Genes Encoding the Phycobilisome Rod Substructure Are Clustered on the *Anabaena* Chromosome: Characterization of the Phycoerythrocyanin Operon

RONALD V. SWANSON,[†] ROBERT DE LORIMIER, AND ALEXANDER N. GLAZER*

*Division of Biochemistry and Molecular Biology, Department of Molecular and Cell Biology,
229 Stanley Hall, University of California, Berkeley, California 94720*

Received 15 October 1991/Accepted 10 February 1992

The phycoerythrocyanin (*pec*) operon, cloned from *Anabaena* sp. strain PCC 7120, encodes four genes, *pecBACE*, located upstream of the C-phycoyanin (*cpc*) operon. This *pec-cpc* cluster includes all the genes for the structural components of the phycobilisome rod. Oligonucleotide probes based on the amino-terminal sequence of the phycoerythrocyanin β subunit were used to clone an 8.0-kbp *EcoRI* fragment which was determined, by sequencing, to partially overlap the previously cloned *cpc* operon. A 5.0-kbp *EcoRI*-*Clal* fragment corresponding to the region upstream of the *cpc* operon was subsequently subcloned and sequenced. Five open reading frames whose polarity of transcription is parallel to that of the *cpc* genes were identified. *pecB* and *pecA* encode the β and α subunits of phycoerythrocyanin, respectively. *pecC* encodes the phycoerythrocyanin-associated linker polypeptide $L_R^{34.5,PEC}$. The identities of these genes are confirmed by agreement with amino-terminal sequences determined from purified phycobilisome components. A gene homologous to *cpcE*, found downstream of *pecC*, has been designated *pecE*. The *cpcE* gene product is involved in the attachment of the phycocyanobilin chromophore to the α subunit of phycocyanin. Three transcripts were observed by Northern (RNA) analyses. The most abundant of these transcripts, 1.35 kbp, corresponds to the β and α subunit genes, whereas the less-abundant transcripts, 2.3 and 3.1 kbp, correspond to *pecBAC* and *pecBACE*, respectively. Phycoerythrocyanin is strongly induced in cells cultured under low light. In parallel, all three transcripts were present at much higher levels in cells cultured under low light.

Phycobilisomes are very large protein complexes which function as light-harvesting antennae in cyanobacteria and red algae. The proteins which compose these highly ordered structures fall into two groups: phycobiliproteins and linker polypeptides. The phycobiliproteins are a large family of colored proteins which serve as the major light-harvesting components of the phycobilisome. These proteins absorb visible light by virtue of bilin prosthetic groups, linear tetrapyrroles covalently attached to the protein through thioether linkages to cysteinyl residues. The phycobiliproteins are subdivided into three major groups, allophycocyanins, phycocyanins, and phycoerythrins, on the basis of sequence homology, bilin content, and location in the antenna complex. The linker polypeptides, which also constitute a homologous family, mediate the ordered interactions of the phycobiliproteins to form the phycobilisome. The phycobilisome is composed of two distinct subdomains: the core, which contains primarily allophycocyanin, and the peripheral rods, which contain phycocyanin and, when present, phycoerythrin. Energy absorbed by the rods, which contribute the majority of the absorption cross section of the phycobilisome, is efficiently transferred to the core from which it migrates to the reaction centers in the thylakoid membrane.

Allophycocyanin invariably contains only phycocyanobilin (PCB). The absorption spectrum of allophycocyanin varies little from one organism to another. In contrast, depending on organismal source, the rod phycobiliproteins, the phycocyanins and phycoerythrins, vary widely with

respect to the types of bilins they carry and, consequently, with respect to spectroscopic properties. The four bilins present in various combinations in cyanobacterial and red algal phycocyanins and phycoerythrins have distinctive absorption spectra. Protein-bound PCB, phycobiliviolin, phycoerythrobilin, and phycourobilin absorb maximally at 620, 568, 565, and 490 nm, respectively.

Phycocyanins are composed of two polypeptide chains: the α subunits (~162 residues) carry one bilin chromophore at position α -84, and the β subunits (~172 residues) carry two bilins, one each at positions β -82 and β -153. Most species of freshwater cyanobacteria produce C-phycoyanin (CPC), which carries PCB at all three positions. Many red algae produce R-phycoyanin, which carries the shorter-wavelength-absorbing phycoerythrobilin at the β -153 position and PCB at the other two positions (7); several marine *Synechococcus* spp. have been found to synthesize R-phycoyanin II, which carries phycoerythrobilin at both the β -153 and α -84 positions and PCB at β -82 (23). At least one marine *Synechocystis* strain synthesizes a phycocyanin which carries phycourobilin at position α -84 and PCB at β -82 and β -153 (27).

Several cyanobacterial strains which contain multiple sets of *cpc* genes are known. In some of these organisms, the additional phycocyanin is induced by changes in light quality, as part of a chromatic adaptation response. *Calothrix* sp. strain PCC 7601 contains three distinct CPCs: one is constitutive, another is induced in red light, and the third, which lacks nonessential cysteine and methionine residues, is induced under conditions of sulfur limitation (21, 22). A relatively small number of cyanobacteria, made up primarily of filamentous heterocyst-forming strains, synthesize both CPC and phycoerythrocyanin (PEC). The latter protein

* Corresponding author.

[†] Present address: Department of Biology, California Institute of Technology, Pasadena, CA 91125.

carries a phycobiliviolin at position α -84 and two PCBs on the β subunit (6, 7). PEC is induced under low-light conditions and occupies the core-distal position in the rods of phycobilisomes. The strains which produce both CPC and PEC are unique in that they synthesize two phycocyanins with different bilins within the same cell and consequently present a special opportunity for studying questions of discrimination between bilins in the course of bilin attachment during phycobiliprotein biosynthesis.

PEC was discovered and originally characterized in two strains of *Anabaena* (6). Subsequently, the protein from *Mastigocladus laminosus* PCC 7603 has been thoroughly characterized. The amino acid sequence of *M. laminosus* PEC has been completely determined (15), and its three-dimensional structure has been solved by X-ray crystallography (13). Recently, a partial sequence of the *pec* operon from this organism has been reported (14). The *pec* operon sequence of *Anabaena* sp. strain PCC 7120 described here adds to the body of data on PEC and to that on phycobiliprotein genetic organization in general. This clone also provides a powerful substrate for genetic manipulations aimed at dissecting the mechanisms of bilin addition and discrimination between bilins, as well as for exploring the molecular basis of regulation by light intensity.

MATERIALS AND METHODS

Strains and culture conditions. *Anabaena* sp. strain PCC 7120 was cultured in BG-11 medium (1) under constant illumination, with either high (200 microeinsteins $m^{-2} s^{-1}$) or low (40 microeinsteins $m^{-2} s^{-1}$) light, at room temperature or at 36°C, as indicated in the text. Cultures were aerated by bubbling with 4% CO_2 -96% N_2 or with 4% CO_2 -96% air. *Escherichia coli* DG-98 (*thi-1 endA1 hsdR17 supE44 lacI^a lacZ Δ M15 proC1::Tn10 F' lacI^a lacZ Δ M15 proC⁺*), obtained from Cetus Corp., was used for cloning of recombinant plasmids. DG-98 was cultured in Luria-Bertan: (LB) liquid or on LB agar plates, supplemented with 100 μ g of ampicillin ml^{-1} when appropriate.

Isolation of PEC subunits for sequencing. PEC was purified from low-light room-temperature cultures by the procedure of Bryant et al. (6) for the purification of PEC from *Anabaena* sp. strain PCC 6411. Subunits were separated by reverse-phase chromatography on a C-4 column (26). After chromatography, solvent was evaporated under a stream of N_2 , and the subunits were redissolved in 44% formic acid. Amino-terminal sequencing was performed with an automated gas-liquid phase sequencer (Applied Biosystems, Inc.) by using 400 pmol of the β subunit.

The α subunit sequence was obtained from material purified by an abbreviated method. *Anabaena* sp. strain PCC 7120 cells (70 mg) were resuspended in 0.7 ml of 20 mM 2-mercaptoethanol by pipetting, 0.7 ml of 9 M urea (pH 2.0) was added, and the solution was mixed by gentle inversion and incubated at room temperature for 30 min. The solution was spun in a microfuge for 2 min, and the supernatant was removed to a clean tube and spun again for 10 min. The supernatant (300 μ l) was injected directly onto the C-4 column and chromatographed according to the subunit separation procedure. Then, 650 pmol of α subunit was recovered and sequenced.

Isolation of chromosomal DNA. Chromosomal DNA was prepared from *Anabaena* sp. strain PCC 7120 cells harvested by centrifugation and resuspended in 50 mM Tris (pH 8.0)-100 mM EDTA-10% (wt/vol) sucrose. This suspension was frozen and thawed three times, lysozyme was added to

10 mg ml^{-1} , and the suspension was incubated at 37°C for 30 min. At this point, pronase and sodium dodecyl sulfate (SDS) were added to final concentrations of 1 mg ml^{-1} and 1% (wt/vol), respectively, and the incubation at 37°C was continued for 60 min. The lysate was extracted sequentially with phenol and chloroform. Finally, DNA was purified by buoyant density centrifugation in a cesium chloride gradient.

Cloning and sequencing. Two 128-fold-degenerate 17-nucleotide (nt) oligonucleotides were synthesized on the basis of two overlapping segments of the amino-terminal sequence of the PEC β subunit, probe B2 with the sequence 5'-G-C-T/C-T-G-T/C-T-C-N-A-C-N-A-C-T/C-T-T3' and probe B4 with the sequence 5'-G-A-T/C-G-C-N-T-T-T/C-T-C-N-A-A-A/G-G-T3'. These oligonucleotides were end labelled with ^{32}P by using T4 polynucleotide kinase and used to probe Southern blots of *Hind*III digests of chromosomal DNA. Hybridizations were performed according to the conditions described by Wallace and Miyada (29) at 46°C for probe B2 and at 42°C for probe B4. Washes were done at room temperature in 0.9 M NaCl-0.18 M Tris (pH 8.0)-6 mM EDTA-0.5% SDS. Both probes hybridized to a 1.3-kbp fragment. A library was constructed from chromosomal DNA, which had been cut with *Hind*III and sized on a 1% low-melting-point agarose gel, and the vector pBluescript KS+ (Stratagene), which had been cut with *Hind*III and treated with calf intestinal alkaline phosphatase. Transformants of strain DG-98 were pooled into 20 groups of ~30 each by being resuspended in LB medium. Plasmid DNA was isolated from these pools by the alkaline lysis method. A portion of each of these plasmid pools was probed by using the dot blot technique described by Costanzi and Gillespie (10). One positive pool was identified, and a small aliquot of the remaining DNA was used to transform *E. coli* DG-98. The dot blot procedure was repeated on 60 individual colonies from this transformation, and two positive clones were recovered. Restriction mapping of these clones suggested that they carried the same insert. Partial sequencing of one clone, pF10, revealed that it carried a portion of *pecB*. A 19-nt oligonucleotide, probe B5 with the sequence 5'-T-C-A-T-C-T-C-A-C-T-A-A-G-A-T-A-A-T3', was synthesized, on the basis of the sequence of *pecB*, for use as a sequencing primer and probe. On the basis of restriction mapping of pF10, an *Eco*RI digest of chromosomal DNA was blotted and probed with B5 at 48°C. The single 8.0-kbp fragment identified by this procedure was cloned in pBluescript by a method similar to that described above to give clone pF400. Double-strand sequencing of pF400 *Hind*III, *Hinc*II, and *Sau*3A subclones was done by the dideoxy method (24) by using the Applied Biosystems dye primer cycle sequencing kit and Model 373A automated DNA sequencer. Some sequences were obtained with the Sequenase Version 2.0 sequencing kit (U.S. Biochemicals) by using pF500 primed from oligonucleotides complementary to the insert.

RNA isolation and Northern (RNA) blotting. RNA was isolated according to Golden et al. (18) from cells grown under either high (200 microeinsteins $m^{-2} s^{-1}$) or low (40 microeinsteins $m^{-2} s^{-1}$) light at 36°C. No significant contamination of the purified RNA by DNA was detected. Cultures were diluted twofold with fresh medium every 12 to 24 h to maintain an optical density at 550 nm of 0.2 to 0.6. This resulted in a maximum variation of 1.5-fold in the amount of light transmitted by a culture. Before being harvested, cultures were grown continuously for at least four generations under these conditions. Culture doubling times were 11 h under high light and 24 h under low light.

For Northern blotting, absolute amounts of high- and

FIG. 1. Genetic organization of the *pec* and *cpc* loci on the chromosome of *Anabaena* sp. strain PCC 7120. The upper portion of the diagram represents the linkage and orientation of the two operons relative to one another; the extent of clone pF400 is shown directly below this. The lower portion shows an enlarged region corresponding to the *EcoRI-ClaI* subclone pF500. The diagram was compiled from data presented in this paper as well as data from references 3 and 8.

low-light RNA were matched to within 5% by A_{260} . RNA was fractionated by electrophoresis in a 1.2% agarose gel containing 6% formaldehyde–50 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid [pH 7.2])–1 mM EDTA and transferred to nitrocellulose. Filters were probed with restriction fragments which had been ^{32}P labelled by using the random primed DNA labelling kit from Boehringer Mannheim. The gene-specific probes used were as follows (numbering according to Fig. 2): *pecBA*, *HindIII* fragment (nt 1102 to 1842); *pecC*, *Sau3A* fragment (nt 2318 to 2677); *pecE*, *XmnI-HindIII* fragment (nt 2900 to 3267); *ORF173*, *AccI* fragment (nt 4090 to 4460); and *cpcBA*, *XbaI-BssHII* fragment corresponding to nt 315 to 712 of the *cpc* operon (numbering according to reference 3). Relative levels of RNA species were quantitated by two methods. In the first, individual bands were excised from the nitrocellulose filters of Northern blots and quantitated by scintillation counting. The second method utilized dot blots of serially diluted RNA samples from high- and low-light cultures. Identical sections of the filter were probed with either the *pecBA* or *cpcBA* restriction fragments. Dot blots were autoradiographed without intensifying screens, and the resulting films were scanned with a densitometer. Peak areas from triplicate samples were used to quantitate relative RNA levels.

Nucleotide sequence accession number. The nucleotide sequence reported in this paper has been submitted to GenBank and assigned the accession number M80357.

RESULTS AND DISCUSSION

Isolation of the PEC genes. PEC was purified from low-light cultures of *Anabaena* sp. strain PCC 7120 grown at room temperature, as previously described (6). The α and β subunits were separated by reverse-phase high-performance liquid chromatography (HPLC) (26), and amino-terminal sequences were determined to provide information for the design of oligonucleotide probes for cloning. Two degenerate probes based on the β subunit sequence were synthesized and used to probe Southern blots of *HindIII*-digested chromosomal DNA. While each probe hybridized to several bands with approximately equal affinity, only one band was recognized by both probes. This 1.3-kbp fragment was cloned and found by sequencing to contain the 5' end of the *pecB* gene and a single *EcoRI* site 750 bp upstream of the

start codon of *pecB*. On the basis of these observations, a nondegenerate oligonucleotide was synthesized and used to probe an *EcoRI* digest of chromosomal DNA. The sole hybridizing fragment (8 kbp) was cloned. This clone, pF400, was determined, by partial sequencing, to overlap the previously cloned *cpc* operon (3) as shown in Fig. 1. An *EcoRI* site used for cloning this fragment was shown to lie within the *cpcE* gene by sequencing. The fact that it is not present in the *cpc* operon sequence reported previously suggests a polymorphism between laboratory strains. The replacement of a T by a C at position 2515 in the *cpc* operon (using the numbering from reference 3) does not result in a change in the translated amino acid sequence.

These results, along with recent further characterization of the *cpc* operon which showed that it contains a small gene family encoding the rod-core linkers (8), show that all the known structural components of the phycobilisome rod are encoded within the 13-kbp region of the *Anabaena* chromosome depicted in Fig. 1. The close linkage of the *pec* and *cpc* operons is reminiscent of the clustering of some phycobilly-protein genes in other cyanobacterial species such as *Calothrix* sp. strain PCC 7601, which contains a cluster encoding allophycocyanin, inducible-phycocyanin, and constitutive-phycocyanin operons (9), *Synechococcus* sp. strain PCC 6301, which carries two closely linked phycocyanin operons (19), and marine *Synechococcus* spp., which have recently been found to possess a cluster containing two phycoerythrin operons along with an R-phycocyanin II operon (12). It is unclear whether these clusters represent a residual consequence of the evolution of these genes by duplication and subsequent divergence or whether they may have some functional significance. It should be noted that many examples of phycobilisome genes which are not clustered are known and that genomic mapping indicates that at least two of the phycobilisome core components in *Anabaena* sp. strain PCC 7120 are not closely linked to one another (2).

Nucleotide sequence of pF500 and identification of *pec* genes. On the basis of the restriction map of the *cpc* operon, a 5-kbp *EcoRI-ClaI* fragment representing the region 5' to the *cpc* genes was subcloned and sequenced. The nucleotide sequence of the entire *EcoRI-ClaI* insert of pF500 is shown in Fig. 2. The region from nt 4711 to 4996 overlaps the previously reported *cpc* operon sequence (3), and no dis-

[illegible]

FIG. 2. Nucleotide sequence of the 4,996-bp *EcoRI*-*Clai* insert of pF500. The translated amino acid sequences of the *pec* genes as well as that of ORF173 are presented. *pecB*, encoding the PEC β subunit, extends from nt 750 to 1265. *pecA*, encoding the PEC α subunit, extends from nt 1344 to 1829. *pecC*, encoding the PEC-associated linker polypeptide L_R^{34.5,PEC}, extends from nt 1979 to 2812. *pecE*, encoding a polypeptide with homology to *cpeE*, extends from nt 2891 to 3565. Residues of these three gene products which have been confirmed by N-terminal sequencing are indicated by bold type. Amino acid sequence data for L_R^{34.5,PEC} is from reference 8. ORF173 extends from nt 4069 to 4587. The sequence was determined on both strands, except for the two end regions from nt 1 to 78 and from nt 4929 to 4996, which were determined on a single strand. DNA sequencing data were managed and analyzed with the IntelliGenetics suite of programs.


```

pecE 1  -----VTAE--PILSPETAIAALSGEDNQIRYYAAWWLGK--HNVQAGFTALCVALFD
cpcE 1  MIEPSVEEFP  NG Q T L  N QSS LSL                      YRVKES AVD  IA  E

pecE 56  ERYRIPSGGYPLRRQAARALGLLKNPQAVPALIAALEC--DEDLRLREAVICSLAAIGDKRA
cpcE 62  AD TEL          N      K G RK  G NC  P FYV EAA QSLEMLKDKTAAP

pecE 116 VTPLLNLQSSQA-----QPYEALIEALATLQVWSARPQIEPFLQHYSERVQCAA
cpcE 123 ALIK LDGGVA  VQVTGRPHLV      VL  GAIGATD I L Q  E PVS

pecE 167 RYMYLLTQSEYIERIVKLNLDNMYLRWAAVFDLGAVAHQQAVQAILTAQVPNSLKLLNL
cpcE 184  A  Q    PV G LL  V AGNDLN  RV LG  IGYLA AE  AN KAE  F  IA

pecE 228 KRIL-----
cpcE 245  GL EHQMSAESNALSISDQAIRVMNLMDSLL

```

FIG. 5. Translated amino acid sequences of *pecE* and *cpcE* from *Anabaena* sp. strain PCC 7120. The translated sequence of *pecE* is 47% identical to that of *cpcE* (3). Blank positions indicate identity and dashes indicate gaps introduced to increase homology.

icantly, PEC differs from CPC in carrying a phycobiliviolin chromophore as opposed to a PCB chromophore at the α -84 position. Consequently, *pecE* might play a role in discrimination between bilins or in bilin isomerization along with bilin attachment. The *pecE* coding region is also followed by a potential stem-loop structure.

The *pec* operon is followed, 104 bp downstream of the *pecE* gene, by 26 direct repeats of the heptanucleotide sequence ATTGGGG. Tandem repeats of this sequence, as well as of other heptanucleotide sequences, have been observed near the coding regions of several genes cloned from heterocystous cyanobacteria (20). The significance of these repeat sequences is not known.

Following these repeats, a fifth parallel open reading frame is present, beginning at nt 4069 (Fig. 2). The 173-amino-acid sequence predicted from this open reading frame shows possible homology to *cpcF* (12).

Identification of transcripts from the *pec* and *cpc* operons. RNA was prepared from cells cultured under both high and low light for Northern blot analysis. Five probes were selected from subclones of pF400 representing sequences internal to *pecBA*, *pecC*, *pecE*, ORF173, and *cpcBA*. Three major *pec* transcripts were detected as shown in Fig. 6. On the basis of their patterns of hybridization to specific probes, the 1.35-kbp transcript corresponds to *pecBA*, the 2.3-kbp transcript corresponds to *pecBAC*, and the 3.1-kbp transcript corresponds to *pecBACE*. As noted in the previous section, large imperfect inverted repeats are present 3' of *pecA*, *pecC*, and *pecE*. This family of transcripts might arise either through incomplete termination at the intergenic hairpins or through partial degradation of a single large transcript. The relative abundance of each of these transcripts is depicted graphically in Fig. 6B. In addition to the three predominant segmental transcripts of the *pec* operon, there is a faint hybridization signal to a 2.6- to 2.7-kbp species with all three *pec* probes. This species apparently contains *pecACE*, perhaps as an independently initiated transcript or as a partially degraded *pecBACE* transcript. There is no evidence of *pecBACE* transcripts longer than 3.1 kbp which could include ORF173.

Both the *pecC* and ORF173 probes hybridize weakly to a 1.5-kbp species. The size of this species and the fact that its abundance is not dependent on light intensity (see below) suggest that this signal is due to spurious hybridization to 16S rRNA. No other major transcripts were observed to hybridize with the ORF173 probe. Thus, it is unclear whether this open reading frame encodes a translated prod-

FIG. 6. (A) Transcripts of the *pec* and *cpc* operons and their dependence on light intensity. A Northern blot of total *Anabaena* RNA from cells grown in high (H)- or low (L)-light intensity, 200 or 40 microeinsteins $m^{-2} s^{-1}$, respectively, was prepared. Each pair of lanes was probed with a restriction fragment specific to the genes denoted above the blot. The positions of RNA markers (lengths in kilobase pairs) are shown to the left of the blot. The 1.5- and 2.8-kbp species were the 16S and 26S rRNAs, respectively, of *Anabaena* sp. The remaining species were from a kit supplied by Bethesda Research Laboratories. (B) *pec* transcripts and their relative abundance. The three major transcripts from the *pec* operon are depicted. Percentages refer to the relative abundance of each transcript in low light estimated from the hybridization signals on the Northern blot shown in Fig. 6.

FIG. 7. HPLC chromatograms monitored at 560 nm of whole-cell extracts prepared from high- (upper chromatogram) and low- (lower chromatogram) light cultures of *Anabaena* sp. strain PCC 7120. High-speed supernatants of whole-cell lysates were injected onto a C-4 column and eluted with a linear gradient of acetonitrile-isopropanol (2:1)–0.1% trifluoroacetic acid (26). Both phycobiliviolin and PCB chromophores absorb at 560 nm. Peaks labelled as corresponding to the α subunits of PEC and CPC have been identified spectroscopically and by injections of these purified proteins. Unlabelled peaks correspond to the β subunits of these proteins and to the α and β subunits of allophycocyanin. The β subunits of PEC and CPC coelute. The scale bar is 0.04 A_{560} unit.

uct, although it is preceded by a potential ribosome binding site. It will be interesting to see whether a homologous open reading frame is present in *M. laminosus* and whether this organism has a similar *pec-cpc* operon arrangement.

The *cpcBA* probe is homologous to transcripts of 1.4, 3.6, and 7.7 kbp (Fig. 6). The predominant species encode *cpcBA* and *cpcBACDE*, respectively (3). The less abundant 7.7-kbp species is of sufficient length to contain the entire *cpc* operon, including all four *cpcG* genes (8).

Regulation by light intensity. The cellular level of PEC has been noted to be strongly influenced by light intensity (4, 17). Quantitation of the hybridization signals of the *pecBA* probe to dot blots of RNA from high- and low-light cultures reveals a 13-fold increase relative to total RNA in low light. The PEC contents of whole-cell extracts prepared from *Anabaena* sp. strain PCC 7120 cultured under high and low light were compared by the reverse-phase subunit separation procedure (26). The results shown in Fig. 7 are consistent with those of the earlier studies and with the observed increase in transcript levels; PEC is present when cells are cultured under low light but is not detectable by this procedure in the high-light cultures. Because the α subunit of PEC is not detectable in cells grown in high light, a value for the relative increase in PEC subunits in low light was not determined. If the α subunit of PEC was present in high-light cells at 1/13 the level in low-light cells, it would, in fact, have been undetectable in this experiment. The level of each of the three major *pec* transcripts increases by approximately the same factor in low light (Fig. 6), although this was not quantitated.

We observed an approximately twofold increase in *cpcBA* transcripts in low light. This correlated with an approximate doubling of CPC, relative to chlorophyll, in cells from

low-light cultures (data not shown). In contrast, Belknap and Haselkorn found that the level of *cpc* transcripts did not change with light intensity (3). The discrepancy could be due to the relatively short time (12 h) that these authors used to culture cells in differing light intensities and also to the use of absolute light intensities that differed from those in the present study. Belknap and Haselkorn did note that the ratio of 3.6- to 1.4-kbp *cpc* transcripts varied with light intensity, increasing by a factor of 10 in low light, and they suggested that transcriptional termination between *cpcA* and *cpcC* was light regulated (3). We also observed an increase in the ratio of these two transcripts in low light. However, the measured increase was twofold. Whether this change is correlated with an increase in CPC-associated linker polypeptides encoded by the 3.6-kbp transcript is unknown. In the cyanobacterium *Synechococcus* sp. strain PCC 7002, the ratio of *cpcBAC* and *cpcBA* transcripts remains constant with light intensity, although the ratio of linker to CPC changes (11).

No extensive sequence similarities which might indicate regulatory regions could be detected in the 5' regions of the *cpc* and *pec* operons. The interspecies comparison of the upstream regions of the *pec* operons from *Anabaena* sp. strain PCC 7120 and *M. laminosus* does, however, reveal similar regions located ~200 bp upstream of the translational start sites. Of the 36 nucleotides from –206 to –171 in the *Anabaena* sequence, 33 match the equivalent sequence from –196 to –161 in the *M. laminosus* sequence. Further experiments will be required to delineate the control elements of these promoters.

ACKNOWLEDGMENTS

We thank Sigurd Wilbanks of this laboratory for performing amino-terminal sequence analyses, Srinu Kadambi and Michael Moore of the University of California at Berkeley Microchemical Facility for the generous gift of all oligonucleotides used in this work, Victor Boyartchuk of the Lawrence Berkeley Laboratory Human Genome Center for help with automated DNA sequencing, and D. A. Bryant for providing us with a preprint of reference 8 and sharing data with us prior to publication.

This research was supported by National Science Foundation grant DMB 8816727, National Institute of General Medical Sciences grant GM 28994, and a predoctoral fellowship from the Department of Health and Human Services training grant GM 08352 to R.V.S.

REFERENCES

1. Allen, M. M. 1968. Simple conditions for growth of unicellular blue-green algae on plates. *J. Phycol.* 4:1–4.
2. Bancroft, I., C. P. Wolk, and E. V. Oren. 1989. Physical and genetic maps of the genome of the heterocyst-forming cyanobacterium *Anabaena* sp. strain PCC 7120. *J. Bacteriol.* 171: 5940–5948.
3. Belknap, W. R., and R. Haselkorn. 1987. Cloning and light regulation of expression of the phycocyanin operon of the cyanobacterium *Anabaena*. *EMBO J.* 6:871–884.
4. Bryant, D. A. 1982. Phycoerythrocyanin and phycoerythrin: properties and occurrence in cyanobacteria. *J. Gen. Microbiol.* 128:835–844.
5. Bryant, D. A. 1989. Genetic analysis of phycobilisome biosynthesis, assembly, structure, and function in the cyanobacterium *Synechococcus* sp. PCC 7002, p. 62–90. In S. E. Stevens and D. A. Bryant (ed.), *Light energy transduction in photosynthesis: higher plant and bacterial models*. American Society of Plant Physiologists, Rockville, Md.
6. Bryant, D. A., A. N. Glazer, and F. A. Eiserling. 1976. Characterization and structural properties of the major phycobiliproteins of *Anabaena* sp. *Arch. Microbiol.* 110:61–75.
7. Bryant, D. A., C. S. Hixson, and A. N. Glazer. 1978. Structural studies on phycobiliproteins. III. Comparison of bilin-containing peptides from the β subunits of C-phycocyanin, R-phycocy-

- anin, and phycoerythrocyanin. *J. Biol. Chem.* **253**:220–225.
8. Bryant, D. A., V. L. Stirewalt, M. Glauser, G. Frank, W. Sidler, and H. Zuber. 1991. A small multigene family encodes the rod-core linker polypeptides of *Anabaena* sp. PCC 7120 phycobilisomes. *Gene* **107**:91–99.
 9. Conley, P. B., P. G. Lemaux, T. L. Lomax, and A. R. Grossman. 1986. Genes encoding the major light-harvesting polypeptides are clustered on the genome of the cyanobacterium *Fremyella diplosiphon*. *Proc. Natl. Acad. Sci. USA* **83**:3924–3928.
 10. Costanzi, C., and D. Gillespie. 1987. Fast blots: immobilization of DNA and RNA from cells. *Methods Enzymol.* **152**:582–587.
 11. de Lorimier, R., R. L. Smith, and S. E. Stevens, Jr. Regulation of phycobilisome structure and gene expression by light intensity. *Plant Physiol.*, in press.
 12. de Lorimier, R., and S. M. Wilbanks. Unpublished data.
 13. Duerring, M., R. Huber, W. Bode, R. Ruemeli, and H. Zuber. 1990. Refined three-dimensional structure of phycoerythrocyanin from the cyanobacterium *Mastigocladus laminosus*. *J. Mol. Biol.* **211**:633–644.
 14. Eberlein, M., and W. Kufer. 1990. Genes encoding both subunits of phycoerythrocyanin, a light harvesting biliprotein from the cyanobacterium *Mastigocladus laminosus*. *Gene* **94**:133–136.
 15. Füglistaller, P., F. Suter, and H. Zuber. 1983. The complete amino-acid sequence of both subunits of phycoerythrocyanin from the thermophilic cyanobacterium *Mastigocladus laminosus*. *Hoppe-Seyler's Z. Physiol. Chem.* **364**:691–712.
 16. Füglistaller, P., F. Suter, and H. Zuber. 1985. Linker polypeptides of the phycobilisome from the cyanobacterium *Mastigocladus laminosus*: amino-acid sequences and relationships. *Biol. Chem. Hoppe-Seyler* **366**:993–1001.
 17. Füglistaller, P., F. Suter, and H. Zuber. 1986. Linker polypeptides of the phycobilisome from the cyanobacterium *Mastigocladus laminosus*. I. Isolation and characterization of phycobiliprotein-linker-polypeptide complexes. *Biol. Chem. Hoppe-Seyler* **367**:601–614.
 18. Golden, S., J. Busslan, and R. Haselkorn. 1987. Genetic engineering of the cyanobacterial chromosome. *Methods Enzymol.* **153**:215–231.
 19. Lind, L. K., S. R. Kalla, A. Lönneborg, G. Öquist, and P. Gustafsson. 1987. Organization of the phycocyanin gene clusters in *Anacystis nidulans*. *Acta Chem. Scand. Ser. B Org. Chem. Biochem.* **41**:112–115.
 20. Mazel, D., J. Houmard, A. M. Castets, and N. Tandeau de Marsac. 1990. Highly repetitive DNA sequences in cyanobacterial genomes. *J. Bacteriol.* **172**:2755–2761.
 21. Mazel, D., J. Houmard, and N. Tandeau de Marsac. 1988. A multigene family in *Calothrix* sp. PCC7601 encodes phycoyanin, the major component of the cyanobacterial light harvesting antenna. *Mol. Gen. Genet.* **211**:296–304.
 22. Mazel, D., and P. Marlière. 1989. Adaptive eradication of methionine and cysteine from cyanobacterial light-harvesting proteins. *Nature (London)* **341**:245–248.
 23. Ong, L. J., and A. N. Glazer. 1987. R-Phycocyanin II, a new phycocyanin occurring in marine cyanobacterial species. Identification of the terminal acceptor bilin in phycocyanins. *J. Biol. Chem.* **262**:6323–6327.
 24. Sanger, F., S. Nicklen, and A. R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA* **74**:5463–5467.
 25. Swanson, R. V. 1991. Ph.D. thesis. University of California, Berkeley, Calif.
 26. Swanson, R. V., and A. N. Glazer. 1990. Separation of phycobiliprotein subunits by reverse-phase high-pressure liquid chromatography. *Anal. Biochem.* **188**:295–299.
 27. Swanson, R. V., L. J. Ong, S. M. Wilbanks, and A. N. Glazer. 1991. Phycoerythrins of marine unicellular cyanobacteria. II. Characterization of phycobiliproteins with unusually high phycourobilin content. *J. Biol. Chem.* **266**:9528–9534.
 28. Tandeau de Marsac, N., D. Mazel, V. Capuano, T. Damerval, and J. Houmard. 1990. Genetic analysis of the cyanobacterial light-harvesting antenna complex, p. 143–153. *In* G. Drews and E. A. Dawes (ed.), *Molecular biology of membrane-bound complexes in phototrophic bacteria*. Plenum Press, New York.
 29. Wallace, R. B., and C. G. Miyada. 1987. Oligonucleotide probes for the screening of recombinant DNA libraries. *Methods Enzymol.* **152**:432–442.