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A Role for *cpeYZ* in Cyanobacterial Phycoerythrin Biosynthesis

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Pigment mutant strain FdR1 of the filamentous cyanobacterium *Fremyella diplosiphon* is characterized by constitutive synthesis of the phycobiliprotein phycoerythrin due to insertional inactivation of the *rcaC* regulatory gene by endogenous transposon Tn*5469*. Whereas the parental strain Fd33 harbors five genomic copies of Tn*5469*, cells of strain FdR1 harbor six genomic copies of the element; the sixth copy in FdR1 is localized to the *rcaC* gene. Electroporation of FdR1 cells yielded secondary pigment mutant strains FdR1E1 and FdR1E4, which identically exhibited the FdR1 phenotype with significantly reduced levels of phycoerythrin. In both FdR1E1 and FdR1E4, a seventh genomic copy of Tn*5469* was localized to the *cpeY* gene of the sequenced but phenotypically uncharacterized *cpeYZ* gene set. This gene set is located downstream of the *cpeBA* operon which encodes the α and β subunits of phycoerythrin. Complementation experiments correlated *cpeYZ* activity to the phenotype of strains FdR1E1 and FdR1E4. The predicted CpeY and CpeZ proteins share significant sequence identity with the products of homologous *cpeY* and *cpeZ* genes reported for *Pseudanabaena* sp. strain PCC 7409 and *Synechococcus* sp. strain WH 8020, both of which synthesize phycoerythrin. The CpeY and CpeZ proteins belong to a family of structurally related cyanobacterial proteins that includes the subunits of the CpcE/CpcF phycocyanin α-subunit lyase of *Synechococcus* sp. strain PCC 7002 and the subunits of the PecE/PecF phycoerythrocyanin α-subunit lyase of *Anabaena* sp. strain PCC 7120. Phycobilisomes isolated from mutant strains FdR1E1 and FdR1E4 contained equal amounts of chromophorylated α and β subunits of phycoerythrin at 46% of the levels of the parental strain FdR1. These results suggest that the *cpeYZ* gene products function in phycoerythrin synthesis, possibly as a lyase involved in the attachment of phycoerythrobilin to the α or β subunit.

Cyanobacteria harvest light energy for photosynthesis with macromolecular antenna complexes, termed phycobilisomes, which are peripherally attached to the photosynthetic membrane (for reviews, see references 18 and 28). These complexes absorb visible light in the range of 500 to 680 nm and efficiently transfer the captured light energy to chlorophylls of the photosynthetic apparatus. Phycobilisomes consist of two structural domains: a core which contacts the photosynthetic membrane and rods, generally six in number, that radiate from the core. Both domains are composed of phycobiliproteins and nonchromophorylated linker polypeptides. The major phycobiliproteins in a number of cyanobacteria are the blue-green-pigmented allophycocyanin (AP) (absorbance maximum [A_{max}] of ~650 nm), the blue-pigmented phycocyanin (PC) (A_{max} of ~620 nm), and the red-pigmented phycoerythrin (PE) (A_{max} of ~560 nm), each consisting of an α and a β subunit. AP is localized to the phycobilisome core in the form of stacked trimeric disks, whereas PC and PE are localized to the rods in the form of stacked hexameric disks. The linker polypeptides direct the assembly of the phycobilisome and modulate the spectral properties of the constituent phycobiliproteins for efficient energy transfer within the complex and to the photosynthetic apparatus.

The filamentous cyanobacterium *Fremyella diplosiphon* UTEX 481 (also referred to as *Calothrix* sp. strain PCC 7601) is one of several strains that respond to changes in spectral light quality by altering the phycobiliprotein composition of the phycobilisome (5, 17, 31). This acclimation, termed complementary chromatic adaptation, allows the cells to modulate the phycobiliprotein composition of the rods for maximum absorbance of incident light. The process of complementary chromatic adaptation is most clearly demonstrated for *F. diplosiphon* by comparing phycobilisomes in cells after growth under red or green illumination. The composition of the core and the invariant core-proximal PC hexamer (contains constitutive PC, designated PC_1) is not affected by the spectral light quality. However, in red light, the rods contain high levels of an inducible phycocyanin (PC_2) and very little PE; cells are pigmented blue-green in red light. Conversely, in green light, the rods contain high levels of PE and very little PC_2; cells are pigmented red in green light. The change in rod phycobiliprotein composition is mediated primarily through differential expression of genes encoding the PC_2 and PE apoproteins (17) and presumably provides the cells an adaptive advantage, since PC_2 efficiently absorbs red light and PE efficiently absorbs green light.

The ability of phycobiliproteins to absorb light is conferred by linear tetrapyrrole chromophores (bilins) attached by thioether linkages to specific cysteinyl residues of the α and β subunits (reviewed in reference 28). Four bilin isomers, each with a different A_{max}, are found in cyanobacterial phycobiliproteins: phycocyanobilin (PCB), phycobiliviolin (PXB), phycoerythrobilin (PEB), and phycourobilin (PUB). In *F. diplosiphon* the subunits of both AP and PC are bonded to PCB; the α and β subunits of AP (designated α^{AP} and β^{AP}, respectively) and the α subunit of PC (designated α^{PC}) contain a single bilin, whereas the β subunit of PC (designated β^{PC}) contains two bilins. In addition, the subunits of PE are bonded to PEB; the α subunit of PE (designated α^{PE}) is bonded to two PEBs,

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TABLE 1. Strains and plasmids used

Strain or plasmid	Description	Reference or source
F. diplosiphon		
UTEX 481	Also referred to as *Calothrix* sp. strain PCC 7601; exhibits complementary chromatic adaptation	Laboratory collection
Fd33	Short filament mutant of UTEX 481; exhibits complementary chromatic adaptation as UTEX 481	11
FdR1	Primary pigment mutant; isolated following electroporation of Fd33; *rcaC::*Tn*5469*	6
FdR1E1	Secondary pigment mutant; isolated following electroporation of FdR1; *rcaC::*Tn*5469 cpeY::*Tn*5469*	This study
FdR1E4	Secondary pigment mutant; isolated following electroporation of FdR1; *rcaC::*Tn*5469 cpeY::*Tn*5469*	This study
E. coli DH5α	F$^-$ ϕ80d*lacZ*ΔM15 Δ(*lacZYA-argF*)U169 *deoR recA1 endA1 hsdR17*(r_K^- m_K^+) *supE44* λ$^-$ *thi*-1 *gyrA96 relA1*	Bethesda Research Laboratories
Plasmids		
pPL2.7	Shuttle vector for *E. coli* and *F. diplosiphon*	10
pFdPE	pBluescript SK (+) with 6.9-kbp *Eco*RI fragment from Fd33 containing *cpeBA* and *cpeYZ*	A. Grossman
pUMC030	pBluescript SK (+) with 3.6-kbp *Cla*I fragment from Fd33 containing intact *rcaC* gene	This study
pUMC227	pGEM3zf(+) with 5.7-kbp *Eco*RI fragment from FdR1 containing intact Tn*5469*	21
pUMC293	pPL2.7 with insert corresponding to the 5.0-kbp *Apa*I-*Eco*RI fragment from pFdPE carrying *cpeBA* and *cpeYZ* sequences	This study
pUMC303	pPL2.7 with insert corresponding to the 2.1-kbp *Hin*dIII fragment from pFdPE carrying *cpeY* sequences	This study
pUMC308	pPL2.7 with insert corresponding to the 3.9-kbp *Sty*I-*Eco*RI fragment from pFdPE carrying *cpeYZ* sequences	This study

whereas the β subunit (designated β^{PE}) is bonded to three PEBs.

Few enzymes involved in the attachment of bilins to the different phycobiliprotein subunits have been identified. The best-characterized form is the CpcE/CpcF lyase from *Synechococcus* sp. strain PCC 7002, which catalyzes the attachment of PCB to the α^{PC} apoprotein. A mutation in *cpcE* or *cpcF* results in cells characterized by a decreased PC level and smaller phycobilisomes (36). In either mutant, the α^{PC} apoprotein is expressed; however, it lacks the native PCB chromophore adduct. Extensive biochemical studies confirmed that the *cpcE* and *cpcF* gene products function as a heterodimeric PCB α^{PC} lyase (14, 15). Recently, the *pecE* and *pecF* gene products in *Anabaena* sp. strain PCC 7120 were implicated as components of a lyase involved in the attachment of PXB to the α subunit (designated α^{PEC}) of the phycobiliprotein phycoerythrocyanin (PEC) (19). Mutants of *Anabaena* inactivated for *pecE* or *pecF* are deficient for PEC and lack the chromophorylated α^{PEC} subunit. Additional results from these studies strongly support the existence of multiple specific bilin lyases. For a strain such as *F. diplosiphon*, which contains at least 13 proteins to which bilins become attached, a significant number of different lyases may be involved in phycobiliprotein assembly.

Mutants of *F. diplosiphon* that exhibit aberrant pigmentation are essential for examining the regulation of complementary chromatic adaptation and phycobilisome structure. Such pigment mutants, which can be identified visually on plates, arise spontaneously at low frequency or at higher frequency in response to exposure of cells to mutagenic agents or electroporation. Pigment mutants classified as phycobilisome regulatory mutants are deficient in the regulation of complementary chromatic adaptation, whereas those classified as phycobilisome structural mutants are deficient in phycobilisome structure, function, or assembly. Of particular interest is regulatory mutant strain FdR1, which is characterized by constitutive synthesis of PE and no synthesis of PC_2 under any illumination; cells of FdR1 are locked in the green light regulatory mode (6). The FdR1 phenotype is due to inactivation of the *rcaC* gene, which encodes a response regulator protein involved in complementary chromatic adaptation (9).

Endogenous transposon Tn*5469* plays a role in the generation of *F. diplosiphon* pigment mutants. We recently showed that Tn*5469* was the mutagenic agent responsible for the FdR1 phenotype (21). The parental strain Fd33 harbors five genomic copies of Tn*5469*, whereas strain FdR1 harbors a sixth copy of the element localized to the *rcaC* gene. By screening for Tn*5469* copy number, we have identified a number of different pigment mutants similarly characterized by an extra genomic copy of the transposon (20). Two such mutants, designated FdR1E1 and FdR1E4, were derived from strain FdR1 and identically exhibit the FdR1 phenotype with significantly reduced levels of PE. Here, we show that the phenotype of strains FdR1E1 and FdR1E4 is due to inactivation of the uncharacterized *cpeYZ* gene set by Tn*5469*. Characterization of the secondary mutant strains suggests that the *cpeYZ* gene products function in PE synthesis, possibly as an enzyme involved in the attachment of PEB to the α^{PE} or β^{PE} apoprotein.

MATERIALS AND METHODS

Strains and growth conditions. The strains and plasmids used in this study are listed in Table 1. Strain Fd33 (referred to as strain SF33 in reference 11) is a short filament mutant of and exhibits the same complementary chromatic adaptation as *F. diplosiphon* UTEX 481 (also referred to as *Calothrix* sp. strain PCC 7601) (11). All of the described pigment mutants are derivatives of strain Fd33. Red mutant strain FdR1, designated here as a primary pigment mutant, was isolated following electroporation of Fd33 cells and has been phenotypically (6) and genotypically (9, 21) characterized. Mutant strains FdR1E1 and FdR1E4, designated here as secondary pigment mutants, were isolated under nonselective conditions following electroporation of FdR1 cells during recomplementation experiments with plasmid pGCMS37 (9); neither mutant harbors plasmid pGCMS37 or exhibits growth on selective media.

Cells were grown in liquid or on solid BG-11 medium (1) as previously described (6, 10). To assay complementary chromatic adaptation, cells were cultured in 50 ml of BG-11 at 15 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of either white (General Electric no. F20T12/CW), red (white light filtered through Acrylite 210-0, maximal transmission at 635 nm, no transmission below 585 nm), or green (white light filtered through Acrylite 545-2, maximal transmission at 540 nm, no transmission below 480 or above 600 nm) illumination. Cells maintained on solid medium received similar intensities of white, red-enriched (Sylvania no. F20T12/R), or green-enriched (Sylvania no. F20T12/G) illumination. For phycobilisome isolation (see below), cells were cultured under 150 to 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of white illumination in 500 ml of BG-11.

Escherichia coli DH5α was purchased from Bethesda Research Laboratories (Gaithersburg, Md.) and used as the host for all plasmids. *E. coli* strains were propagated in liquid or on solid Luria-Bertani medium with antibiotics at standard concentrations (27).

DNA methods. DNA restriction endonucleases and modifying enzymes were purchased from Promega (Madison, Wis.). [α-^{32}P]dCTP was purchased from DuPont/NEN (Boston, Mass.). DNA manipulations, including restriction digests, agarose gel electrophoresis, ligations, transformation of *E. coli*, and plasmid minipreparations, were performed according to Sambrook et al. (27). Cyanobacterial genomic DNA was extracted by the procedure of Kahn and Schaefer (21). For DNA hybridization analysis, genomic DNA was transferred to a charged nylon membrane (Magnagraph; Micron Separations, Westboro, Mass.) by the method of Reed and Mann (26). DNA probes for *rcaC* (3.6-kbp *Cla*I fragment of pUMC030), Tn*5469* (3.8-kbp *Xba*I-to-*Eco*RI fragment of pUMC227), and *cpeY* (2.2-kbp *Hin*dIII fragment of pFdPE) were generated from gel-purified DNA fragments by random-primer labeling using a kit from Promega. Hybridizations were performed at 62°C as described by Sambrook et al. (27).

Spectral analysis of cellular phycobiliproteins. For each strain examined, six 1.5-ml samples were collected from an exponentially growing culture and the cells were harvested by centrifugation at 13,000 × *g* for 5 min. Three of the cell pellets were assayed for chlorophyll content by the method of Tandeau de Marsac and Houmard (32). The remaining three pellets were frozen in liquid nitrogen, thawed at room temperature, resuspended in 1 ml of STES (50 mM Tris [pH 8.0], 50 mM NaCl, 10 mM EDTA, 250 mM sucrose) supplemented with lysozyme at 5 mg · ml^{-1}, and incubated at room temperature for 30 min. Cell debris was removed from the lysates by centrifugation at 13,000 × *g* for 5 min. Absorption spectra for the supernatants were obtained with an HP 8452A spectrophotometer (Hewlett Packard, Palo Alto, Calif.) and normalized to equal chlorophyll (8.0 μg · ml^{-1}). Phycobiliprotein concentrations were calculated according to the method of Tandeau de Marsac and Houmard (32).

Phycobilisome isolation. Intact phycobilisomes were isolated from strains FdR1, FdR1E4, and FdR1E4/pUMC308 for spectral analysis and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). All procedures were carried out at 4°C unless indicated otherwise. Cells from an exponentially growing 500-ml culture were harvested by centrifugation at 10,000 × *g* for 5 min, resuspended in buffer A (0.75 M NaK–phosphate buffer [pH 7.0]), and reharvested. The cells were once more washed with buffer A and then washed with buffer B (buffer A adjusted to 1 mM EDTA and 1 mM benzamidine). The cells were resuspended in 25 ml of buffer B with a tissue homogenizer and disrupted by passage through a French press at 18,000 lb/in^2 (~124 MPa). The cell lysate was brought to 1 mM phenylmethylsulfonyl fluoride (PMSF) and 4% (vol/vol) Triton X-100, incubated in darkness with agitation for 30 min, and cleared of cellular debris by centrifugation at 40,000 × *g* for 15 min at 10°C. The upper layer of the supernatant containing lipids, pigments, and detergent was siphoned away, and the intermediate layer containing the phycobilisomes was brought to 2% (vol/vol) Triton X-100. The supernatant (10 ml) was layered on top of a discontinuous sucrose gradient with the following concentrations (in buffer A) from top to bottom: 0.5 M (5 ml), 0.75 M (7 ml), 1.0 M (5 ml), and 1.5 M (5 ml). Intact phycobilisomes were recovered from the 0.75 M layer after centrifugation at 200,000 × *g* for 3 h at 20°C and dialyzed overnight against buffer C (buffer A adjusted to 1 mM EDTA and 0.8 mM PMSF) to remove sucrose. After dialysis, the suspension of intact phycobilisomes was aliquoted, frozen, and stored at −20°C.

Analysis of phycobilisomes. For each strain, the phycobiliprotein composition was spectroscopically determined for dissociated phycobilisomes by the method of Tandeau de Marsac and Houmard (32). The phycobilisome phycobiliprotein and linker protein composition was analyzed by SDS-PAGE. Samples of phycobilisomes in buffer B containing 50 μg of AP were precipitated by dropwise addition of 0.67 volumes of saturated ammonium sulfate followed by centrifugation at 11,000 × *g* for 20 min. Each pellet was resuspended in 100 μl of 1× SDS-PAGE sample loading buffer (3), solubilized by heating at 100°C for 3 min, and aliquots of the solubilized phycobilisomal proteins were separated on a 32-cm linear gradient polyacrylamide (15 to 20% acrylamide; acrylamide-bis acrylamide, 30:0.8) gel to resolve the closely migrating subunits of PE, PC, and AP. Chromophorylated phycobiliprotein subunits were visualized in the gel prior to Coomassie staining by incubating in 10 mM zinc acetate for 5 min and illuminating on a UV transilluminator (25).

Complementation analysis. Plasmid pFdPE carries the 6.9-kbp *Eco*RI fragment of Fd33 genomic DNA that contains the *cpeBA* and *cpeYZ* gene sets. For the complementation experiments, different DNA fragments were excised from pFdPE and sequentially subcloned into one or more cloning vectors to generate flanking *Bam*HI sites. This cloning strategy provided for ligation of the DNA fragments into the unique *Bam*HI site on shuttle vector pPL2.7. Three plasmids were generated for this study: pUMC293 (insert contains intact *cpeBA* and *cpeYZ* gene sets), pUMC308 (insert contains intact *cpeYZ* gene set), and pUMC303 (insert contains intact *cpeY* gene) (Table 1). Each of the plasmids was introduced into cells of strain FdR1E1 and FdR1E4 by electroporation, and transformants were isolated as described by Chiang et al. (10).

DNA and protein sequence analysis. DNA and protein sequences were analyzed and compared with sequences in the GenBank and CyanoBase databases by using the BLAST (2) or MacVector (Eastman Kodak, Rochester, N.Y.) sequence analysis program. The structural relatedness of CpeY or CpeZ to reported homologous cyanobacterial gene products was determined by alignment against the following sequences: CpcE and CpcF from *Synechocystis* sp. strain PCC 6803 (23), CpcE and CpcF from *Synechococcus* sp. strain PCC 7002 (36), CpcE, CpcF, PecE, and PecF from *Anabaena* sp. strain PCC 7120 (4, 8, 29, 35, 36), CpcE, CpcF, CpeY, and CpeZ from *Pseudanabaena* sp. strain PCC 7409 (12, 13), CpcE and CpcF from *F. diplosiphon* UTEX 481 (33), CpcE and CpcF from *Synechococcus* sp. strain PCC 7942 (22), CpcF, PecE, and PecF from *Mastigocladus laminosus* (16), CpeY, CpeZ, MpeU, MpeV, RpcE, and RpcF from *Synechococcus* sp. strain WH 8020 (35), and CpeE and CpeF from *Synechococcus elongatus* (GenBank database accession number D13173).

Nucleotide sequence accession number. The complete DNA sequence of *cpeYZ* from *F. diplosiphon* has been deposited in the GenBank database under accession number X04592.

TABLE 2. Cellular phycobiliprotein levels and ratios in the parental and pigment mutant strains

Strain	Light quality[a]	Phycobiliprotein level (μg/μg of chlorophyll)			Ratio		
		PE	PC	AP	PE/AP	PC/AP	PE/PC
Fd33	R	1.15	4.80	2.17	0.53	2.21	0.24
	G	4.83	1.52	1.28	3.78	1.19	3.19
	W	4.84	1.76	1.43	3.39	1.23	2.76
FdR1	R	5.83	1.82	1.76	3.30	1.03	3.21
	G	5.20	1.66	1.42	3.67	1.17	3.14
	W	4.50	1.50	1.32	3.41	1.13	3.01
FdR1E1	R	1.65	2.10	1.89	0.87	1.11	0.79
	G	2.91	1.94	1.83	1.59	1.06	1.50
	W	2.74	1.81	1.39	1.97	1.30	1.52
FdR1E4	R	1.78	2.14	2.25	0.79	0.95	0.83
	G	2.72	2.02	1.80	1.51	1.12	1.35
	W	2.49	1.83	1.52	1.64	1.21	1.36
FdR1E1/pUMC308	R	4.42	1.79	2.03	2.18	0.88	2.47
	G	4.83	1.68	1.24	3.91	1.36	2.87
	W	3.79	1.67	1.67	2.27	1.00	2.28
FdR1E4/pUMC308	R	4.78	1.95	1.92	2.50	1.02	2.46
	G	4.14	1.48	1.11	3.73	1.34	2.79
	W	3.56	1.68	1.41	2.52	1.19	2.12

[a] R, red light (635 nm), G, green light (540 nm), W, white light.

RESULTS

Isolation of pigment mutant strains FdR1E1 and FdR1E4. Cells of mutant strain FdR1 are pigmented red in any illumination (6, 9) due to inactivation of the *rcaC* gene by a sixth genomic copy of transposon Tn*5469* (21). Electroporation of FdR1 cells yielded 15 secondary (second-site) mutants, each characterized by a pigmentation phenotype epistatic to that of strain FdR1. DNA hybridization analysis showed that five of the secondary pigment mutants contained seven genomic copies of Tn*5469*, suggesting that transposition of the element was responsible for the new pigmentation phenotype. Two of these, designated strains FdR1E1 and FdR1E4, identically exhibited the FdR1 phenotype with visibly reduced levels of PE, suggesting a lesion in a common gene involved in PE synthesis. In order to correlate transposition of Tn*5469* with the reduced PE phenotype, which would provide a direct means to identify the affected gene(s), mutant strains FdR1E1 and FdR1E4 were analyzed in detail.

Cellular phycobiliprotein composition. Cellular phycobiliprotein levels were determined in extracts from the parental and pigment mutant strains cultured in red, green, or white light. The phycobiliprotein concentrations (normalized to equal chlorophyll) and ratios for the analyzed strains are presented in Table 2. In the standard red-green light photoassay, the parental strain Fd33 exhibited the characteristic comple-

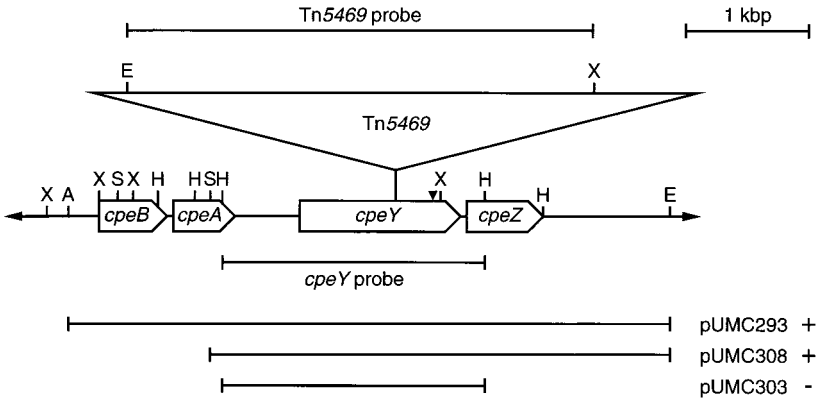

FIG. 1. Physical map of the *cpeBAYZ* locus for *F. diplosiphon*. Open boxes indicate the size and orientation of the *cpeBAYZ* genes as determined by sequence analysis. The site and orientation of Tn*5469* inserted into the *cpeY* gene of strain FdR1E1 is depicted by the large open triangle (drawn to scale) above map. Small closed triangle on map indicates site of Tn*5469* insertion into the *cpeY* gene of strain FdR1E4. Labeled horizontal bars above and below map identify regions that correspond to probes used in DNA hybridization analysis. Labeled horizontal bars at bottom of figure indicate corresponding regions harbored in the pPL2.7 shuttle vector for complementation experiments. Complementation (+) or noncomplementation (−) of strains FdR1E1 and FdR1E4 by the respective constructs is indicated. Flanking and internal restriction sites are shown for enzymes used in cloning and mapping experiments. A, *Apa*I; E, *Eco*RI; H, *Hin*dIII; S, *Sty*I; X, *Xba*I.

mentary chromatic adaptation response; in red light, cells accumulated high levels of PC and low levels of PE, whereas in green light, cells accumulated high levels of PE and low levels of PC. The ratio of PE to PC changed by a factor of 13 when the two cultures were compared. In the red-green photoassay, the level of AP was elevated in Fd33 cells cultured in red light. These measurements also showed that in white light, strain Fd33 essentially exhibits the green light phenotype. The pigmentation phenotype for cells of strain FdR1 cultured in either red, green, or white light was identical to that for the parental strain cultured in green light (Table 2). The lack of change in the ratio of PE to PC for the different light environments demonstrates that strain FdR1 is unresponsive to light quality. The consequence of this phenotype is that on solid medium colonies of FdR1 are pigmented red under any illumination.

Secondary mutant strains FdR1E1 and FdR1E4 were independently identified on solid medium as brown-green-pigmented colonies among the red-pigmented colonies of the primary pigment mutant strain FdR1. The cellular phycobiliprotein assay showed that strains FdR1E1 and FdR1E4 exhibited the FdR1 phenotype, but with a reduced level of PE (Table 2). In red, green, and white light, strains FdR1E1 and FdR1E4 contained levels of PC and AP roughly equal to those measured for strain FdR1. In contrast, they contained between 28 and 61% of the PE measured for strain FdR1 at the different light environments. Surprisingly, the secondary pigment mutants exhibited a red-green photoresponse. The ratio of PE to AP for strains FdR1E1 and FdR1E4 in green light was nearly twice the ratio measured for the same strains in red light, suggesting that a red-green photoregulated component of PE synthesis is not under the control of the *rcaC* regulatory circuit. A consequence of this effect was that in contrast to strain FdR1, the ratio of PE to PC in strains FdR1E1 and FdR1E4 changed by an averaged factor of 1.76 when the red and green cultures were compared.

Genotype of mutant strains FdR1E1 and FdR1E4. The reduced PE phenotype of mutant strains FdR1E1 and FdR1E4 suggested a lesion in a common gene involved in PE synthesis or assembly. Hybridization analysis of FdR1E1 and FdR1E4 genomic DNA against probes for *cpeBA* (encode β and α subunits of PE), *cpeCDE* (encode PE linker polypeptides), and Tn*5469* ruled out transposition of the element into known PE structural genes for the two mutants (data not shown). However, for both mutants, this analysis localized the seventh genomic copy of Tn*5469* to a sequenced but phenotypically uncharacterized locus immediately downstream of the *cpeBA* gene set. Previous DNA sequence analysis for the *cpeBA* downstream region predicted two adjacent open reading frames (ORFs), designated ORFY and ORFZ (33), which we now designate *cpeY* and *cpeZ*, respectively (Fig. 1). The clustered arrangement and orientation of *cpeY* and *cpeZ* with respect to *cpeBA* is common for cyanobacterial phycobilisome structural genes. In both strains FdR1E1 and FdR1E4, the seventh genomic copy of Tn*5469* was localized to the *cpeY* gene at sites 1.3 and 1.6 kbp downstream of the *cpeBA* gene set, respectively (Fig. 1).

Transposition of the 4.9-kbp Tn*5469* into the *cpeY* gene of strains FdR1E1 and FdR1E4 was examined by DNA hybridization analysis. Total DNA from strains Fd33, FdR1, FdR1E1, and FdR1E4 was digested with *Xba*I and *Sal*I and hybridized to a probe for *rcaC*, Tn*5469*, or *cpeY* (Fig. 2). With the *rcaC* probe, the 15-kbp fragment for the parental strain Fd33 was detected as fragments of 18.7 and 1.2 kbp in primary mutant strain FdR1 (Fig. 2A; compare lanes 1 and 2) as a result of the introduction of an *Xba*I site in Tn*5469*, which interrupted the *rcaC* gene in the latter strain (21). Hybridization with the Tn*5469* probe showed that the 18.7-kbp fragment detected with the *rcaC* probe corresponded to the sixth genomic copy of Tn*5469* (the 8.5-kbp fragment identified in Fig. 2B represents a doublet) in strain FdR1 (Fig. 2B; compare lanes 1 and 2). As independent derivatives of strain FdR1, secondary pigment mutant strains FdR1E1 and FdR1E4 exhibited the altered restriction fragment pattern obtained with the *rcaC* probe (Fig. 2A; compare lanes 3 and 4 with lane 2) and the 18.7-kbp fragment corresponding to the sixth genomic copy of Tn*5469* (Fig. 2B; compare lanes 3 and 4 with lane 2).

Hybridization with the Tn*5469* probe detected an additional 6.2-kbp fragment for strain FdR1E1 which corresponded to the seventh genomic copy of Tn*5469* (Fig. 2B; compare lanes 2 and 3). Insertion of this additional copy into the *cpeY* gene altered the restriction pattern obtained with the *cpeY* probe. Note that there are no *Sal*I sites in the region of the *cpeBAYZ* genes (see Fig. 1). The 2.5-kbp *Xba*I fragment for strains Fd33 and FdR1 was detected as two fragments of 6.2 and 1.2 kbp for strain FdR1E1 (Fig. 2C; compare lane 3 with lanes 1 and 2) due to introduction of the *Xba*I site in Tn*5469* (see Fig. 1). For

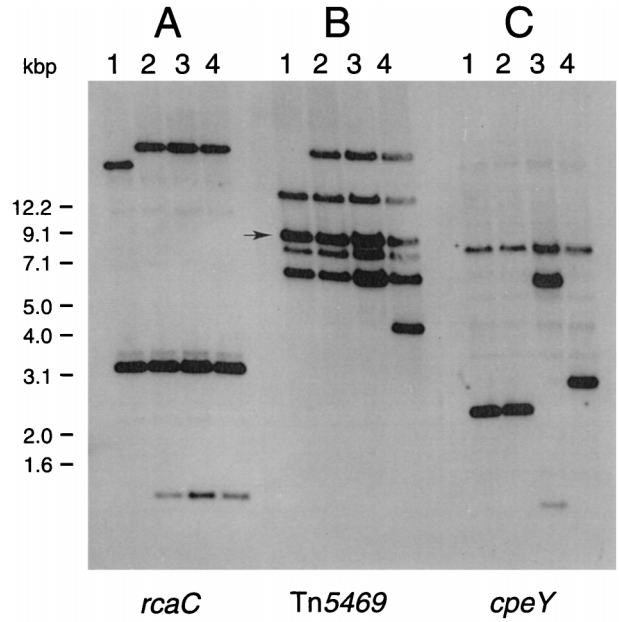

FIG. 2. Transposition of Tn*5469* into the *cpeY* gene of *F. diplosiphon*. Total DNA (5 μg per lane) was isolated from strain Fd33 (lane 1), primary mutant strain FdR1 (lane 2), and secondary mutant strains FdR1E1 (lane 3) and FdR1E4 (lane 4), digested with *Sal*I and *Xba*I, and subjected to DNA blot hybridization with a probe for *rcaC*, Tn*5469*, or *cpeY*. Note that there are no *Sal*I sites in the region of the *cpeBAYZ* genes (see Fig. 1). (A) Blot hybridized with the *rcaC* probe; (B) identical blot hybridized with the Tn*5469* probe; (C) identical blot hybridized with the *cpeY* probe. The arrow indicates the location of a doublet.

strain FdR1E4, the seventh genomic copy of Tn*5469* was detected as a new 4.1-kbp fragment (Fig. 2B; compare lanes 2 and 4) with the Tn*5469* probe. The restriction fragment pattern obtained for strain FdR1E4 with the *cpeY* probe is attributed to insertion of Tn*5469* in an orientation opposite to that observed for strain FdR1E1. The 2.5-kbp *Xba*I fragment detected for strains Fd33 and FdR1 increased to a 3.2-kbp fragment (Fig. 2C; compare lane 4 with lanes 1 and 2) for strain FdR1E4 due to the *Xba*I site in Tn*5469*. The 4.1-kbp fragment generated by insertion of the element contained mostly transposon DNA and therefore hybridized to the Tn*5469* probe but not the *cpeY* probe. Finally, the 8.0-kbp fragment detected for all strains with the *cpeY* probe (Fig. 2C; lanes 1 through 4) corresponds to the downstream *Xba*I fragment which originates in the *cpeY* gene and overlaps the *cpeY* probe.

Complementation of mutant strains FdR1E1 and FdR1E4. For this study, three different DNA fragments (see Fig. 1) were subcloned from plasmid pFdPE (carries the 6.9-kbp *Eco*RI fragment of Fd33 genomic DNA that contains the *cpeBA* and *cpeYZ* gene sets) into shuttle vector pPL2.7, and the resulting constructs were assayed for their abilities to restore the FdR1 phenotype to strains FdR1E1 and FdR1E4. Plasmids pUMC293 (carries both intact *cpeBA* and *cpeYZ* gene sets), pUMC308 (carries the intact *cpeYZ* gene set), and pUMC303 (carries the intact *cpeY* gene) were capable of transforming the two strains; however, only pUMC293 and pUMC308 were capable of complementing the mutant phenotype. Collectively, these results suggest that the products of both *cpeY* and *cpeZ*, as opposed to just *cpeY*, are involved in the reduced PE phenotype of the mutant strains and that expression of the two genes is independent of transcription from *cpeBA*.

To characterize complementation of the secondary mutants, the phycobiliprotein composition of strains FdR1E1/pUMC308 and FdR1E4/pUMC308 was assayed for cells cultured in red, green, and white light. This analysis showed that transformation of strain FdR1E1 with pUMC308 resulted in a 2.7-fold increase in PE for cells grown in red light, a 1.7-fold increase in PE for cells grown in green light, and a 1.4-fold increase in PE for cells grown in white light (Table 2). Nearly identical increases in PE were measured for complemented strain FdR1E4/pUMC308. The levels of PE measured for the complemented strains at the specific light qualities ranged from 76 to 93% of the corresponding levels measured for strain FdR1, indicating that introduction of the *cpeYZ* gene set on pUMC308 essentially restored the FdR1 phenotype to the two mutants. The decreased PE levels of strains FdR1E1/pUMC308 and FdR1E4/pUMC308 relative to those for strain FdR1 are attributed to the continuous loss of pUMC308 (plasmid curing) from a small fraction of the complemented cells in liquid culture, which is routinely observed for these transformants.

Sequence analysis of *cpeYZ*. A map of the *cpeBAYZ* locus is presented in Fig. 1. The *cpeY* gene (nucleotide positions 2054 to 3340) initiates 530 bp downstream of *cpeA* and predicts a protein of 429 amino acids with a molecular mass of 48.2 kDa and a pI of 5.54. The *cpeZ* gene (nucleotide positions 3395 to 4009) initiates 55 bp downstream of *cpeY* and predicts a protein of 205 amino acids with a molecular mass of 21.8 kDa and a pI of 4.3. Like many characterized cyanobacterial genes, neither of the *cpeY* nor *cpeZ* coding regions is preceded by an obvious ribosome-binding sequence.

A BLAST (tblastn) search of the GenBank database indicated that the CpeY and CpeZ proteins from *F. diplosiphon* share significant sequence identity with homologous gene products reported for two PE-containing cyanobacteria: *Pseudanabaena* sp. strain PCC 7409 (12), which like *F. diplosiphon* belongs to chromatic adaptors of group III (31), and *Synechocystis* sp. strain WH 8020 (34). The genes predicting the *Pseudanabaena* sp. strain PCC 7409 CpeY and CpeZ homologs, originally designated ORFY and ORFZ (12), have been designated *cpeY* and *cpeZ*, respectively (7). An alignment of the CpeY and CpeZ proteins is shown in Fig. 3. The CpeY protein from *F. diplosiphon* shares 68.8% amino acid sequence identity with the CpeY protein from *Pseudanabaena* sp. strain PCC 7409 and 31.7% sequence identity with the CpeY protein from *Synechocystis* sp. strain WH 8020 (Fig. 3A). Similarly, the *F. diplosiphon* CpeZ protein is 67.9% identical to the CpeZ protein from *Pseudanabaena* sp. strain PCC 7409 and 28.9% identical to the CpeZ protein from *Synechocystis* sp. strain WH 8020 (Fig. 3B). In comparison, the genomic structure of the *F. diplosiphon cpeBAYZ* locus is nearly identical to the corresponding locus reported for *Pseudanabaena* sp. strain PCC 7409 (12); for both strains, the *cpeBA* gene set is closely followed by the tandemly arranged gene set encoding the CpeY and CpeZ proteins. In *Pseudanabaena* sp. strain PCC 7409, a small ORF, designated ORFW, is located immediately downstream of *cpeZ*. Whether a similar ORF is present at the same location for *F. diplosiphon* is not known.

The BLAST search also showed that the CpeY and CpeZ proteins belong to a family of structurally related cyanobacterial proteins (28, 35), several of which have been implicated as enzymes involved in the chromophorylation of phycobiliprotein subunits (15, 19). An extensive sequence analysis of many members of this family, including the CpeY and CpeZ proteins, was recently reported by Wilbanks and Glazer (35). An alignment against an updated collection of 29 member protein sequences (see Materials and Methods) showed that in addition to the CpeY and CpeZ proteins described above, the *F.*

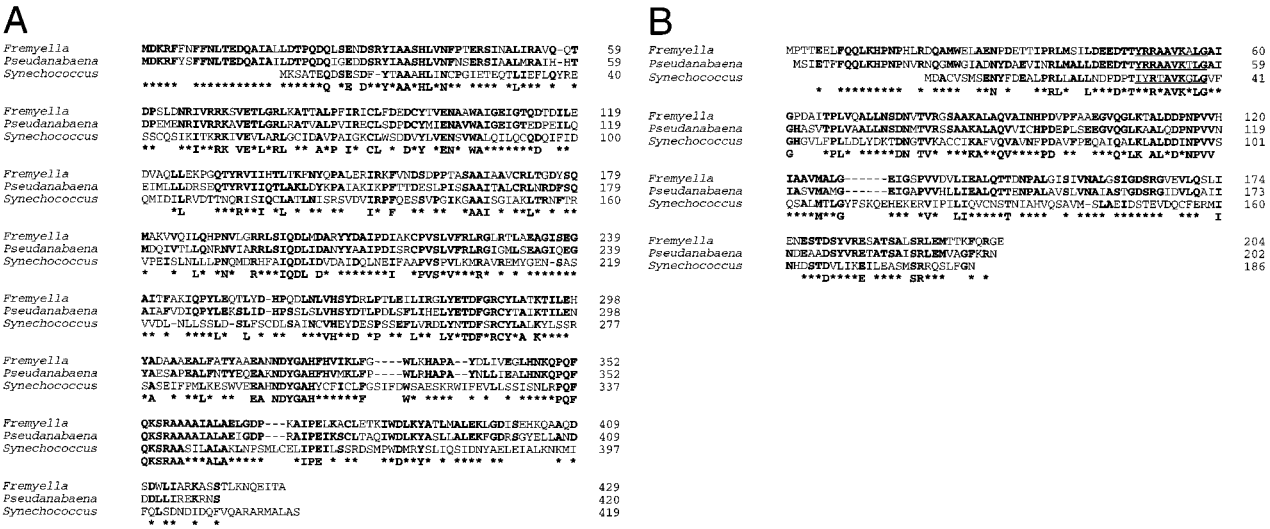

FIG. 3. Comparison of the *F. diplosiphon* CpeY and CpeZ amino acid sequences with corresponding sequences from *Pseudanabaena* sp. strain PCC 7409 (12) and *Synechococcus* sp. strain WH8020 (34). Alignment of the CpeY (A) and CpeZ (B) forms is shown. The numbering of the amino acids for the individual proteins is shown to the right of the sequences. Residues identical to at least two proteins are indicated by boldface letters. Amino acid identities between the *F. diplosiphon* form and an aligned sequence are marked by an asterisk below the sequences. Boldface letters below the sequences identify residues identical to all three cyanobacterial forms. The designated E-V motif (LVYI)X(RE)X(AS)(AV)(KR)(ASGT)L(GANT) (35) for each of the CpeZ proteins is underlined.

diplosiphon CpeY and CpeZ proteins share significant sequence identity with a majority of different members of the protein family (data not shown). The three members most similar to CpeY were PecE from *M. laminosus*, CpcE from *Synechococcus* sp. strain PCC 7942, and RpcE from *Synechococcus* sp. strain WH 8020, whereas the three members most similar to CpeZ were MpeV and MpeU from *Synechococcus* sp. strain WH 8020 and PecF from *Anabaena* sp. strain PCC 7120. In addition, the CpeZ protein was equally similar to the hypothetical protein slr1687 from *Synechocystis* sp. strain PCC 6803. The sequence analysis also showed that the *F. diplosiphon* CpeZ sequence contains the designated E-V motif (LVYI)X(RE)X(AS)(AV)(KR)(ASGT)L(GANT) (Fig. 3B) within a domain of significant sequence identity, which characterizes many members of the protein family (35).

Analysis of phycobilisome composition. The sequence similarity between the *cpeYZ* gene products and the subunits of the defined chromophore lyases described above suggested a similar role for CpeY and CpeZ in *F. diplosiphon*. To explore this possibility the phycobilisome composition was examined for representative mutant and complemented strains. For this analysis, phycobilisomes were isolated from strains FdR1, FdR1E4, and FdR1E4/pUMC308 cultured in white light and purified on sucrose step gradients. The phycobilisomes from all three strains sedimented into the 0.75 M layer, with those from strains FdR1 and FdR1E4/pUMC308 sedimenting slightly farther than those of strain FdR1E4. The phycobiliprotein ratios for the examined strains, determined following dissociation of the phycobilisomes and normalization to a constant level of AP, are presented in Table 3. The phycobilisomes from strain FdR1E4 contained 46% of the PE present in the phycobilisomes from strain FdR1. Transformation of strain FdR1E4 with plasmid pUMC308 restored the PE content of the phycobilisomes to 87% of the level determined for strain FdR1. The low and constant ratio of PC to AP determined for the three examined strains reflects the constitutive green light phenotype of the primary mutant strain FdR1. These data indicate that under conditions supporting PE expression, the loss of *cpeYZ* results in the synthesis of phycobilisomes with significantly reduced levels of spectrally detectable PE.

The molecular composition of the phycobilisomes isolated from strains FdR1, FdR1E4, and FdR1E4/pUMC308 was examined by SDS-PAGE on a 32-cm linear gradient gel. For all three strains, visualization of the pigmented subunits in the unstained gel revealed two closely migrating, red-pigmented bands for the β subunit of PE. Coomassie staining of the gel showed that the two β subunit forms (designated β^{PE*} and β^{PE}) were present in roughly equal amounts (Fig. 4). The structural difference between the two β^{PE} forms is unknown. In comparison to strain FdR1, the phycobilisomes from strain FdR1E4 were significantly reduced for the native PE subunits and slightly reduced for the 27.9-kDa PE linker polypeptide in an otherwise indistinguishable protein profile (Fig. 4, compare lanes 1 and 2). The apparent reduction in the level of β^{PC} in phycobilisomes from strain FdR1E4 was not observed in replicate gels or the spectral analysis of phycobiliprotein composition (Table 3). The protein profile for the phycobilisomes from complemented strain FdR1E4/pUMC308 was indistinguishable from that for strain FdR1. The same results were observed in a related experiment to examine the relative chromophorylation state of the individual phycobiliprotein subunits by a method based on the fluorescence of a biliprotein following the formation of the Zn-bilin chelate (25). For all three strains, the amount of fluorescence from the α^{PE} and β^{PE}

TABLE 3. Phycobiliprotein ratios from phycobilisomes purified from strains FdR1, FdR1E4, and FdR1E4/pUMC308 cultured in white light

Strain	Ratio		
	PE/AP	PC/AP	PE/PC
FdR1	2.48	0.71	3.50
FdR1E4	1.15	0.62	1.87
FdR1E4/pUMC308	2.16	0.69	3.12

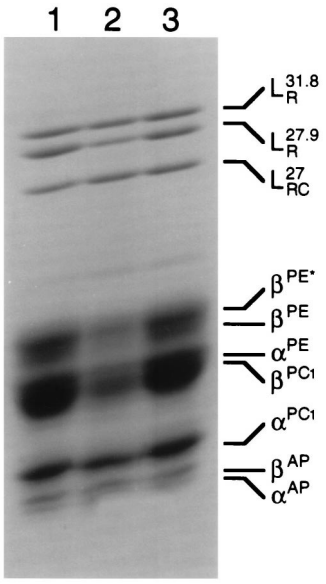

FIG. 4. Molecular composition of phycobilisomes isolated from mutant and complemented strains. Shown is an SDS-PAGE gel of phycobilisomes isolated from primary mutant strain FdR1 (lane 1), secondary mutant strain FdR1E4 (lane 2), and complemented strain FdR1E4/pUMC308 (lane 3) cultured in white light. Each lane was loaded with 50 μg of AP. Following electrophoresis, the gel was stained with Coomassie blue. Major phycobilisome polypeptides are identified.

forms matched the levels of the corresponding subunits visualized after Coomassie staining of the gel (data not shown). The unaltered migration and fluorescence of the α^{PE} and β^{PE} subunits for strain FdR1E4 indicated that the PE incorporated into the phycobilisomes of the mutant strain is composed of chromophorylated subunits.

DISCUSSION

Previous work has suggested that endogenous transposon Tn*5469* may provide a unique opportunity to identify new genes involved in complementary chromatic adaptation by *F. diplosiphon*. We have determined that like strain FdR1, a significant number of pigment mutants in our strain collection are characterized by an extra genomic copy of Tn*5469*, suggesting a link between the transposition event and the mutant phenotype. To assess the utility of tracking Tn*5469* transposition as a means to identify new genes involved in complementary chromatic adaptation, we analyzed two such pigment mutants, designated FdR1E1 and FdR1E4, which were derived from strain FdR1 and identically exhibit the FdR1 phenotype with a reduced level of PE. Here, we correlate the PE deficiency of strains FdR1E1 and FdR1E4 to inactivation of the *cpeYZ* gene set by Tn*5469* and propose that the products of *cpeY* and *cpeZ* may function as a heterodimeric PEB lyase involved in the chromophorylation of α^{PE} or β^{PE}.

A previous report has described the tandemly arranged ORFs (originally designated ORFY and ORFZ) comprising the *cpeYZ* gene set downstream of *cpeBA* on the *F. diplosiphon* genome (33). That study also included an RNA-DNA hybridization analysis showing that *cpeY* is transcribed as a monocistronic unit. For mutant strains FdR1E1 and FdR1E4, the seventh genomic copy of Tn*5469* was localized to the *cpeY* gene of the *cpeYZ* gene set, supporting the earlier hypothesis that the *cpeY* gene product functions in PE synthesis or assembly (33). However, complementation of the mutant strains was achieved only by plasmid pUMC308, which carries the intact *cpeYZ* gene set, and not by plasmid pUMC303, which only carries the intact *cpeY* gene. Two conclusions can be drawn from this result: (i) the products of both *cpeY* and *cpeZ*, as opposed to the product of *cpeY* alone, play a role in the reduced PE phenotype of the mutant strains. The significant degree of sequence identity between the *cpeYZ* gene products and the heterologous subunits of the characterized chromophore lyases adds further support to this conclusion (see below). (ii) The *cpeZ* gene is cotranscribed with *cpeY*. This conclusion contradicts the earlier observation of a monocistronic *cpeY* transcript (33); however, that analysis does not rule out the possibility that *cpeY* and *cpeZ* are cotranscribed. For example, the detected *cpeY* transcript might represent a stable degradation product of an unstable, and therefore difficult to detect, *cpeYZ* transcript. Furthermore, cotranscription of *cpeYZ* was not unexpected; many of the characterized genes involved in phycobilisome structure and biosynthesis are present in polycistronic gene clusters.

The polypeptides predicted by *cpeY* and *cpeZ* belong to a family of structurally related cyanobacterial proteins (28, 35). Most often, the genes encoding the protein members of this family are present as a tandem heterologous pair linked to a specific phycobiliprotein operon. For example, the best-characterized members are the products of the *cpcE* and *cpcF* genes, which are located on the *cpc* operon containing the genes encoding the constitutive PC subunits. To date, *cpcE* and *cpcF* genes have been identified in eight cyanobacterial strains. For *Synechococcus* sp. strain PCC 7002, it has been shown that the CpcE and CpcF proteins comprise a heterodimeric (CpcE-CpcF [1:1]), α^{PC} phycocyanobilin lyase that functions in the attachment of PCB to the α^{PC} apoprotein (14, 15, 30, 36). In addition to the products of the *cpcE* and *cpcF* homologs, the protein family includes the products of the *pecE* and *pecF* genes from *Anabaena* sp. PCC 7120 (4, 8, 29, 35, 36), the *rpcE*, *rpcF*, *mpeU*, and *mpeV* genes from *Synechococcus* sp. strain WH 8020 (35), and the *cpeY* and *cpeZ* genes from *Pseudanabaena* sp. strain PCC 7409 (12) and *Synechococcus* sp. strain WH 8020 (35). Within each heterologous protein pair from a given strain, one or both proteins share significant sequence identity with CpcE (28, 35) and contain the designated E-V motif that characterizes many members of the protein family (35). For the CpeY and CpeZ heterologous pair, it is the CpeZ protein that shares these features (28, 35). Given that the PecE and PecF polypeptides for *Anabaena* sp. strain PCC 7120 were recently implicated as the subunits of an α^{PEC} lyase that functions in the attachment of PXB to the αPEC apoprotein (19), it is inferred that the remaining heterologous pairs, including the CpeY and CpeZ proteins, similarly function as phycobiliprotein subunit bilin lyases.

Homologs to *cpeY* and *cpeZ* have been identified in two cyanobacterial strains that share with *F. diplosiphon* the capacity to synthesize PE. On the basis of sequence identity, the CpeY and CpeZ proteins from *F. diplosiphon* are most similar to the corresponding proteins from *Pseudanabaena* sp. strain PCC 7409, which is characterized by complementary chromatic adaptation. This similarity extends to the genomic level; the *cpeBAYZ* locus in *F. diplosiphon* is structurally identical to the *cpeBAYZ* locus reported for *Pseudanabaena* sp. strain PCC 7409 (12). The *F. diplosiphon* CpeY and CpeZ proteins also share significant sequence identity with the CpeY and CpeZ proteins reported for the marine cyanobacterium *Synechococcus* sp. strain WH 8020 (34). The latter strain is unique in that it synthesizes two distinct PE forms, designated PE I and PE II, that differ in the number of attached bilins (24). In *Synechococcus* sp. strain WH 8020, the *cpeY* and *cpeZ* genes are lo-

cated downstream of the *cpeBA* gene set that encodes the subunits of PE I, which is the form similar to the PE of freshwater cyanobacterial strains (34). Similarly, the *mpeBA* gene set that encodes the subunits of PE II is followed by the *mpeU* and *mpeV* genes, which encode heterologous members of the protein family described above. Excluding the *Pseudanabaena* sp. strain PCC 7409 and *Synechococcus* sp. strain WH 8020 CpeZ forms, the *F. diplosiphon* CpeZ protein shares greatest sequence identity with the MpeV and MpeU proteins from *Synechococcus* sp. strain WH 8020. Collectively, these similarities establish a link between the CpeY and CpeZ proteins and the synthesis of PE.

At the phycobilisome level, the phenotype of strain FdR1E4 differed from that of the *Synechococcus* sp. strain PCC 7002 and *Anabaena* sp. strain PCC 7120 bilin lyase mutants. Phycobilisomes from strain FdR1E4 contained equal amounts of chromophorylated α^{PE} and β^{PE} at levels of 46% that of strain FdR1. The remaining protein components of the phycobilisome in strain FdR1E4 were unchanged, and no nonchromophorylated α^{PE} or β^{PE} apoproteins were detected. Mutants of *Synechococcus* sp. strain PCC 7002 inactivated for *cpcE* or *cpcF* (or both) are characterized by significantly reduced levels of PC and smaller phycobilisomes (36). The mutants contain normal levels of chromophorylated β^{PC}; however, no wild-type chromophorylated α^{PC} is detectable. Biochemical analysis showed that the mutants express the α^{PC} apoprotein at levels similar to those for β^{PC} and that a fraction becomes chromophorylated with an unnatural PCB adduct, possibly due to the activity of a different PCB lyase (30). Both the α^{PC} apoprotein and the altered α^{PC} form are incorporated into phycobilisomes, presumably in the native $\alpha\beta$ conformation with the normal β^{PC}. Inactivation of *pecE* or *pecF* in *Anabaena* sp. strain PCC 7120 yields cells that lack native PEC (19). In either case, the phycobilisomes contain approximately 30% of the β^{PEC} found in the wild-type strain and virtually no form of α^{PEC}. It is believed that the β^{PEC} forms a hybrid monomer with α^{PC} and is incorporated into the phycobilisome. Interestingly, when both *pecE* and *pecF* are mutated, α^{PEC} is detected in the phycobilisome at the same level as β^{PEC}, and the α^{PEC} carries the PCB chromophore. This matches the phenotype of strain FdR1E4 with respect to α^{PE} and β^{PE}. For the *pecE* and *pecF* double mutant, it is hypothesized that the addition of PCB to the α^{PEC} apoprotein is catalyzed by the intact CpcE/CpcF PCB α^{PC} lyase. The lack of a similar activity in the *pecE* or *pecF* mutant may be due to the formation of nonfunctional PecE/CpcF or CpcE/PecF hybrids that complex the α^{PEC} apoprotein, making it unavailable for chromophorylation by the CpcE/CpcF PCB α^{PC} lyase or facilitating its degradation. The phenotypes of the *Synechococcus* sp. strain PCC 7002 and *Anabaena* sp. strain PCC 7120 mutants suggest that the loss of a specific bilin lyase can be partially compensated for by the activity of a different bilin lyase. In a manner similar to that for α^{PEC} in the *pecE* and *pecF* double mutant of *Anabaena* sp. strain PCC 7120, the presence of chromophorylated α^{PE} in strain FdR1E4, demonstrated here by complementation analysis to be a *cpeY* and *cpeZ* double mutant, could be attributed to the activity of a different PEB or PCB lyase in *F. diplosiphon.*

The precise role of the CpeY and CpeZ proteins in PE synthesis remains to be determined. The data presented here suggest that CpeY and CpeZ function as a heterodimeric PEB α^{PE} lyase responsible for attaching one or both of the PEB chromophores bonded to α^{PE}. Nonetheless, these data do not rule out the possibility that CpeY and CpeZ function as a PEB β^{PE} lyase. Because all of the phycobiliproteins in mutant strains FdR1E1 and FdR1E4 are spectrally indistinguishable from those of the wild-type strain, it is unlikely that the *cpeYZ* gene products function in bilin biosynthesis. It is therefore anticipated that in vitro experiments with purified forms will confirm a PEB lyase mechanism for the CpeY and CpeZ proteins.

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