from the Integrated Microbial Genomes of the DOE Joint Genome Institute (img.jgi.doe.gov/cgi-bin/pub/main.cgi) and from Cyanobase. Searches for protein homologs in databases were carried out with the blastP program at www.ncbi.nlm.nih.gov/BLAST/. Analyses of DNA and protein sequences were conducted using MacVector (Accelrys, San Diego, CA). Phylogenetic analyses were performed using the PAUP Phylogenetic Analysis program (Sinauer Associates, Inc., Sunderland, MA).

**Construction of the Mutant Strains of the cpcS-I, cpcU, and cpcV Genes**—For mutagenesis of the cpcS-I gene, a 1.4-kb EcoRI/PstI DNA fragment encoding the cpcS-I gene was amplified by PCR with primers 5'TCTTTGGCTTGTCGAG-GCTCAGGTC-3' and 5'GTAAAGAATTTAACGGAT-TCCGAC-3' from the genome of Synechococcus sp. PCC 7002, cloned, and re-sequenced. To generate a construct for insertional inactivation of the cpcS-I gene, a 1.1-kb gentamicin-resistance cartridge, encoding the aacCl gene, was inserted into the unique Nhel site within the cpcS-I gene (supplemental Fig. S1A, left panel). The construct was used to transform wild-type cells of Synechococcus sp. PCC 7002 as previously described (29, 30). Segregation of the wild-type and mutant alleles for cpcS-I was verified by PCR amplification (supplemental Fig. S1A, right panel). To generate a construct for insertional inactivation of cpcU, an 1140-bp DNA fragment encoding the cpcU gene was amplified with primers 5'GTATCTATGCTGGAT-CGGGCTCAAT-3' and 5'CCTCAGCGCGAAAAGCTTATTATG-3' from the genome of Synechococcus sp. PCC 7002 and cloned into pUC19. A 1.3-kb DNA fragment, encoding the aphII gene and conferring kanamycin resistance, was subsequently inserted into the unique EcoRV site within the cpcU coding sequence (supplemental Fig. S1B, left panel). The cpcU:aphII construct was used to transform wild-type cells of Synechococcus sp. PCC 7002. Segregation of the wild-type and mutant alleles for cpcU was verified by PCR amplification (supplemental Fig. S1B, right panel).

To generate the cpcS-I cpcU mutant, an 810-bp DNA fragment, encoding the cpcv gene, was amplified by PCR with primers 5'TGAAGACCTTGATCCCTGG-GGCTCC-3' and 5'GCCGATCCGATAAGCCTTACACAA-C3' from the genome of Synechococcus sp. PCC 7002 by PCR and cloned into pUC19. A 1.0-kb cartridge, encoding the aaadA gene and conferring spectinomycin and streptomycin resistance, was inserted at the unique Stul site in the cpcv gene (supplemental Fig. S1C, left panel). The cpcv::aadA construct was used to transform wild-type cells of Synechococcus sp. PCC 7002. Segregation of the wild-type and mutant alleles for cpcv was verified by PCR amplification (supplemental Fig. S1C, right panel).

To generate the cpcS-I cpcU mutant, the cpcU:aphII construct was used to transform cells of the cpcS-I::aadCl mutant, and transformants resistant to both kanamycin and gentamicin were selected. Segregation of the wild-type and mutant alleles for cpcU in the cpcS-I inactivation background was verified by PCR analysis of the cpcU locus (supplemental Fig. S1B, right panel). The cpcS-I cpcU mutant was constructed by transforming the cpcS-I cpcU mutant with a cpcT::aadA construct and selecting for resistance to kanamycin, gentamicin, and spectinomycin (24). Segregation of the cpcT and cpcT::aadA alleles in the cpcS-I cpcU mutant background was confirmed by PCR analysis (data not shown). The cpcS-I cpcU cpcv mutant was constructed by transforming cpcS-I cpcU mutant cells with the cpcv::aadA construct described above and selecting for resistance to kanamycin, gentamicin, and spectinomycin (supplemental Fig. S1C, right panel).

**DNA Isolation and RNA Purification**—Chromosomal DNA from Synechococcus sp. PCC 7002 wild-type and mutant strains was isolated as described previously (29, 30). DNA sequencing was carried out at the Penn State Nucleic Acid Facility. Total
RNA was extracted from cells harvested from liquid cultures of *Synechococcus* sp. PCC 7002 strains by using the Micro-to-Midi Total RNA Purification Kit from Invitrogen as described by the manufacturer. The procedure was modified to include the use of a bead-beater and glass beads to disrupt cells. The sample was incubated with RNase-free DNase I for 1 h at room temperature to remove contaminating DNA. RNA concentrations were determined by absorbance, and the absence of DNA in the RNA samples was confirmed by PCR analyses.

The OneStep RT-PCR kit (Qiagen) was used to examine mRNA abundance by reverse transcription-PCR (RT-PCR). RT-PCR reactions were programmed with a 30-min reverse-transcription reaction at 50 °C, a 15-min initial heating step at 95 °C, and 35 three-step cycles (92 °C, 1 min; 55 °C, 1 min; and 72 °C, 1 min). The RT-PCR products were separated on 1.0% (w/v) agarose gels.

Isolation of Phycobilisomes and Phycobiliproteins—Intact PBSs were isolated on sucrose gradients as described previously (24). Blue-colored PBS bands (see supplemental Fig. S2) resolved on the sucrose gradients were collected and further purified on DEAE-Sepharose columns as described (24).

Absorption and Fluorescence Spectroscopy—Absorption and fluorescence spectra of isolated PBS, PBP fractions, and cell suspensions measured as previously described (24). Fluorescence excitation and emission spectra were measured with an upgraded SLM Model 8000C spectrophotometer (ThermoSpectronic, Rochester, NY) as described (24, 29). Absorption spectra of whole cells and the purified PBS and PBP were measured using a GENESYS 10 spectrophotometer (ThermoSpectronic, Rochester, NY).

Quantitation of Chlorophyll and PBP—Pigments were extracted from cells with 100% methanol to determine the chlorophyll (31, 32) and carotenoid (33) contents of cells. Concentrations were determined on the basis of equivalent cell numbers (equal $A_{730}$ nm values). To measure cellular PBP contents, cells in late-exponential growth phase ($A_{730}$ nm = 1.5–2.0) were collected and adjusted to 0.5 $A_{730}$ nm per milliliter. The reduction in the PBP absorbance at 635 nm of liquid-culture samples that had been heated at 65 °C for 5 min was used to calculate the PBP content as described (34, 35).

Gel Electrophoresis and Immunoblotting Analyses—SDS-PAGE was performed on 10–22% (w/v) acrylamide gradient gels as described (36). Resolved proteins were visualized by staining with Coomassie Blue. PBP were also specifically visualized by zinc-enhanced fluorescence emission as described (24, 37). Immunoblotting was performed as described previously (36). Proteins separated by SDS-PAGE were transferred electrophoretically onto nitrocellulose membranes (Schleicher & Schuell, Keane, NH). The resulting membranes were incubated with rabbit polyclonal antibodies against *Synechococcus* sp. PCC 7002 CpcA, CpcB, or ApcB.

Quantitation of PBP by Immunoblotting—To compare the PBP contents of cells, liquid cultures of wild-type and mutant strains of *Synechococcus* sp. PCC 7002, cells were grown to late-exponential phase, harvested by centrifugation, and resuspended in 50 mM Tris-HCl, pH 8.0. Cells of the wild type and mutants were loaded at 0.08 $A_{730}$ nm (corresponding to $\approx 1.1 \times 10^7$ cells) per lane as the 1× dilution. The wild-type cells were loaded at several further dilutions. The proteins were resolved by SDS-PAGE and transferred onto nitrocellulose membranes as described above. Specific polypeptides were detected by using the polyclonal antibodies directed against the ApcB, CpcA, and CpcB proteins of *Synechococcus* sp. PCC 7002. The staining intensities of specific protein bands were measured using the program ImageJ (National Institutes of Health, Bethesda, MD) and were compared with the dilutions of the wild type to estimate the PBP contents of mutants.

HPLC-Mass Spectrometry—Mass spectrometry (MS) was performed on a Quattro II–Z Mass Spectrometer (Micromass, Beverly, MA) equipped with LC10ADvp pumps (Shimadzu, Columbia, MD) and an HTC Pal autosampler (Leap Technologies, Carrboro, NC). The analyses were performed using electrospray ionization (ESI) in positive ion mode with a cone potential of 20 V, and spectra were acquired over $m/z$ 500–20,000 at 3.6 s/scan. Proteins were separated by HPLC with a reverse-phase 1 × 150 mm BioBasic C$_18$–reverse-phase column (Keystone Scientific, Bellefonte, PA) and step water/isopropanol gradient (plus 0.15% formic acid).

RESULTS

Comparative Bioinformatics Analysis—Paralogs of the CpeS and CpeT proteins of *F. diplosiphon* are found among the core set of proteins common to all cyanobacteria that produce PBSs, and most interestingly, paralogs occur in strains that do not synthesize PE (Fig. 1 and supplemental Fig. S3). CpeS is also similar to the products of conserved hypothetical genes ycf58 (orf149) of the red algal chloroplast genomes of *Porphyra purpurea* (38) and *Porphyra yeezoensis*, which synthesize PE, but a homolog is also found in the chloroplast genome of *Cyanidium caldarium*, which can only synthesize PC and AP (39). Using the sequence of the CpeS protein of the PE-containing cyanobacterium *F. diplosiphon* as the query (22), three unlinked open reading frames with 40–51% sequence similarity to CpeS were identified in the genome of *Synechococcus* sp. PCC 7002, a strain that only synthesizes AP and PC (supplemental Fig. S3). Based on phylogenetic analyses and the characterization of mutant strains described below, we designate these genes as *cpcS-I*, *cpcS-II*, and *cpcS-III*.

Using the products of these three genes to search the databases, orthologs and paralogs from different cyanobacteria were identified, aligned, and clustered to produce a more complete phylogenetic analysis. As shown in Fig. 1, homologs of the CpcS and CpeS proteins can be classified into five groups. Two of these groupings, Groups A and B, only include strains that can synthesize one or more PEs. This observation, and the further observation that these genes are usually clustered with other genes known to play a role in PE biosynthesis or assembly, suggests that these proteins, denoted CpeS and CpeU, respectively, probably play roles in phyceroerythrobilin (PEB) attachment to PE subunits. All of the other groups contain at least some members that only synthesize proteins with PCB chromophores, and thus these proteins cannot be involved in PE synthesis, at least in some organisms. The largest of these groups, Group C, can be further divided into three clades, which we have denoted CpcS-I, CpcS-II, and CpcS-III. *Synechococcus* sp. PCC 7002 has a protein within the CpcS-I clade. With only a single exception, *Nostoc punctiforme*, all organisms
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FIGURE 1. Phylogenetic analysis of the CpcS-I, CpcS-II, CpcS-III, CpcU, CpcV, CpeU, and CpeS proteins from different cyanobacteria. Sequence alignments and comparisons were made on CpeS-like homologs from Synechococcus sp. PCC 7002 (7902), Synechocystis sp. PCC 6803 (6893), Thermosynechococcus elongatus BP-1 (BP-1), Gloeobacter violaceus PCC 7421 (7421), Synechococcus elongatus PCC 7942 (7942), Synechococcus sp. PCC 6301 (6301), Synechococcus sp. WH8102 (8102), Synechococcus sp. WH8103 (8103), Synechococcus sp. BL107 (8107), Synechococcus sp. CC9311 (9311), Synechococcus sp. CC9902 (9902), Synechococcus sp. CC9605 (9605), Synechococcus sp. JA-3-3Ab (JA3Ab), Synechococcus sp. JA-2-3Ba (JA23Ba), Synechococcus sp. WH7803 (7803), Synechococcus sp. WH7805 (7805), Synechococcus sp. RJ9916 (9916), Synechococcus sp. CCY9414 (9414), Synechococcus sp. WH7501 (7501), Crocosphaera watsonii WH8501 (8501), Nostoc sp. PCC 7120 (7120), N. punctiforme PCC 73102 (73102), Anaabaena variabilis ATCC 29413 (29413), Trichodesmium erythraeum IMS101 (IMS101), Lyngbya sp. PCC 8106 (8106), Cyanotothece sp. CCC1 0110 (0110), Nodularia spumigena CCY9414 (9414), Calothrix PCC 7601 (Fremyella diplosiphon) (7601), Prochlorococcus marinus SS120 (8102), P. marinus MIT9312 (9312), P. marinus MIT9313 (9313), P. marinus MIT9301 (MIT9301), P. marinus MIT9303 (MIT9303), Prochlorococcus sp. AS9601 (AS9601), P. marinus MIT9515 (MIT9515), P. marinus MIT9211 (MIT9211), P. marinus SS120 (CCMP1375) (8120), P. marinus NATL1A (NATL1A), and P. marinus NATL2A (NATL2A). The sequence alignment was made using the ClustalW feature of MacVector version 9.0 (MacVector, Inc., Cary, NC). The phylogenetic tree was generated using the PAUP phylogenetic analysis program.

with a protein in the CpcS-I clade also have a protein in Group D, which we have designated as CpeU. As will be shown below, CpcS-I and CpeU together form a heterodimeric PCB lyase for β-PC and AP subunits. Clade III of Group C includes the Nostoc sp. PCC 7120 open reading frame alr0617, the product of which has been shown by Zhao et al. (25, 26) to ligate PCB to Cys-82 of CpcB, PeCB, ApC, ApC, ApC, and ApC. With the exception of N. punctiforme, no organism with a CpcS-III protein also has a protein in Group D (CpeU). The CpcS-II clade includes a variety of marine Synechococcus sp., some of which produce PC with PEB chromophores (40). Finally, Group E includes several divergent sequences, and the organisms that produce the members of this clade do not have an obvious PCB pigmentation pattern. Synechococcus sp. PCC 7002 produces one of the members of this clade; we have provisionally designated these proteins as CpcV.

1, the PBP contents of the cpcS-I, cpcU, cpcS-I cpcU, and cpcS-I cpcU cpcT mutants were significantly reduced compared with the PC content in the wild-type cells. The chlorophyll contents of these mutants were also slightly reduced (~30%) relative to that of wild-type cells. On the other hand, the carotenoid contents of these mutant strains were significantly higher (46–93%) than in wild-type cells (see Table 1). In all cases, the cpcV mutant was most similar to the wild type in the properties measured. No obvious additional difference could be observed when the cpcV gene was insertionally inactivated in a cpcS-I cpcU double mutant background (data not shown).

To check whether inactivation of the cpcS-I gene had any effect on the transcript levels for genes encoding other PBS components, RT-PCR was used to compare the expression of the cpcA and cpcB genes encoding for α-PC and β-PC subunits, the cpcA and cpcB genes encoding the α-AP and β-AP sub-
units, and the pcyA gene, encoding phycocyanobilin:ferredoxin oxidoreductase (supplemental Fig. S4). There was no significant difference in the mRNA levels for the cpcBA, apcAB, and pcyA genes in the wild-type and cpcS-I mutant cells. These results suggested that the greatly decreased PBP content of the cpcS-I mutant was not due to lower transcript levels for the structural genes encoding the major PBP subunits or to a reduced transcript level for the enzyme that produces PCB but is more likely due to a post-transcriptional defect in translation or maturation of the PBP.

**Fluorescence Emission Spectra**—Changes of PBP levels within cells can be revealed and compared by fluorescence emission spectroscopy at low temperature. Fluorescence emission spectra at 77 K were measured for intact cells of the wild-type and the single, double, and triple mutants (Fig. 2). Upon excitation of the PBP at 590 nm, the wild-type cells displayed three major fluorescence emission peaks (Fig. 2, A and B). The emission peak at 645 nm is mostly due to PC, the 665 nm emission peak is mostly due to AP, and the 680 nm emission peak is mostly due to fluorescence emission from the terminal emitters of the PBS (ApCD and ApCE) as well as chlorophylls associated with PS II (11). Based upon a comparison of the fluorescence emission from equal numbers of cells, the fluorescence amplitude was obviously reduced, especially from the 645 nm emission from PC, in cells of the cpcS-I and cpcU mutants, but not in cpcV mutant cells (Fig. 2A). In the emission spectra of the cpcS-I and cpcU mutants, a new, small emission peak was present at ~620 nm; as will be shown below, this likely originates from one of the “donor” bilins from PC (i.e. the PCB chromophore at α-Cys-84 or β-Cys-153). The reduction in the 680 nm peak in the mutant cells suggests that the efficiency of light energy transfer from PC to the terminal emitter and PS II reaction centers is significantly impaired in the CpcS-I and CpcU mutants. Although the effect on PC emission was most pronounced in the mutants, the reduction of the fluorescence emission amplitude at 665 nm and 680 nm in the mutant cells might also indicate that the number of PBS cores assembled in the cpcS-I and cpcU mutants was lower than in wild-type cells. Therefore, a deficiency of CpcS-I and CpcU leads to a significantly decreased capacity for light harvesting and impairment of excitation energy-transfer efficiency in PBS.

As shown in Fig. 2B, upon excitation of the PBP, the fluorescence emission spectrum of the cpcS-I cpcU double mutant was similar to those of the cpcS-I and cpcU single mutants. However, upon the additional inactivation of the cpcT, the cpcS-I cpcU cpcT triple mutant exhibited a further significant depletion of fluorescence emission from PBP. This is clearly indicated by the small emission peak at 665 nm from AP and the appearance of two emission peaks at 683 nm and 695 nm from chlorophylls of PS II due to excitation of chlorophylls (Fig. 2B).

Low temperature fluorescence emission spectra, measured with excitation of chlorophyll at 440 nm, showed that the ratio of PS II to PS I was altered in cells of the cpcS-I, cpcU, cpcV, cpcS-I cpcU, and cpcS-I cpcU cpcT mutant strains (Fig. 2, C and D). For wild-type cells, the 715 nm emission occurs from chlorophylls associated with PS I complexes; the 695 nm emission is mostly from chlorophylls associated with CP47 (PsbB) of PS II, and the 685 nm emission is mostly from

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**TABLE 1**

Physiological properties of *Synechococcus* sp. PCC 7002 wild-type and mutant strains

| Strain            | Doubling time (h) | PBP content (relative to WT) | Chlorophyll content µg ml⁻¹ | Carotenoid content µg ml⁻¹ |
|-------------------|-------------------|------------------------------|-----------------------------|---------------------------|
| Wild type         | 3.6 ± 0.3         | 100                          | 4.5 ± 0.1                   | 1.5 ± 0.1                 |
| cpcS-I            | 31.4 ± 0.8        | 29                           | 3.2 ± 0.1                   | 2.2 ± 0.2                 |
| cpcU              | 33.7 ± 1.2        | 31                           | 3.3 ± 0.2                   | 2.4 ± 0.1                 |
| cpcV              | 3.9 ± 0.4         | 96                           | 4.3 ± 0.2                   | 1.6 ± 0.1                 |
| cpcS-I cpcU       | 32.6 ± 0.9        | 25                           | 3.1 ± 0.1                   | 2.5 ± 0.2                 |
| cpcS-I cpcU cpcT  | 48.1 ± 1.2        | 125                          | 2.7 ± 0.2                   | 2.9 ± 0.1                 |
| cpcS-I cpcU cpcV  | 31.8 ± 1.1        | 29                           | 3.6 ± 0.2                   | 2.3 ± 0.2                 |

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**FIGURE 2.** Low temperature fluorescence emission spectra of the *Synechococcus* sp. PCC 7002 wild-type and mutant strains. A, 77 K fluorescence emission spectra from the wild-type (solid line), cpcS-I mutant (dotted), cpcU mutant (short dashes), and cpcV mutant (long dashes) with excitation of mostly PBP at 590 nm. B, 77 K fluorescence emission spectra from the wild-type (solid line), cpcS-I cpcU mutant (dotted), cpcS-I cpcU cpcT mutant (short dashes), and cpcS-I cpcU cpcV mutant (long dashes) with excitation of mostly PBP at 590 nm. C, 77 K fluorescence emission spectra from the wild-type (solid line), cpcS-I cpcU mutant (short dashes), and cpcS-I cpcU cpcT mutant (short dashes), and cpcS-I cpcU cpcV mutant (long dashes) with excitation of mostly chlorophyll a at 440 nm. D, 77 K fluorescence emission spectra from the wild-type (solid line), cpcS-I cpcU mutant (dotted), cpcS-I cpcU cpcT mutant (short dashes), and cpcS-I cpcU cpcV mutant (long dashes) with excitation of mostly chlorophyll a at 440 nm. Cells were adjusted to same cell densities at 0.5 A₃₄₀ nm, ml⁻¹. Each spectrum is the average of four independent measurements.
CP43 (PsbC) of PS II (29). Reflecting a reduction in PS I content relative to wild-type cells, the fluorescence emission amplitude at 715 nm was lower in cells of the cpcS-I, cpcU, and cpcS-I cpcU mutants. Consistent with the observed reduction in chlorophyll content noted above, the cells of the cpcS-I cpcU cpcT triple mutant exhibited a greatly decreased fluorescence emission from PS I (Fig. 2D). However, increases in the amplitudes of the PS II-related chlorophyll fluorescence emission peaks were also obvious in all mutant cells except for the cpcV mutant. This might be due to an increase in the cellular content of PS II complexes to compensate for the loss of light-harvesting capacity due to the lowered PBP content of the cells (Table 1). A similar phenotype was previously observed in cells of a PC-less mutant (∆cpcBAC) of Synechococcus sp. PCC 7002 (41).

Composition of PBS—PBS were isolated from the wild type and each of the mutant strains of Synechococcus sp. PCC 7002. As shown in supplemental Fig. S2, intact PBS from the wild-type cells were recovered in the lower regions of the sucrose gradients, and only a small amount of free PBP was recovered in the upper gradient fractions. However, for the cpcS-I, cpcU, and cpcS-I cpcU mutant strains, only a very small amount of PBP was recovered from the lower blue band of the sucrose gradients. Suggesting that these PBP assemblies were smaller than wild-type PBS, the PBP associated with this band did not migrate as far into the sucrose gradients as wild-type PBS. These results showed that PBS assembly was severely impaired by a deficiency of either CpcS-I and/or CpcU. Demonstrating that no intact PBS could be assembled when the cpcS-I, cpcU, and cpcT genes were inactivated, PBP were exclusively recovered from the upper gradient fraction for the triple mutant.

The isolated PBS complexes and PBP from wild-type and cpcS-I, cpcU, cpcS-I cpcU, and cpcS-I cpcU cpcT mutant strains were further analyzed by SDS-PAGE to compare their PBP compositions. Compared with wild type, the PBS complexes isolated from the cpcS-I mutant strain contained a new polypeptide that migrated slightly faster than β-PC. This polypeptide, visualized by Coomassie Blue staining, is labeled β-PC* in Fig. 3A; it contained at least one PCB chromophore, because it was fluorescent after zinc enhancement (Fig. 3B).

### TABLE 2

| Subunit* | Wild type | cpcS-I mutant | Calculated mass |
|----------|-----------|---------------|-----------------|
| α-AP     | 17,741    | 17,741        | ApaC ± 1 PCB = 17,742 |
| β-AP<sup>a</sup> | 17,817 | 17,808        | ApaB + (CH3) + 1 PCB = 17,824 |
| β-PC<sup>b</sup> | 18,208 | 18,207        | Cpa + 1 PCB = 18,210 |
| β-AP<sup>c</sup> | 19,522 | 19,520        | CpaB + (CH3) + 1 PCB = 19,526 |
| β-PC<sup>d</sup> | ND<sup>e</sup> | 18,933 | CpaB + (CH3) + 1 PCB = 18,938 |
| PCB      | ND<sup>e</sup> | 587.5         | 588.3           |

<sup>a</sup> The mass data indicate that the N-terminal Met residue is removed from ApaC (α-AP) but not from the other polypeptides.

<sup>b</sup> ApaB and CpaB polypeptides are methylated on the amide nitrogen of Asn residue 72, so 14 Da was added to ApaB and CpaB (42–44).

<sup>c</sup> ND, not detected.

The identity of this polypeptide as β-PC/CpaB was shown by immunoblot analysis with anti-CpaB antibodies (Fig. 4). Two β-PC polypeptides were detected for all PBP fractions from the cpcS-I mutant, but the α-PC/CpaA subunit was unaffected in these mutants. The same results described for the cpcS-I mutant were observed for the PBS complexes isolated from the cpcU and cpcS-I cpcU mutant strains (Fig. 3C). These results suggested that the cpcS-I and cpcU genes play one or more important roles in the specific maturation of the β-PC protein and further suggested that the β-PC* protein might be missing a PCB chromophore. The cpcS-I cpcU cpcT triple mutant did not accumulate any detectable PC; neither the α-PC nor the β-PC subunits were detected by immunoblot analysis (Fig. 4B).

**HPLC-ESI-MS Analyses—**HPLC separation of polypeptides with mass analysis by ESI-MS was performed to identify the masses of the components of the PBS complexes isolated from the cpcS-I mutant. As shown in Table 2 for wild-type PBS, two PC subunits and two AP subunits were identified with molec-
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FIGURE 5. Absorption and room temperature fluorescence emission spectra. Absorption (A) and room temperature fluorescence emission spectra (B) of purified PC from wild-type and the mutant strains. The excitation wavelength in (B) was 590 nm. To facilitate the comparison, all spectra were normalized to the same amplitude as the wild type. PC from the wild-type (solid line), cpcS-I mutant (dotted), cpcU mutant (small dashes), and cpcS-I cpcU mutant (long dashes).

ular masses of 19,522 Da (β-PC), 18,208 Da (α-PC), 17,817 Da (β-AP), and 17,741 (α-AP), respectively. Each of these masses matched the expected masses for these polypeptides when all known post-translational modifications, i.e. when the appropriate number of PCB chromophores (588 Da each), asparagine N-methylation of the β-72 residues (42–44), and removal of the N-terminal methionine residue for ApcA were taken into account (Table 2). For the PBS complexes of the cpcS-I mutant isolated from the lower band of sucrose gradients, an extra polypeptide with a molecular mass of 18,933 Da was detected (Table 2). The molecular mass of this new component matched that expected for β-PC minus the mass of one PCB chromophore. Interestingly, a compound with a mass of 587.5, which corresponds to the mass of the free PBS chromatophore, was detected in the PBS preparation from the wild type and from the mutant; no compound with this mass was detected in PBS preparations from the wild type (Table 2). Therefore, the HPLC-ESI-MS analyses showed that the β-PC polypeptide identified by SDS-PAGE of PBS preparations from the cpcS-I mutant was missing one PCB chromophore. In addition, the HPLC-ESI-MS results suggest that at least some of the β-PC* within the PBS of the cpcS-I mutant carried a PCB chromophore that was tightly bound but not covalently attached to the protein.

Purification and Characterization of the β-PC* Protein—To analyze the β-PC* protein further, PC from the cpcS-I mutant was purified by anion-exchange chromatography. The purity of the PC in the fractions was analyzed by SDS-PAGE; subunits were diagnosed by zinc-enhanced bilin fluorescence (supplemental Fig. S5A) and immunodetection using antibodies to CpcB (supplemental Fig. S5B). For wild type, only the α-PC and β-PC proteins were detected in fractions 1–4 eluted from the column with increasing NaCl concentrations. However, for the cpcS-I mutant, fractions 1 and 2 contained PC complexes (αβ-PC*) formed from the α-PC and (β-PC*) subunits, whereas later fractions contained some apparently wild-type PC complexes (αβ-PC). The same procedure was employed to purify the modified PC (αβ-PC*) from the PBS preparations of the cpcU and cpcS-I cpcU mutants (data not shown).

Absorption and fluorescence emission spectroscopy was employed to compare the purified PC complexes of wild type (αβ-PC) and the αβ-PC* proteins of the cpcS-I, cpcU, and cpcS-I cpcU mutant strains. As shown in Fig. 5A, the PC purified from wild type exhibited an absorption spectrum with a maximum at 625 nm. However, the absorption maxima for the αβ-PC* complexes of the cpcS-I, cpcU, and cpcS-I cpcU mutants were blue-shifted by 20 nm to 605 nm. The missing chromophore on β-PC* in the cpcS-I, cpcU, and cpcS-I cpcU mutants is likely responsible for this 20 nm blue-shift. Room temperature fluorescence emission spectra were also recorded for the purified PC of the wild type and mutants. As shown in Fig. 5B, two obvious differences were observed. The emission maxima of the mutant αβ-PC* complexes had a 4 nm blue-shift to 644 nm from 648 nm of the wild-type αβ-PC, and there was a significant increase in the emission at 620 nm. These results suggested that the PCB at Cys-82 was missing. Energy from the donor bilin attached to Cys-153 was not transferred efficiently within the trimer, resulting in the increased fluorescence observed at 620 nm (45). If there had been a non-covalently bound PCB in the binding site for the Cys-82 chromophore, the fluorescence emission from this bilin should have been red-shifted as previously observed for site-directed mutants of ApoE (10, 11, 20). Because this was not observed, it is likely that the non-covalently bound PCB that had been to the β-PC* in the PBS had been removed during the purification procedure.

Formic Acid Cleavage of the β-PC and β-PC* Proteins—The β-PC subunit has a unique peptide bond between residues Asp-144 and Pro-145 that can be cleaved by dilute formic acid. Cleavage at this site was previously used to verify which PCB chromophore was missing in the β-PC* of a cpcT mutant (24). The purified αβ-PC* proteins were incubated overnight in 70% (v/v) formic acid and analyzed by SDS-PAGE (Fig. 6). Peptides with covalently bound PCB were visualized by zinc-enhanced bilin fluorescence. For wild-type β-PC, the polypeptide was cleaved into two peptides, and each carried one PCB chromophore (Fig. 6, lane 1): a 144-residue peptide was produced from the N terminus, and a 28-residue peptide from the C terminus. However, for β-PC* from the cpcS-I mutant, the larger, 144-residue, N-terminal peptide was not fluorescent (Fig. 6, lane 2), indicating that no PCB chromophore was attached to the Cys-82 site of β-PC*. The remaining PCB was covalently bound to Cys-153 on the 28-residue, C-terminal peptide derived from β-PC*. Identical results were obtained after formic acid cleavage analysis of the αβ-PC* complexes purified from the cpcU mutant (Fig. 6, lane 3) and from the cpcS-I cpcU double mutant (Fig. 6, lane 4). Thus, the absence of either CpcS-I, CpcU, or both proteins resulted in a defect in PCB chromophore attachment to Cys-82 of the β-PC subunit.

Quantitation of β-AP (ApcB) Levels—In the fluorescence emission spectra described above, it appeared that AP fluorescence emission was significantly reduced in the mutants in comparison to that for wild-type cells (Fig. 2A). To verify if this was indeed the case, the AP contents of wild-type and mutant cells were compared by immunoblotting with antibodies to
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β-AP (ApcB) (Fig. 7). The cpcS-I cpcU mutant cells contained only ~27% of wild-type levels of β-AP; the cpcS-I cpcU cpcT mutant contained only ~7% of wild-type ApcB levels; and the cpcS-I cpcU cpcV mutant contained only ~13% of wild-type β-AP levels. In contrast, a ΔcpcBAC mutant, which cannot synthesize PC apoproteins at all, contained ~70% of wild-type levels of β-AP (6, 41). If the CpcS-I and CpcU proteins were only involved in PCB attachment to Cys-82 on β-PC, there should have been a much smaller effect on β-AP levels than was observed. The ΔcpcBAC mutant produces relatively normal levels of AP in the complete absence of PC, and this AP can be assembled into stable PBS cores that are functional in light harvesting (6, 20, 41). Thus, these results strongly suggest that CpcS-I and CpcU are involved in PCB chromophore attachment to both AP and PC.

**DISCUSSION**

CpcS-I and CpcU: a Heterodimeric Lyase—Mutants lacking CpcS-I, CpcU, or both proteins displayed a similar phenotype physiologically: all strains had slower growth rates due to a 70–75% reduction of PBP, and all had impairment in PBS assembly. Additionally, these mutant strains accumulated a form of the β-PC (denoted β-PC*) that lacked a covalently bound PCB chromophore at Cys-82. These results indicate that CpcS-I and CpcU function together as the phycobilin lyase in attachment of the chromophore at the Cys-82 for β-PBPs in *Synechococcus* sp. PCC 7002, because neither gene product alone could attach the PCB chromophore to this binding site. These data are completely consistent with *in vitro* biochemical characterization of the recombinant proteins. CpcS-I and CpcU together are required to attach PCB to Cys-82 on β-PC, and the two proteins co-purify as a heterodimeric complex (27, 46). We have also shown that CpcS-I and CpcU from *Synechocystis* sp. PCC 6803 are also required for PCB addition to the β-PC of this strain in vitro (47).

These results are different from the results of Zhao et al. (25), who recently reported that a single CpeS-like protein, Alr0617, alone could catalyze the attachment of PCB to Cys-82 of β-PC and β-phycoerythrocyanin as well as several AP subunits (26) in *Nostoc* sp. PCC 7120. Similarly, in *Synechococcus* sp. PCC 7002 we show that the cpcS-I cpcU mutants are also affected in AP synthesis (Fig. 7), and in vitro assays show that the CpcS-I-CpcU lyase is responsible for PCB addition to α-AP and β-AP subunits (27). AP subunits only carry a single PCB, which is attached at Cys-81; these PCB chromophores are bound at the homologous positions to the addition site on β-PC and have the same R stereochemistry at C-3’ of the PCB (12).

These observations are consistent with the phylogenetic analyses that are presented in Fig. 1. Cyanobacteria can be divided into two groups on the basis of their PCB lysases for the Cys-82 position of β-PC. Some strains, including *Nostoc* sp. PCC 7120 and three thermophilic *Synechococcus* sp. (clade CpcS-III) and marine *Synechococcus* sp. (clade CpcS-II), apparently produce lyases composed of a single subunit (25, 26), whereas many other cyanobacteria produce a heterodimeric lyase composed of a CpcS-I subunit and CpcU. For the moment, only *N. punctiforme* (strain PCC 73102) appears to have proteins from two separate CpcS clades (clades I and III) and no CpcU protein. This is interesting, because this organism also has multiple copies of genes encoding PC and might use different lysases for these different substrate proteins or under specific growth conditions. Although no cpcU gene was found in the *N. punctiforme* genome, it should be noted that the genome sequence data presently in the databases are incomplete for this organism.

Based on sequence alignment analyses (supplemental Fig. S3), CpcS-I and CpcU share a high level of sequence similarity (~53%), especially in the N-terminal region containing the conserved FFXSXGXXWXXR motif and C-terminal region containing the conserved ERXWFXXPXRXR motif. These two conserved motifs within CpcS-I and CpcU might play important roles in catalyzing PCB binding or attachment or PBP substrate binding.

As shown in Fig. 1, the branch lengths for the CpcV sequences are rather long, and this indicates that the CpcV
sequences and function(s), which are currently unknown, are likely to be quite distinct from those of the other members of this protein family. A cpev single mutant showed no visible phenotype. Its PBP levels were unaffected (Table 1), and the mutant did not exhibit any defects in PBP degradation under nutrient starvation conditions (data not shown). However, the amount of ApCB in a cpcs-I cpcU cpev triple mutant was only ~50% that for the cpcs-I cpcU double mutant (Fig. 7); this suggests that the Cpev protein might participate in AP biosynthesis in the absence of Cpcs-I and CpcU. However, in vitro biochemical experiments with Cpev did not support this conclusion (27). Although Cpev is clearly a member of the Cpcs/CpeS protein family, this protein differs in two ways from Cpcs. Cpev carries a truncation of ~22 amino acids at its C terminus as well as an internal deletion of 7 amino acids. These differences could explain the structural and functional divergence of this protein from Cpcs and CpcU. Based on data base searches, the cpev gene is not universally distributed among PC-producing cyanobacteria (Fig. 1, Group E). For example, although Synechocystis sp. PCC 6803 has both cpcs-I and cpcU genes, it does not have a cpev gene. One possibility is that Cpev participates in PCB attachment to some protein that is not a PBP (e.g. a phytochrome-like protein) and has a limited distribution.

Cpcs in PE-containing Cyanobacteria—In the cyanobacterium Fremyella diplosiphon, the cpeS gene is encoded in an operon, cpcDESTR, which includes the genes encoding PBS-linker proteins for PE (22). Cpev was shown to be an activator for expression of the cpeBA (encoding the β and α subunits of PE) operon, and the cpeS and cpeT genes were proposed to be important for expression of cpeAB (22, 48). As shown in the phylogenetic analysis (Fig. 1), the Cpcs-like protein group together with other CpeS proteins from other PE-containing cyanobacteria. Because Cpcs-I and CpcS-III have been shown to be PC lyases, it is probable that the Cpcs protein is a lyase that attaches PEB to the Cys-82 position of β-PE. Zhao et al. (26) have reported that Cpcs-III of Nostoc sp. PCC 7120 can attach a PEB chromophore to both PE subunits. However, it seems unlikely that Cpcs-III-like proteins would perform this function in cells in which both PC and PE are being made at the same time, and Nostoc sp. PCC 7120 does not naturally synthesize proteins with PEB chromophores. Interestingly, a second, divergent group of CpeS-like proteins only occurs in strains that produce PE. The function of this clade of proteins, which are denoted CpeU in Fig. 1, is presently unknown; however, given the distribution of this protein among cyanobacterial strains and its co-localization with the genes for other PBP-related functions, CpeU almost certainly plays a role in PEB attachment to α-PE or β-PE. By analogy to the results for Cpcs-I and CpcU presented here and in the accompanying report (27), it seems plausible to suggest that a subgroup of CpeS proteins combine with CpeU to form a heterodimeric PEB lyase for the Cys-82 position of β-PE.

Assembly of PBS in Absence of Cpcs-I and CpcU—The bilin chromophores of PBP play extremely important functions in light-harvesting and energy transfer. As shown by site-directed mutagenesis of PBP genes and inactivation of bilin lyase genes, bilin attachment also plays an important role in the subunit structure, stability, and assembly of PBP (24, 45, 49–51). Characterization of the cpeE and cpeF mutants demonstrated that the absence of PCB on α-PC significantly reduces cellular PC content (17, 18). This suggests that the PCB bound to Cys-84 of the α-PC subunit is important for folding and assembly of PC. Similarly, it has been shown that the two PCB chromophores associated with β-PC subunit have a structural role in PBP assembly. The absence of PC at these positions reduces the monomer interaction between αβ, the unassembled and non-chromophorylated subunits are targeted for rapid degradation (49, 51).

Attachment of PCB to Cys-82, the chromophore binding site that is common to all PBP, is the most critical step in biogenesis, because this chromophore promotes proper subunit folding and αβ protomer stability (49). In vitro protein folding studies with apomyoglobin, a structurally related protein, have shown that helices E and F are disordered until insertion of the heme group in the region analogous to where PCB is attached at Cys-81/82/84 (52); helices E and F contain amino acids, which are involved in interaction with the PCB chromophore (14, 53). The role of the peripheral PCB at Cys-153 on β-PC is less critical. Examination of a site-directed mutant showed that PCB levels were reduced, but that more PC could be isolated from these mutants than from a mutant missing PCB at Cys-82 (45, 49). Similar results were observed when the cpeT gene, encoding the Cys-153 β-PC lyase, was inactivated; a 40% reduction in PCB occurred in the cpeT mutant cells (24). A more serious effect on PC and AP levels was observed in the cpcs-I and cpcU mutants (Table 1).

The PCB chromophore bound to Cys-82 plays a critical role in αβ monomer stability, and in PC trimers, hexamers, and rods, it is also the acceptor (fluorescing) bilin (54). This could provide an explanation for why we detected small amounts of chromophorylated Cys-82-PC significantly reduces cellular PC content (17, 18). This suggests that the PCB bound to Cys-84 of the α-PC subunit is important for folding and assembly of PC. Similarly, it has been shown that the two PCB chromophores associated with β-PC subunit have a structural role in PBP assembly. The absence of PC at these positions reduces the monomer interaction between αβ, the unassembled and non-chromophorylated subunits are targeted for rapid degradation (49, 51).

In conclusion, a bioinformatics and reverse genetics approach (this report) and in vitro biochemical
studies (27) together demonstrate that a heterodimer of CpcS-I and CpcU comprises the PC lyase for Cys-82 of β-PC and Cys-81 for the α and β subunits of AP in the cyanobacterium *Synechococcus* sp. PCC 7002. A gene duplication event gave rise to this heterodimeric lyase in some but not all cyanobacteria. With the completion of this study, the three required lyases for PC biogenesis in *Synechococcus* sp. PCC 7002 have been identified: CpcE/CpcF for Cys-84 on α-PC (17–21), CpcT for Cys-153 of β-PC (24), and CpcS-I/CpcU for Cys82 of β-PC (this study, 27). In a *cpcS-I cpcU cpcT* triple mutant, no intact PBS could be assembled, and although small amounts of chromophorylated Cys-82 of β-PC could be detected in the *cpcS-I* and *cpcU* mutants, no chromophorlated β-PC or apo-β-PC was detected in the triple mutant. Our results further show that the heterodimeric CpcS-I/CpcU-PC lyase attaches chromophores to the Cys-81 positions of ApCα and ApCβ (this study and the companion report (27)). It is not clear how the activities of these three distinct lyases are coordinated in vivo, or if there is a preferred order in which these enzymes must act in the synthesis of PC.

### REFERENCES

1. Glazer, A. N. (1989) *J. Biol. Chem.* **264**, 1–4
2. Sidler, W. A. (1994) in *The Molecular Biology of Cyanobacteria* (Bryant, D. A., ed.) pp. 139–216, Kluwer Academic Press, Dordrecht, The Netherlands
3. Bryant, D. A. (1991) in *Cell Culture and Somatic Cell Genetics of Plants, Vol. 7B: The Molecular Biology of Chloroplasts and Mitochondria* (Bograd, L., and Vasil, I., eds) pp. 255–298, Academic Press, New York
4. Gómez-Lojero, C., Pérez-Gómez, B., Shen, G., Schluchter, W. M., and Bryant, D. A. (2003) *Biochemistry* **42**, 13800–13811
5. de Lorimier, R., Bryant, D. A., Porter, R. D., Liu, W.-Y., Jay, E., and Stevens, S. E., Jr. (1990) *Arch. Microbiol.* **153**, 550–560
6. de Lorimier, R., Guglielmi, G., Bryant, D. A., and Stevens, S. E., Jr. (1990) *Arch. Microbiol.* **153**, 541–549
7. de Lorimier, R., Guglielmi, G., Bryant, D. A., and Stevens, S. E., Jr. (1990) *Biochim. Biophys. Acta* **1019**, 29–41
8. Maxson, P., Sauer, K., Bryant, D. A., and Glazer, A. N. (1989) *Biochim. Biophys. Acta* **974**, 40–51
9. Gindt, Y. M., Zhou, J., Bryant, D. A., and Sauer, K. (1992) *J. Photochem. Photobiol. B* **15**, 75–89
10. Gindt, Y. M., Zhou, J., Bryant, D. A., and Sauer, K. (1994) *Biochim. Biophys. Acta** **1186**, 153–162
11. Brejc, K., Ficner, R., Huber, R., and Steinbacher, S. (1995) *J. Mol. Biol.* **249**, 424–440
12. Reuter, W., Wiegang, G., Huber, R., and Than, M. E. (1999) *Proc. Natl. Acad. Sci. U.S.A.* **96**, 1363–1368
13. Schmidt, T., Bode, W., and Huber, R. (1987) *J. Mol. Biol.* **196**, 677–695
14. Schmidt, T., Bode, W., Huber, R., Sidler, W., and Zuber, H. (1985) *J. Mol. Biol.* **184**, 257–277
15. Schirmer, T., Huber, R., Schneider, M., Bode, W., Miller, M., and Hackert, M. L. (1986) *J. Mol. Biol.* **188**, 651–676
16. Zhou, J., Gasparich, G. E., Stirewalt, V. L., de Lorimier, R., and Bryant, D. A. (1992) *J. Biol. Chem.* **267**, 16138–16145
17. Swanson, R. V., Zhou, J., Leary, J. A., Williams, T., de Lorimier, R., Bryant, D. A., and Glazer, A. N. (1992) *J. Biol. Chem.* **267**, 16146–16154
18. Fairchild, C. D., Zhao, J., Zhou, J., Colson, S. E., Bryant, D. A., and Glazer, A. N. (1992) *Proc. Natl. Acad. Sci. U.S.A.* **89**, 7017–7021
19. Zhou, J. (1992) *Mutational Analysis of the Genes Encoding Phycobilisome Components in the Cyanobacterium Synechococcus* sp. PCC 7002, Ph.D. thesis, Pennsylvania State University, University Park, PA
20. Fairchild, C. D., and Glazer, A. N. (1994) *J. Biol. Chem.* **269**, 8686–8694