The cpcE and cpcF Genes of Synechococcus sp. PCC 7002

CONSTRUCTION AND PHENOTYPIC CHARACTERIZATION OF INTERPOSON MUTANTS*

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The 3' region of the cpc operon of Synechococcus sp. PCC 7002 has been sequenced, transcriptionally characterized, and analyzed by interposon mutagenesis. The cpc operon contains six genes, 5' cpcB-cpcA-cpcC-cpcD-cpcE-cpcF 3', and gives rise to at least eight (more likely ten) discrete mRNA transcripts. The steady-state levels of transcripts for the cpcE and cpcF genes are very low and are estimated to represent only about 1–2% of the total transcripts arising from the cpc locus. The cpcE gene predicts a protein of 268 amino acid residues, whereas the cpcF gene predicts a protein of 205 amino acid residues. The deduced amino acid sequences of these proteins are about 50% identical and 70% similar to the predicted products of homologous genes which have been identified in other cyanobacterial cpc operons. Interposon insertion mutations were constructed in the cpcE and cpcF genes, and an interposon deletion mutation affecting both genes was constructed. The phenotypes of all mutant strains were similar. These strains were yellow-green in color, had doubling times approximately twice that of the wild-type strain, and failed to accumulate normal levels of phycocyanin. Further analyses indicated that these strains contained substantial amounts of apparently normal phycocyanin β subunits; however the majority of the phycocyanin α subunit (about 90%) did not carry a phycocyanobilin chromophore. During serial subculturing of the mutant strains, suppressor mutations, which allowed cells to regain the ability to synthesize phycocyanin, arose at significant frequency. Based upon the results reported here, as well as those presented in the accompanying paper (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), we propose that the CpcE and CpcF polypeptides are the two subunits of a phycocyanobilin lyase specifically required for chromophorylation of the phycocyanin α subunit.

Phycobilisomes serve as the major light-harvesting antennae for photosynthesis in cyanobacteria, red algae, cryptomonads, and phylogenetically ambiguous cyanelle-containing flagellates such as Cyanophora paradoxa (for reviews, see Glazer, 1985, 1987, 1989; Bryant, 1991). Phycobilisomes are composed of the brightly colored phycobiliproteins (PBP), which account for approximately 85% of the protein in these structures, and a small number of typically nonchromophor-ylated linker polypeptides, which are responsible for directing the assembly of the phycobilisome and modulating the spectroscopic properties of the constituent PBPs. The PBPs have been extensively characterized biochemically, and the structures for three phycocyanins (PC; Schirmer et al., 1985, 1986, 1987; Duerring et al., 1991) and a phycocerythrocyanin (Duerring et al., 1990) have been determined by x-ray crystallography. Protein sequences for all types of PBPs have been determined, and these and other studies have shown that PBPs are an homologous family of proteins derived from a single ancestral gene by gene duplication and divergence processes (see Zuber, 1987; Bryant, 1991).

Although much is known about the structure of PBPs and their chromophore contents (see Glazer, 1989), much less is known about how the phycobilin chromophores are synthesized and how these linear tetrapyrroles become attached to the PBP apoproteins. Beale and co-workers (1991a, 1991b, 1991c) have recently proposed a biosynthetic pathway leading from heme to the presumed precursor of polypeptide-bound phycocyanobilin, 3(E)-phycocyanobilin, in the acidothermophilic red alga Cyanidium caldarium. In vitro studies performed with apoPC subunits demonstrated that a variety of linear tetrapyrroles could be added by simple chemical addition to the α-82 and β-84 positions, but no addition to the β-155 position was observed in these studies (Aric et al., 1988a, 1988b, 1988c). The chemical addition of 3(E)-phycocyanobilin resulted in a mixture of products with differing stereochemistry and reduction states. These observations suggested that the chromophore attachment reactions must be enzymatic, much like the attachment of heme to apocytochromes c and c₁.

In characterizing the cpc operon of Synechococcus sp. PCC 7002, two genes, denoted cpcE and cpcF, were identified which did not encode structural components of the phycobilisomes. Nonetheless, these two genes were cotranscribed with the other four genes of the operon (cpcB, cpcA, cpcC, and cpcD (see de Lorimier et al., 1984; de Lorimier et al., 1990a, 1990b). Similar genes have been localized downstream from the genes encoding the subunits of PC in Anaabaena sp. PCC 7120 (Belknap and Haselkorn, 1987), Calothrix sp. PCC 7601 (Maz et al., 1988), and Pseudanabaena sp. PCC 7409 (Dubbs, 1990). The characterization of these genes in Synechococcus sp. PCC 7002 as well as the phenotypic characterization of...

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†The abbreviations used are: PBP, phycobiliprotein; bp, base-pair(s); kbp, kilobase pair(s); ORF(s), open reading frame(s); PC, phycocyanin; PCC, Pasteur culture collection; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

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experimental procedures

results

nucleotide sequence and deduced amino acid sequences for the cpe and cpf genes—a physical map of the region surrounding the cpeBACDEF operon of Synechococcus sp. PCC 7002 is shown in Fig. 1A. In the present work the nucleotide sequence of the region extending from the HindIII site to the cpcD gene to the right-most PstI site was completely determined on both strands. The nucleotide sequence data obtained have been deposited in GenBank under the accession number M39569. The cpeE gene initiates 137 bp downstream from the cpcD gene (de Lorimier et al., 1990a) and predicts a protein of 268 amino acids (Fig. 2) with a predicted molecular mass of 29,172 Da and a calculated isoelectric point of 4.6. The predicted protein contains about 49% nonpolar amino acids, and secondary structure algorithms suggest that the protein should be largely composed of α-helical secondary structure. The start codon is preceded by a polypurine motif 5'-AAAGGAGAA-3' which strongly resembles typical prokaryotic ribosome-binding (Shine-Dalgarno) sites. The cpcF gene initiates 54 bp downstream from the cpeE gene and predicts a protein of 205 amino acids with a calculated molecular mass of 22,303 Da and a calculated isoelectric point of 5.4. The start codon is preceded only by the purine trinucleotide 5'-GAA 3' which might play a role in ribosome binding. The protein contains 51% nonpolar amino acids and is also predicted to consist of predominantly α-
helical segments. Two imperfect inverted repeat structures, which have the potential to form energetically favorable stem-loop structures, occur downstream from the cpcF gene. One or both of these structures could play a role in transcription termination or mRNA stabilization. Analyses of the 2472-bp region downstream from the cpcF gene revealed several potential ORFs on both strands. The largest of these ORFs occur on the strand opposite the cpcF gene (see Fig. 1A). However, database searches revealed no significant homology to any of these putative ORFs. An insertion mutation constructed at the XhoI restriction site within the first ORF downstream from cpcF (see Fig. 1) did not appear to affect PBP levels in the cell and produced no other discernable effects. "

**Fig. 2. Comparison of the deduced amino acid sequences of CpcE and CpcF from several cyanobacteria.** Comparison of the deduced amino acid sequence of the cpcE and cpcF genes of *Synechococcus sp. PCC 7002, Pseudanabaena sp. PCC 7120, Calothrix sp. PCC 7601* (Mazel et al., 1988, and Bryant 1991). Only residues which differ from those of the *Synechococcus sp. PCC 7002* proteins are shown. Hyphens indicate insertions/deletions included to optimize the homology. The *Anabaena* sp. PCC 7120 CpcF sequence is not complete; the region not sequenced occurs between the cpcE and cpcF genes; when the 0.884-kbp XhoI-Hind111 fragment which in some experiments could be resolved into two species, containing an equivalent amount of total RNA isolated from cells starved for nitrogen for 5 h. When the 3.01-kbp HindIII fragment encoding cpcD (Fig. 3A) was used as the hybridization probe, at least eight transcripts were detected (Fig. 3C). Less abundant transcripts of approximately 1350 and 1500 nucleotides in length, encode the cpcB and cpcA genes and differ in length by 161 nucleotides at their 5′ ends. The pair of transcripts of approximately 2350 and 2500 nucleotides encode the cpcBACDEF operon—Fig. 3 shows the results of Northern blot hybridization experiments using a variety of DNA fragments from the cpc operon as probes. In each panel, lane 1 contains total RNA isolated from cells grown in nitrogen-replete medium and lane 2 contains an equivalent amount of total RNA isolated from cells which had been starved for nitrogen for 5 h. When the 3.01-kbp HindIII fragment encoding the cpcB, cpcA, and cpcC genes was used as the hybridization probe, at least eight transcripts could be detected (Fig. 3, A and B); the levels of all transcripts were approximately 50- to 100-fold lower in cells starved for nitrogen. The smallest pair of transcripts, approximately 1500 and 1600 nucleotides in length, encode only the cpcB and cpcA genes and differ in length by 161 nucleotides at their 5′ ends. The pair of transcripts of approximately 2350 and 2500 nucleotides encode the cpcB, cpcA, and cpcC genes; when the 0.884-kbp XhoI-HindIII fragment specific for cpcC was used as probe, only transcripts of this size were detected (Fig. 3C). Less abundant transcripts of approximately 2900, 3200, and 4600 nucleotides were also detected with the cpcBAC probe. The 2900-nt transcripts, which in some experiments could be resolved into two species, hybridized specifically to the 0.364-kbp HindIII fragment encoding cpcD (Fig. 3D). This result indicates that these transcripts encode the cpcB, cpcA, cpcC, and cpcD genes. Hybridization with the 1.989-kbp HindIII-XhoI fragment en-

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\(^4\) G. Gasparich, unpublished data.
the products of these two genes, insertional mutations within the 23 probe fragment to transcripts of approximately 1400-1600 panel, exponentially in replete medium. With the 1.992-kbp HindIII-XhoI fragment encoding the of cpcE and cpcF genes were constructed (see “Experimental Procedures”). The eight transcript species detected (also see Fig. 1A). Hybridization with the 3.01-kbp HindIII fragment encoding cpeB cpeA, and cpcC. The arrowheads to the left of lane 1 show indicate the DNA fragment sizes in kilobase pairs. In each lane 1 contains 20 µg of total RNA isolated from cells growing exponentially in replete medium. Lane 2 contains an equivalent amount of total RNA isolated from cells that had been starved for nitrogen for 5 h. The positions of the 23 S (approximately 2900 nucleotides) rRNA and the 16 S (approximately 1500 nucleotides) rRNA are indicated to the right. The unlabeled arrows to the right indicate the positions of two naturally occurring cleavage products of the 23 S rRNA. The source of the hybridization probe fragments can be seen in Fig. 1A. Hybridization with the 3.01-kbp HindIII fragment encoding cpeB cpeA, and cpcC. The arrowheads to the left of lane 1 show indicate the eight transcript species detected (also see Fig. 1A). B, same experiment as A, but the film was heavily overexposed to show the minor transcript species cpeBACDE and cpeBACDEF as well as to emphasize the low-abundance of all cpc transcripts in the nitrogen-starved cells. C, hybridization with 0.884-kbp HindIII-XhoI fragment specific for the cpcC gene. D, hybridization with 0.364-kbp HindIII-HindIII fragment specific for the cpcD gene. E, hybridization with the 1.992-kbp HindIII-XhoI fragment encoding the cpeD and cpcF genes.

coding cpeE and cpcF (Fig. 3E) demonstrated that the extremely low-abundance transcripts of approximately 3900 and 4600 nucleotides encoded the cpeBACDE and cpeBACDEF genes, respectively. A smear of hybridization for this latter probe fragment to transcripts of approximately 1400-1600 nucleotides could indicate that a secondary promoter might exist somewhere in the 3' region of the cpc operon. Alternatively, primary transcripts encoding the cpeBACDE genes might be endonucleolytically processed to produce low-abundance transcripts encoding only the cpeE and cpcF genes. Hybridization experiments with probe fragments derived from the region 3' to cpcF did not identify transcripts from this region (data not shown).

Construction of Interposon Mutants in the cpeE and cpcF Genes—A previous study (Bryant et al., 1990) had shown that the phycobilisomes of Synechococcus sp. PCC 7002 did not contain the polypeptide products of the cpeE and cpcF genes. However, the results described above indicate that the cpeE and cpcF genes are cotranscribed with the cpeBACD genes, a result which suggested that these genes might encode proteins functionally related to PC. In order to reveal the function of the products of these two genes, insertional mutations within each of the genes and a deletion mutation inactivating both genes were constructed (see “Experimental Procedures”). The mutant constructions are shown diagramatically in Fig. 1, B and C. The constructions indicated were introduced into Synechococcus sp. PCC 7002 by transformation; homozygous mutations for each construction were obtained by appropriate selection and streak purification. Each mutant construction was confirmed by Southern blot hybridization experiments (Fig. 4). Fig. 4A shows a comparison of the hybridization patterns for strains PR6000 (wild type) and PR240 (cpeE-). The HindIII fragment hybridizing to a probe fragment encoding the cpeE and cpcF genes (1.98-kbp HindIII-XhoI fragment, see Fig. 1A) is 1.3 kbp larger than in the wild type as expected. After digestion with HindIII and BglII, two fragments of 1.6 and 3.27 kbp hybridized to this probe (Fig. 4A, lane 4) since the interposon has a single BglII site. These results confirm the insertion of the aphII gene into the EcoRI site and indicate that the segregation of the mutant allele from the wild-type allele is complete (Fig. 4A, lanes 1 and 2).

FIG. 3. Fluorograms of Northern blot hybridization studies of the cpe operon of Synechococcus sp. PCC 7002. In each panel, lane 1 contains 20 µg of total RNA isolated from cells growing exponentially in replete medium. Lane 2 contains an equivalent amount of total RNA isolated from cells that had been starved for nitrogen for 5 h. The positions of the 23 S (approximately 2900 nucleotides) rRNA and the 16 S (approximately 1500 nucleotides) rRNA are indicated to the right. The unlabeled arrows to the right indicate the positions of two naturally occurring cleavage products of the 23 S rRNA. The source of the hybridization probe fragments can be seen in Fig. 1A. Hybridization with the 3.01-kbp HindIII fragment encoding cpeB cpeA, and cpcC. The arrowheads to the left of lane 1 show indicate the eight transcript species detected (also see Fig. 1A). B, same experiment as A, but the film was heavily overexposed to show the minor transcript species cpeBACDE and cpeBACDEF as well as to emphasize the low-abundance of all cpc transcripts in the nitrogen-starved cells. C, hybridization with 0.884-kbp HindIII-XhoI fragment specific for the cpcC gene. D, hybridization with 0.364-kbp HindIII-HindIII fragment specific for the cpcD gene. E, hybridization with the 1.992-kbp HindIII-XhoI fragment encoding the cpeD and cpcF genes.

FIG. 4. Fluorograms of Southern hybridization experiments to confirm mutant strain constructions. Numbers to the right indicate the DNA fragment sizes in kilobase pairs. In each panel, lanes 1 and 3 contain total DNA isolated from the wild-type strain PR6000 and lanes 2 and 4 contain total DNA isolated from the mutant strain. The DNAs in lanes 1 and 2 were digested with HindIII, and the DNAs in lanes 3 and 4 were digested with HindIII and BglII. The probe fragment employed in all cases was the 1.98-kbp HindIII-XhoI fragment encoding both the cpeE and cpcF genes (see Fig. 1). A, confirmation of strain PR6240. The probe hybridized to the 3.56-kbp HindIII fragment in the wild-type (lane 1) and a 4.88-kbp HindIII fragment in the mutant (lane 2). The 1.3-kbp increase in size is due to the insertion of the aphII gene in the EcoRI site of the cpeE gene (see Fig. 1B). Correspondingly, after digestion with HindIII and BglII, the same results are obtained with the wild-type strain, but two fragments (1.6 and 3.27 kbp) hybridize in the mutant DNA because of the presence of a single BglII site in the cpeE gene. After digestion with HindIII and BglII, hybridization of a single 3.56-kbp HindIII fragment was still observed in the wild-type strain (lane 3), whereas in the mutant strain two fragments of 3.4- and 1.46-kbp hybridized because of the introduction of the BglII site in the aphII interposon (see Fig. 1). C, confirmation of strain PR6260. In the wild-type strain, the probe hybridizes to two HindIII-SalI fragments of 0.98 and 2.58 kbp (lane 1), whereas in the mutant strain only a single fragment of 4.35 kbp hybridizes, since the SalI site was deleted during this construction (see Fig. 1). Digestion of the wild-type DNA with HindIII and BglII produced a single hybridizing fragment of 3.56 kbp, whereas digestion of strain PR6260 DNA with HindIII and BglII produced two hybridizing fragments of 2.74 and 1.6 kbp hybridize because of the introduction of the BglII site in the aphII interposon (see Fig. 1).
produce PC because the structural genes for its two subunits were wild type. Nonetheless, the relative growth rates were significantly faster than that of strain PR6230, which does not contain the mutant allele from the wild-type strain PR6000 and the three mutant strains PR6240, PR6250, and PR6260. Similar results were obtained with mutant strain PR6054 (data not shown). This identification has also been confirmed by isolation and tryptic peptide mapping of the polypeptide (Swanson et al., 1992). A sensitive method for detecting polypeptides carrying linear tetrapyrrole chromophores takes advantage of the fluorescence of these polypeptides after the formation of the Zn-bilin chelate (Raps, 1990). No fluorescence could be detected from the apoPC α subunit, but the fluorescence of the β subunit and the subunits of allophycocyanin appeared to be normal (Fig. 6A, lane 1). Interestingly, the polypeptide-containing fractions 2 from all three mutants also contained some apoPC α subunit. In addition, a small amount of a polypeptide comigrating with the chromophorylated form of the α subunit could be detected (Fig. 6, B, lane 2, and C, lanes 2 and 4).

During serial subculturing of the three mutant strains, the color phenotypes of the strains became noticeably more blugreen than those of the original mutant strains. Whole-cell phycobilisomes were significantly smaller than those of the wild-type strain, which are typically recovered from the interface of the 1.0 and 2.0 M sucrose layers. The absorption spectra of these fractions suggested the presence of a small amount of PC in the mutant cells. As shown in Fig. 5C, varying amounts of absorbance at 630 nm, characteristic of PC, could be observed (also see below) but did not exceed about 10% of the level found in wild-type cells.

Further analyses of the gradient fractions by SDS-PAGE indicated that fractions 1 contained a blue-colored polypeptide which comigrated with authentic PC subunit (Fig. 6). In addition, the three fractions 1 contained a polypeptide which migrated slightly more rapidly than the wild-type PC α subunit. The behavior of this polypeptide upon SDS-PAGE was identical to that observed for the apoPC α subunit expressed in Escherichia coli (Bryant et al., 1985) and immunoblotting experiments with antibodies directed against the α subunit of Synechococcus sp. PCC 6301 PC confirmed that this polypeptide was the PC α subunit apoprotein (data not shown). This identification has also been confirmed by isolation and tryptic peptide mapping of the polypeptide (Swanson et al., 1992). A sensitive method for detecting polypeptides carrying linear tetrapyrrole chromophores takes advantage of the fluorescence of these polypeptides after the formation of the Zn-bilin chelate (Raps, 1990). No fluorescence could be detected from the apoPC α subunit, but the fluorescence of the β subunit and the subunits of allophycocyanin appeared to be normal (Fig. 6A, lane 1). Interestingly, the polypeptide-containing fractions 2 from all three mutants also contained some apoPC α subunit. In addition, a small amount of a polypeptide comigrating with the chromophorylated form of the α subunit could be detected (Fig. 6, B, lane 2, and C, lanes 2 and 4).

During serial subculturing of the three mutant strains, the color phenotypes of the strains became noticeably more blugreen than those of the original mutant strains. Whole-cell
absorption spectra of such cultures indicated that the PC levels were substantially increased (compare Figs. 5A and 7A). As much as 50% of the absorbance lost at 630 nm was recovered in some subculturing experiments (Fig. 7A). Phycobilisomes isolated from such strains had substantially greater absorbance at 630 nm as well (Fig. 7B). Low light intensity (≤100 microeinsteins m⁻² s⁻¹) and elevated temperature (≥39 °C) favored increased PC content, whereas high light intensity (≥200 microeinsteins m⁻² s⁻¹) and lower temperature (24–28 °C) favored the original mutant phenotype (lower apparent PC content).

**DISCUSSION**

The cpc operon of *Synechococcus* sp. PCC 7002 is shown to contain six genes which are transcribed into at least eight, but more likely ten (see Fig. 1A) transcript species. The steady-state levels of the transcripts which include the cpcE and cpcF genes, which occur at the 3' end of the operon, are quite low and are estimated to account for only about 1–2% of the total transcripts arising from this locus. Previous studies have demonstrated that the CpcE and CpcF products are not structural components of the phycobilisomes of *Synechococcus* sp. PCC 7002 (Bryant et al., 1990). The low steady-state levels of the transcripts encoding these proteins are consistent with this observation and suggest that these proteins are only required in catalytic amounts. The transcription patterns observed here differ from those reported for *Calothrix* sp. PCC 7601 (Mazel et al., 1988) and *Anabaena* sp. PCC 7120 (Belknap and Haselkorn, 1987; also see Bryant et al., 1991).

![Fig. 7. Absorption spectra of whole cells and fractions obtained from phycobilisome isolation. A, whole-cell absorption spectra normalized at 681 nm of of mutant strains PR6240, PR6250, and PR6260 after many serial subculturings. B, phycobilisome fractions isolated from the serially subcultured mutant cells whose spectra are shown in A. The wild-type and mutant spectra were normalized at 650 nm to facilitate their comparison.](image-url)

In *Calothrix* sp. PCC 7601, the cpcE gene is cotranscribed with the cpcB1 and cpcA1 genes, but the cpcF gene is transcribed as a monocistronic mRNA. Transcription of the cpc operon in *Anabaena* sp. PCC 7120 is more complex; at least some transcripts occur in which cpcE and cpcF are cotranscribed with the cpcABCD structural genes as well as the cpcG1G2G3G4 genes (Belknap and Haselkorn, 1987; Bryant et al., 1991). At present it is not known how the complex family of transcripts observed for *Synechococcus* sp. PCC 7002 arises. Sequences capable of forming energetically stable stem-loop structures occur between the cpcA-cpcC, cpcC-cpcD, cpcD-cpcE, and cpcE-cpcF gene pairs as well as downstream from the cpcF gene, and such sequences could play a role in mRNA stabilization or transcription termination or both processes. Transcription might partially terminate after the cpcA, cpcC, cpcD, cpcE, and cpcF genes in a sequential and additive fashion such that the longest transcripts would therefore be least abundant. Alternatively, primary transcripts might initially encode all six genes but could be rapidly processed by endonucleolytic or exonucleolytic processing of the full-length primary transcripts to the shorter species. Transcripts stabilized by the largest number of secondary structures (i.e., those encoding the cpcB and cpcA genes) would thus be most abundant as observed. Additional experiments will be required to distinguish between these two possibilities.

The deduced amino acid sequences of the CpcE and CpcF proteins did not exhibit significant homology to other proteins in databases which could suggest their function, and a retrogenetics approach was thus adopted for examining their possible functions. The mutations in the cpcE gene reported here are the first mutations identified or constructed in this gene. However, Tandeau de Marsac and co-workers (Tandeau de Marsac et al., 1988, 1990) have previously isolated a spontaneous deletion mutant as well as mutants in which insertion elements (IS701 and IS703) inactivated the cpcF gene. These mutants are phenotypically similar to those reported here; they are defective in PC accumulation and have levels that are only about 10% those of the wild-type strain. Although it was initially proposed that these mutations affected the transcription of the cpcBA mRNA, a reinvestigation of the properties of these mutants, based upon preliminary results from observations in *Synechococcus* sp. PCC 7002, revealed that these mutants are also defective in the chromophorylation of the PC α subunit (Tandeau de Marsac et al., 1990). Interestingly, the *Calothrix* sp. PCC 7601 mutants are also defective in their ability to transcribe the genes encoding phycoerythrin when the cells are grown in green light.

Insertional inactivation of either the *Synechococcus* sp. PCC 7002 cpcE or cpcF genes, or a deletion affecting both genes, produced strains with identical phenotypes. The yellow-green color phenotypes of these mutants suggested that the cells did not accumulate normal levels of PC. However, biochemical studies revealed that significant levels of normally chromophorylated PC β subunit were present in all three mutant strains (Swanson et al., 1992). However, only small amounts of chromophorylated PC α subunit along with substantial amounts of PC α apoprotein were found. All other PBPs appeared to be normally chromophorylated with phycocyanobilin; this indicates that these mutations did not affect phycocyanobilin synthesis, since phycocyanobilin is the only chromophore found in this cyanobacterium (Bryant et al., 1990). These observations strongly suggest that the CpcE and CpcF proteins are involved in the specific attachment of chromophores to the PC α subunit. Other gene products are apparently involved in chromophore attachment to other PBP subunits. It could be argued that only the CpcF polypeptide...
is required and that the phenotype of strain PR6240 strain arises from a polarity effect of the interposon insertion in the cpcE gene. However, the chromophorylation of the PC α subunit is normal in mutant strains PR6009, PR6011, and PR6014 which have the same interposon inserted in the cpcC or cpcD genes which occur 5' to the cpcE and cpcF genes (see Fig. 1 and de Lorimier et al., 1990a, 1990b). Hence, it is unlikely that the inability to chromophorylate the PC α subunit in strain PR6240 is due to a polarity effect on the cpcE gene; therefore, it is probable that both the CpcE and CfpF product are specifically required for this process.

The small amounts of chromophorylated PC α subunit detected in strains PR6240, PR6250, and PR6260 could arise from a spontaneous chemical reaction between phycocyanobilin and the apoPC α subunit (Arciero et al., 1988a, 1988b, 1988c). Alternatively, the apoPC α subunit could be a poor substrate for a chromophore attachment enzyme normally acting on another PBP substrate (e.g. an enzyme involved in the chromophore attachment to the PC β subunit or an allophycocyanin subunit). Studies presented in the accompanying manuscript (Swanson et al., 1992) suggest both of these processes can occur. The plasticity of the phenotypes of serial subcultures of these mutants is consistent with the notion that secondary mutations can arise in the cells which can partially or even completely restore the ability to chromophorylate the PC α subunit (Swanson et al., 1992).

In summary, results presented here and in the accompanying paper (Swanson et al., 1992) suggest that the CpcE and CfpF proteins form a lyase which specifically attaches phycocyanobilin to the PC α subunit. Recently, these proteins have been individually overproduced in E. coli. The availability of large amounts of the CpcE and CfpF proteins allows in vitro experiments to test this hypothesis and possibly reveal details of the mechanism of this process.

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**SUPPLEMENTARY MATERIAL**

**EXPERIMENTAL PROCEDURES**

**Stages and Growth Conditions.** Synechococcus sp. PCC 7002 strain FR8000 (formerly known as Anacystis taylorii strain FR-800) was grown at 30 °C in medium A supplemented with 1.5 mM sodium succinate as previously described (Lamer et al., 1977). Cells were used for experiments when cultures had reached late exponential phase of growth. Growth rates were monitored by absorbance at 750 nm as a measure of total cellular material at each time point. Growth on solid media, medium A plus sucrose, was supplemented with 1.5 mM sodium succinate as previously described (Lamer et al., 1977).

**DNA and RNA Manipulations and Transformation of Synechococcus sp. PCC 7002.** DNA was isolated from Synechococcus sp. PCC 7002 (formerly known as Anacystis taylorii strain FR-800) as described by Lamer et al. (1977). Plasmid DNA was purified from 1.5% agarose gels in the presence of 1% sodium laurel sarcosyl and 0.05 M sodium acetate. DNA concentrations were determined from UV absorbance at 260 nm. DNA was stored at -70 °C.

**Transformation of Synechococcus sp. PCC 7002.** Transformation of Synechococcus sp. PCC 7002 was performed by electroporation as described by Lamer et al. (1977). Transformation efficiency was about 1 transformant per 10^6 cells.

**Phycobilisome Isolation, Abrogation Spectroscopy, and Phycobiliprotein Gel Electrophoresis.** Phycobilisomes were prepared from Synechococcus sp. PCC 7002 strain FR8000 as described by Shen et al. (1981) and modified for complementation analysis by GoLee (Genius, 1982). Phycobilin pigments were extracted by 30% methanol at 90 °C for 30 min and used for absorption measurements.

**TABLE 1**

| Strain | Genotype | Detection Method | Relative Growth Rate |
|--------|----------|------------------|----------------------|
| FR8000 | wild type | F^+/+ | 1.0 (3.8) 1.60 |
| FR796 | cpeE::tnaA | A^+/+ | 1.38 (10.3) 1.49 |
| FR240 | cpeF::tnaA | A^+/+ | 3.5 (10.5) 2.32 |
| FR242 | cpeF::tnaA | A^+/+ | 3.5 (10.5) 2.32 |
| FR244 | cpeF::tnaA | A^+/+ | 3.5 (10.5) 2.32 |
| FR250 | cpeF::tnaA | A^+/+ | 3.5 (10.5) 2.32 |
| FR251 | cpeF::tnaA | A^+/+ | 3.5 (10.5) 2.32 |
| FR252 | cpeF::tnaA | A^+/+ | 3.5 (10.5) 2.32 |

**Notes:**

* All cultures were grown under a strong light source, which allows for maximum photosynthetic growth rates. Growth rates are normalized to the maximum growth rate observed for the wild-type strain.

* Relative growth rate is given for different strains as calculated by the peak absorbance in each graph column.

* High light intensity (0.5 × S800) was used for all experiments.