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Structure and Light-Regulated Expression of Phycoerythrin Genes in Wild-Type and Phycobilisome Assembly Mutants of *Synechocystis* sp. Strain PCC 6701†

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Phycoerythrin is a major pigmented component of the phycobilisome, a cyanobacterial light-harvesting complex. It contains bilin-type chromophores that absorb and transfer light energy to chlorophyll protein complexes of the photosynthetic membranes. In many cyanobacteria, phycoerythrin expression is regulated by light wavelength in a response known as chromatic adaptation. Green light-grown cells contain higher levels of this biliprotein than do cells grown in red light. The phycoerythrin gene set from the unicellular cyanobacterium *Synechocystis* sp. strain PCC 6701 was cloned and sequenced, and the 5′ end of the phycoerythrin mRNA was localized. The amino acid sequences of the phycoerythrin subunits from *Synechocystis* strain 6701 and *Fremyella diplosiphon* were 90% identical. As observed in *F. diplosiphon*, the *Synechocystis* strain 6701 phycoerythrin transcript accumulated to high levels in green light-grown cells and low levels in red light-grown cells. Similar nucleotide sequences, which might control gene expression, occurred upstream of the transcription initiation sites of the phycoerythrin genes in both organisms. While the phycoerythrin structure and light-regulated transcript accumulation were similar in *Synechocystis* strain 6701 and *F. diplosiphon*, the steady-state levels of phycoerythrin subunits during growth in red light were quite different for the two organisms. This observation suggests that control of phycoerythrin levels in *Synechocystis* strain 6701 is complex and may involve posttranscriptional processes. We also characterized the phycoerythrin genes and mRNA levels in two phycobilisome assembly mutants, UV16-40 and UV16.

The phycoerythrins represent one class of biliproteins that are found in the phycobilisome, an abundant light-harvesting antennae complex present in the procaryotic cyanobacteria and eucaryotic red algae (15). Intensely red in color because of their high chromophore content, the phycoerythrin (PE) absorbance and fluorescence properties are a consequence of interactions between covalently bound bilin chromophores and the PE apoprotein. PE is located at the periphery of the phycobilisome as part of the rod substructures and is coupled to the phycobilisome core by association with the biliprotein phycocyanin (PC). Both PE and PC are composed of dissimilar α and β subunits and are associated with specific nonchromophoric linker proteins in hexameric aggregates, $(\alpha\beta)_6$-linker. The PE and PC hexamers are stacked to form the rod substructures which are attached to the phycobilisome core. The core contains the allophycocyanin class of biliproteins and is energetically coupled to chlorophyll protein complexes in the photosynthetic membranes (16). PE extends the light-harvesting capability of the phycobilisome into the green region of the visible light spectrum, and its location at the phycobilisome periphery reflects the position of PE in the sequential energy transfer pathway, PE > PC > AP > chlorophyll.

In many cyanobacteria, expression of the PE genes is controlled by light wavelength in a process known as chromatic adaptation (9, 27, 43). Increased transcription of the PE gene set in green light and depressed transcription in red light (27) allow the cyanobacterium to adjust the phycobilisome composition to optimize absorbance by the antenna complex in specific light environments. Two types of chromatic adaptations in cyanobacteria have been defined on the basis of variation of biliprotein content in red or green light (43). The filamentous cyanobacterium *Fremyella diplosiphon* represents one class in which both PE and PC are regulated in a complementary fashion. Red light increases the PC content while it decreases the PE content, and green light stimulates the synthesis and incorporation of PE into the phycobilisome as PC levels decline (24). *F. diplosiphon* has been the focus of molecular studies on complementary chromatic adaptation and is the only cyanobacterium for which a PE gene or protein sequence has been published (17, 27, 42). In the second type of chromatic adaptation, exhibited by the unicellular cyanobacterium *Synechocystis* sp. strain 6701, PE levels are regulated by light wavelength but PC is constitutively expressed (8, 14). Analyses of phycobilisomes from *F. diplosiphon* and *Synechocystis* strain 6701 grown in red and green light show that these two organisms suppress PE accumulation to different extents in red light (3, 14, 24). *F. diplosiphon* has very little PE in its phycobilisomes after growth in red light (about 5% of the green light level), while red light-grown *Synechocystis* strain 6701 has a variable PE content that ranges from 12 to 40% of that in green light-grown cells (3, 8, 14, 43). A comparison of regulation of the PE gene sets from *Synechocystis* strain 6701 and *F. diplosiphon* may help to establish biochemical events that occur as part of the chromatic adaptation response.

Isolation of the *Synechocystis* strain 6701 PE genes has facilitated the investigation of the phycobilisome assembly mutants UV16-40 and UV16. Earlier work showed that 90% of the PE β subunit in UV16-40 is missing one of the three phycoerythrobilin chromophores normally associated with this protein. This mutant has a defect in PE assembly at the

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hexamer stage and a PE content that is 10% of the wild-type (WT) level (4). UV16, the parent of UV16-40, has a lesion that blocks PC assembly, and its phenotype includes decreased PE content (55% of WT) and an altered chromatic adaptation response (2–4).

We characterized the PE genes from *Synechocystis* strain 6701 WT and phycobilisome assembly mutants, localized the transcription initiation site of *cpeBA*, and used gene-specific DNA fragments to quantitate mRNA in cells grown in green and red light. The PE subunits of *Synechocystis* strain 6701 and *F. diplosiphon* show extensive homology. There were similar patterns of PE mRNA accumulation in the two organisms in response to light wavelength, which may be the consequence of homologous nucleotide sequences just upstream of the transcription initiation sites. While no lesion in the coding region of the UV16-40 PE gene set was observed, characterization of these genes suggests which chromophore is missing in the PE β subunits of this mutant. Quantitation of PE transcript accumulation in UV16-40 and its parent, UV16, has furnished data on possible pleiotropic effects in these phycobilisome assembly mutants.

MATERIALS AND METHODS

Bacterial strains, media, and growth conditions. The WT *Synechocystis* strain 6701 has been described by Rippka et al. (34). The UV16-40 mutant is a product of two UV mutagenesis steps beginning with *Synechocystis* strain 6701 WT. The first step produced a mutant, UV16, with defective PC assembly (3, 4). Mutagenesis of UV16 resulted in the UV16-40 that is defective in both PC and PE assembly (4). Cyanobacteria were grown as previously described (2). The *Escherichia coli* strains used in this study were JM101, for M13 cloning and the isolation of sequencing templates (31); DH5α, for transformation and propagation of plasmids (Bethesda Research Laboratories, Inc., Gaithersburg, Md.); and BB4 and XL1-Blue, for library construction, screening, and the preparation of sequencing templates from the λZAP phage (Stratagene, La Jolla, Calif.).

DNA isolation, library construction, cloning, and sequencing. Chromosomal DNA was isolated from *Synechocystis* strain 6701 WT (1) and partially digested with *Sau*3AI. A genomic expression library was constructed from this DNA by using the λZAP cloning system (Stratagene). DNA fragments (1 to 4 kilobase pairs [kbp]) of the partially digested chromosomal DNA were eluted from an agarose gel (26), and the *Sau*3AI ends were partially filled in with A and G residues by using the Klenow fragment of DNA polymerase I. The λZAP vector was cut with *Xho*I and partially filled in with T and C residues. The chromosomal fragments were ligated with the vector and packaged into λ phage by using Gigapak Plus (Stratagene), and plaques were screened for expression of PE (47) by using polyclonal antibodies raised to B-PE from the eucaryotic red alga *Porphyridium cruentum*. For phage that expressed PE, the pBluescript plasmid was excised and single-stranded templates were prepared (46) and sequenced. Clones containing part of *cpeB* were used as hybridization probes to screen the library to obtain DNA fragments containing the complete PE gene set, *cpeBA*. The isolated genes were sequenced as previously described (2). The UV16-40 PE genes were amplified by the polymerase chain reaction (PCR) by using primers (see Fig. 2) and *Thermus aquaticus* DNA polymerase (Cetus, Emeryville, Calif.). The PCR reactions contained components specified in the product instructions plus 3 μg of UV16-40 DNA. Reactions were cycled through 94°C (1.5 min), 37°C (3 min), and 72°C (5.5 min) for 25 to 30 cycles. The DNA from PCR was then incubated with a restriction enzyme (*Hin*dIII or *Eco*RI) that cut near one of the primer-binding sites, resulting in amplified fragments with one end blunt (where the oligonucleotide primed) and the other end with a 5′ overhang. The *Hin*dIII site used to clone PCR-1 (see Results) was present in *cpeA*, while the *Eco*RI site used to clone PCR-2 was introduced as part of one of the primers used during amplification. The fragments were ligated into mp18 and mp19 cut with appropriate restriction endonucleases and were sequenced as described above for the WT PE gene set.

Transcription mapping and mRNA analysis. RNA was isolated from *Synechocystis* strain 6701 WT, UV16-40, and UV16 grown in red or green light by a protocol modified from those of Conley et al. (9) and Krawetz and Anwar (21). Cells (50 ml) were harvested during the exponential phase of growth (3×10^7 to 6×10^7 cells per ml), rapidly chilled by swirling them in a flask immersed in liquid nitrogen, and pelleted by centrifugation for 3 min at 5,500 rpm and 4°C in a rotor (SS-34; Ivan Sorvall, Inc., Norwalk, Conn.). The cells were suspended in 4 ml of 6.0 M guanidine hydrochloride–1% *N*-laurylsarcosyl–10 mM dithiothreitol–100 mM Tris (pH 6.5; freshly prepared) and immediately passed through a French pressure cell at 14,000 lb/in^2. Cell debris was pelleted by a 10-min centrifugation at 10,000 rpm at −10°C in a rotor (HB-4; Sorvall). The supernatant was stored overnight at −20°C after the addition of 16 μl of 4.5 M sodium acetate (pH 5.5) and 3.0 ml of ice-cold ethanol. The RNA was collected by a 60-min centrifugation at 4,000 rpm and 0°C in a rotor (HB-4; Sorvall). Pellets were suspended in 2 ml of CsCl solution (1.0 g of CsCl per 2.5 ml of TE buffer [10 mM Tris, 1 mM EDTA; pH 8.0]) and layered on top of a 9.5-ml cushion of 5.7 M CsCl in TE buffer. The gradients were centrifuged in a swinging bucket rotor (TST41.14; Sorvall) for 16 h at 32,000 rpm and 20°C. The supernatant was carefully removed with a pipette; and the RNA pellet was suspended in 0.5 ml of 100 mM NaCl–20 mM EDTA–1% sodium dodecyl sulfate–100 mM Tris hydrochloride (pH 9.0) followed by extraction with phenol, phenol-chloroform (1:1), and chloroform-isoamyl alcohol (24:1). To precipitate the RNA, 0.7 volume of isopropanol was added to the aqueous phase, which was then stored overnight at −20°C. The RNA was collected by centrifugation (13,000 × *g* for 30 min), dried under a vacuum, suspended in 0.2 ml of distilled water, and stored at −80°C.

The 5′ end of the PE transcript was determined by both S1 nuclease treatment of RNA-DNA hybrids (5) and primer extension (11). The probe for S1 nuclease mapping was generated from a single-stranded template by priming chain elongation with an end-labeled oligonucleotide that terminated 3 bp upstream of the *cpeB* translation start codon (see Fig. 2). The extension product was cut with *Dra*II (a restriction site in the polylinker of the pBluescript vector), and the resultant 500-base fragment was denatured and isolated on a 6% polyacrylamide–urea gel (28). The labeled strand (approximately 150,000 cpm) was hybridized to 10 μg of RNA (isolated from green or red light-grown *Synechocystis* strain 6701) at 37°C for 4 h and treated with S1 nuclease at 700 U/ml for 30 min at 37°C. Glycogen (1 μg; Boehringer Mannheim Biochemicals, Indianapolis, Ind.) was added as a carrier, and 2 volumes of ethanol were added for precipitation. After centrifugation, the precipitate was dried and suspended in 10 μl of water. The protected fragment was sized on a 6% sequencing gel (28) by using a sequence ladder generated with the same oligonucleotide primer. Primer

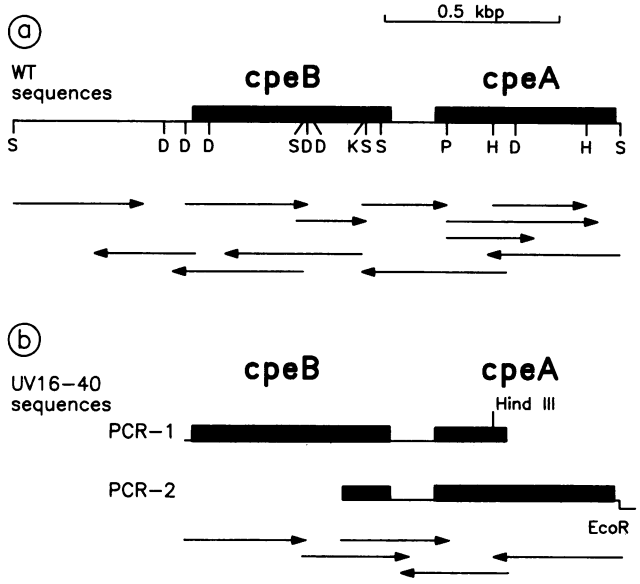

FIG. 1. (a) Restriction map and sequencing strategy for *cpeB* and *cpeA* from WT *Synechocystis* strain 6701. The letters S, D, K, P, and H denote the restriction endonucleases *Sau*3A, *Dra*I, *Kpn*I, *Pst*I, and *Hin*dIII, respectively. (b) Clones of cpeBA (PCR-1 and PCR-2) obtained from UV16-40 by using the PCR and sequencing strategy. The *Hin*dIII site in PCR-1 was part of *cpeA*, while the *Eco*RI site in PCR-2 was introduced at the end of one of the primers for PCR. Both sites were used for cloning the amplified sequences. The sequenced regions of both the WT and UV16-40 PE gene sets and the sequenced strand are indicated by arrows.

extension was performed essentially as described by Curtis (11), except an end-labeled primer was used and the incubation temperature was 42°C.

Northern and slot blot analyses were performed with total RNA isolated from WT, UV16-40, and UV16 cells grown in either green or red light. For Northern hybridizations, RNA (2.5 μg per lane) was resolved by electrophoresis on formaldehyde-agarose gels (9), transferred to nitrocellulose, and hybridized at 67°C (22) to a ^{32}P-labeled 270-bp *Dra*I fragment of the *cpeB* gene. Slot blot hybridizations were performed as described by Thompson et al. (45). Quantitation of PE mRNA levels was determined from a series of twofold dilutions of RNA, ensuring that measurements were within the linear response range of the film. Autoradiograms of slot blots were scanned on a gel scanning apparatus (GS 300; Hoefer).

RESULTS AND DISCUSSION

Nucleotide and deduced amino acid sequences of PE genes from *Synechocystis* strain 6701. Based on hybridizations of the cloned PE genes to restriction endonuclease digests of chromosomal DNA from *Synechocystis* strain 6701, there appears to be a single set of PE genes in this organism (data not shown). The gene organization is *cpeB* followed by *cpeA*, as observed for the *cpeBA* operon of *F. diplosiphon* (27). A restriction map and the strategy used to sequence *cpeBA* from *Synechocystis* strain 6701 WT are shown in Fig. 1a. The sequenced region includes both *cpeB* and *cpeA*, a 124-bp intergenic region, 511 bp 5′ of the initiation codon of *cpeB*, and 20 bp 3′ from the end of the coding region of *cpeA* (Fig. 2). All of the coding regions and the intergenic region were sequenced on both strands, while most of the upstream region represents sequence from a single strand. A probable ribosome-binding site (40) in front of *cpeB* is underlined. The *Synechocystis* strain 6701 *cpeB* and *cpeA* genes are 79 and 80% identical, respectively, to the homologous genes from *F. diplosiphon* (27). The deduced amino acid sequences for PE β and α subunits (186 and 164 amino acids, respectively) are shown beneath the nucleotide sequence in Fig. 2 and are aligned with the published PE sequences from *F. diplosiphon* (deduced from the nucleotide sequence [27]) and the red alga *P. cruentum* (a direct protein sequence [41]) (Fig. 3).

The amino acid sequences of the PE subunits from *F. diplosiphon* and *Synechocystis* strain 6701 were 87% (PE β subunit) and 90% (PE α subunit) identical. In general, the primary structures of biliproteins within a given class are highly conserved. PC and AP amino acid sequences from many organisms have been compared, showing primary structure identity between 67 and 78% for PC subunits (10) and 73 and 83% and 82 and 93% for allophycocyanin α and β subunits, respectively (18). Thus, the homology between PE subunits from *Synechocystis* strain 6701 and *F. diplosiphon* is among the highest observed for any of the biliproteins.

A comparison of algal and cyanobacterial phycoerythrins may reveal structural features specific to eucaryotic or procaryotic phycobilisomes. The B-PE amino acid sequences from the eucaryotic red alga *P. cruentum* showed less homology with the *Synechocystis* strain 6701 subunits (74% for the PE β subunit and 68% for the PE α subunit; Fig. 3) than the *F. diplosiphon* sequences. The cyanobacterial sequences were nearly identical in all regions of chromophore attachment, while the algal sequence showed significant divergence near the β155, α83, and α140 cysteine residues. The cyanobacterial β subunits were also mostly identical (8 of 9 residues) where residues were deleted in the red algal sequence (see *Synechocystis* strain 6701 residues β149 to β155 and β159 to β160 in Fig. 3). The few differences between the cyanobacterial amino acid sequences were mostly conservative substitutions that were randomly distributed throughout both subunits. However, a region of 12 amino acid residues in the *Synechocystis* strain 6701 PE β subunit (Lys-15 to Gln-26) exhibited considerable divergence from that of *F. diplosiphon*; the region contained two identical residues, two conserved substitutions, six nonconserved substitutions, and a two-residue deletion in the *F. diplosiphon* protein (Gly-20 and Gly-21). The algal β sequence was also variable in this region, but it aligned with the *Synechocystis* strain 6701 sequence and did not have a two-residue deletion.

Differences between the algal and cyanobacterial phycoerythrins may reflect structural differences in the phycobilisomes present in these organisms. The phycobilisome of *P. cruentum* is hemiellipsoidal (13) and has a much higher PE content (33) than the hemidiscoidal cyanobacterial phycobilisomes. Furthermore, as discussed previously (41), the algal PE interacts with a linker that contains chromophores and may have sequence differences from cyanobacterial PE that accommodate this structural interaction. A comparison of PE sequences from the hemidiscoidal phycobilisomes of the red alga *Rhodella violacea* (30) may reveal the extent to which relative PE homologies reflect phycobilisome structural differences or a differentiation between procaryotes and eucaryotes.

PE mRNA accumulation and transcription initiation. Isolation of *cpeBA* from *Synechocystis* strain 6701 allowed us to examine light wavelength-regulated PE mRNA accumulation and to define the site of transcription initiation. A 270-bp

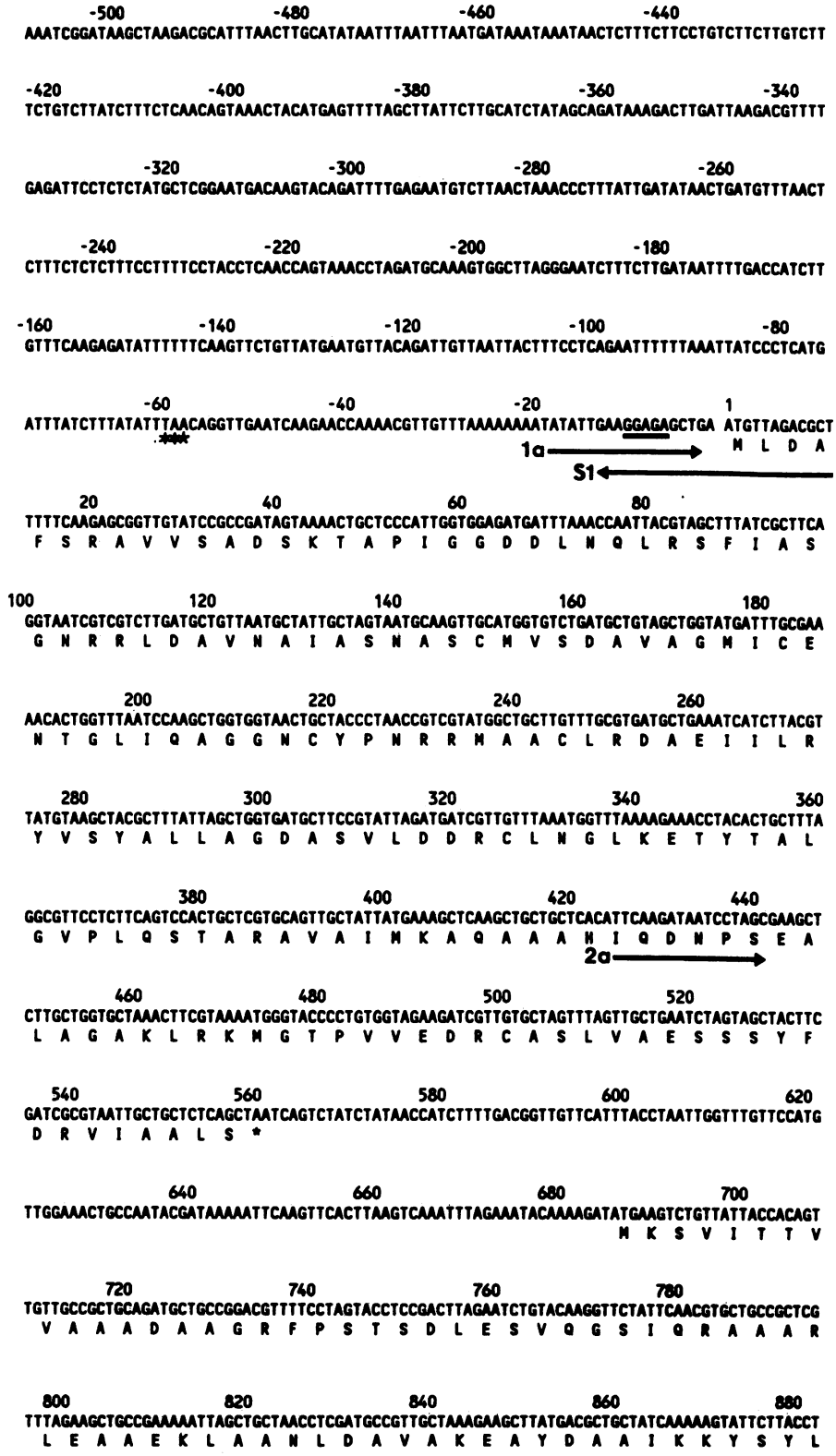

FIG. 2. Nucleotide sequence *cpeBA* and the deduced amino acid sequences of PE β and α subunits from *Synechocystis* strain 6701. Underlined regions indicate a possible ribosome-binding site (25) for *cpeB*. The synthetic oligonucleotides used to amplify the UV16-40 PE genes (1a, 1b, 2a, and 2b) and in primer extension-S1 nuclease experiments (S1) are shown as arrows under the sequence they represent. The *cpeBA* transcription initiation site is marked by three asterisks.

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                900                 920                 940                 960
CAATAATGCAGGTGAAGCTAATTCTACTGATACTTTTAAAGCAAAATGTCTTCGTGACATCAAACACTACTTACGTTTAATCAACTA
 N  N  A  G  E  A  N  S  T  D  T  F  K  A  K  C  L  R  D  I  K  H  Y  L  R  L  I  N  Y
1b<------

        980                1000                1020                1040
CTCCTTAGTTGTTGGTGGCACTGGTCCTCTTGATGAGTGGGGTATTGCTGGTCAGCGTGAAGTTTATCGTACTTTAGGATTACCTAC
 S  L  V  V  G  G  T  G  P  L  D  E  W  G  I  A  G  Q  R  E  V  Y  R  T  L  G  L  P  T

1060                1080                1100                1120                1140
TGCTCCTTACGTTGAAGCTCTTAGCTTTGCTCGTAATCGTGGTTGTTCTCCTCGTGACCTTTCCGCTCAAGCTTTAACTGAGTATAA
 A  P  Y  V  E  A  L  S  F  A  R  N  R  G  C  S  P  R  D  L  S  A  Q  A  L  T  E  Y  N

            1160                1180                1200
CTCTCTCCTTGACTATGTCATCAACTCTTTATCTTAATTTTGATAGAGTAAGTTGATC
 S  L  L  D  Y  V  I  N  S  L  S  * 2b<------
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FIG. 2—*Continued*

DraI fragment of *cpeB* (Fig. 1) was labeled and hybridized to PE mRNA from red and green light-grown cultures in both Northern and slot blot analyses. The Northern hybridizations showed that very little PE mRNA accumulates in WT cells maintained in red light, while growth in green light causes the appearance of an abundant transcript of 1.4 kilobases that encodes both PE β and α subunits (Fig. 4a). The difference in the PE mRNA content between red and green light-grown cells was quantitated by slot blot hybridizations. Two independent RNA preparations from both green and red light-grown cells were examined. One of these preparations gave the results shown in Fig. 4b, in which the level of PE mRNA was 28-fold higher in green light-grown than in red light-grown cells. The other preparation showed 18-fold more PE mRNA in cells from cultures maintained in green light. Similar results have been observed for *F. diplosiphon* (A. Grossman, unpublished data), in which there is a 20-fold difference in PE mRNA between cells grown in red and green light. For *F. diplosiphon* the PE mRNA level is primarily controlled by the transcriptional activity of the PE gene set; the rate of mRNA turnover is similar in red and green light-grown cells (32). While transcript turnover has not been measured in *Synechocystis* strain 6701, the comparable differential accumulation of PE transcripts suggests that similar mechanisms govern expression of the PE gene sets in *Synechocystis* strain 6701 and *F. diplosiphon*. The

PE β

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                                                          *
6701 MLDAFSRAVV SADSKTAPIG GDDLNQLRSF IASGNRRLDA VNAIASNASC 50
F.d.               AS STV- -S IAA  A  V
P.c.         V  NS A A YV   S  QA K     D  K       S V
                *                      *
6701 MVSDAVAGMI CENTGLIQAG GNCYPNRRMA ACLRDAEIIL RYVSYALLAG 100
F.d.            Q                             V     T
P.c.       S    P      SR      T           G
6701 DASVLDDRCL NGLKETYTAL GVPLQSTARA VAIMKAQAAA HIQDNPSEAL 150
F.d.                   A      TT  V    Q             T    R
P.c.      E            I      TN SI    S      V  F TNTAT --
                      *
6701 AGAKLRKMGT PVVEDRCASL VAESSSYFDR VIAALS 186
F.d.                          A          S
P.c. -----   -- SFAAGD T   AS VA       G  I
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PE α

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6701 MKSVITTVVA AADAAGRFPS TSDLESVQGS IQRAAARLEA AEKLAANLDA 50
F.d.     V   I                                         N I
P.c.          S            N     I  N    S             G HE
                                        *
6701 VAKEAAYDAA IKKYSYLNNA GEANSTDTFK AKCLRDIKHY LRLINYSLVV 100
F.d.   T  - N C     P    S               A           Q
P.c.  V    G  C FA  A  K P    GENQEKI N  Y  VD   M  V  D
                                               *
6701 GGTGPLDEWG IAGQREVYRT LGLPTAPYVE ALSFARNRGC SPRDLSAQAL 150
F.d.                     A                       A   M
P.c.               A        N   SA  A SIAYT D L  V   M    G
6701 TEYNSLLDYV INSLS 164
F.d.     A    A
P.c. V FSAY   L   A
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FIG. 3. Comparison of amino acid sequences of the PE subunits from *Synechocystis* strain 6701, *F. diplosiphon*, and *P. cruentum*. The complete *Synechocystis* strain 6701 sequences (designated *6701*) are shown, with differences in the *F. diplosiphon* sequences (designated *F.d.*) and *P. cruentum* (designated *P.c.*) indicated below. The horizontal lines in the *F. diplosiphon* and *P. cruentum* sequences represent deleted residues relative to the 6701 sequence. Attachment sites for the phycoerythrobilin chromophores are marked by asterisks. The Cys residues at β-50 and β-61 are the attachment sites for the doubly linked chromophore in PE (20).

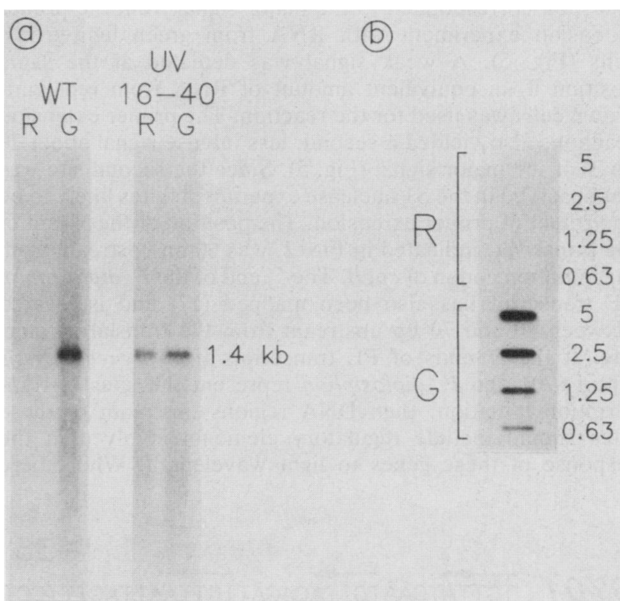

FIG. 4. (a) Northern blot analysis of mRNA from *Synechocystis* strain 6701 strains WT and UV16-40. The signals were obtained by using a 270-bp *DraI* fragment of the *cpeB* gene as a hybridization probe. R, mRNA isolated from cultures grown in red light; G, mRNA from cultures grown in green light. kb, Kilobases. (b) Slot blot analysis of WT mRNA. Numbers on the right are total RNA (in micrograms) loaded in each slot for the twofold dilution series.

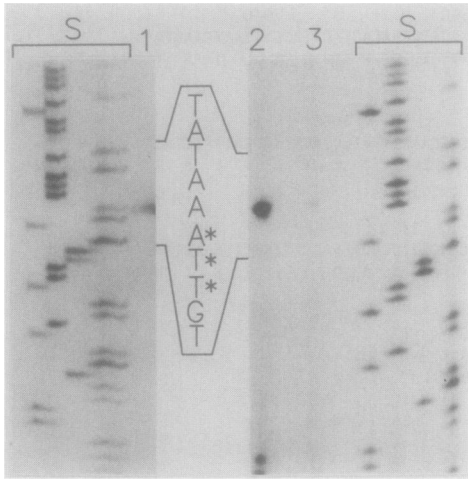

FIG. 5. Transcription initiation site for *cpeBA*. Lane 1, S1 nuclease protection experiment with mRNA from green light-grown WT cells; lanes 2 and 3, primer extension experiments with mRNA from green and red light-grown WT cells, respectively; lanes S, sequence ladders (from left to right, G, A, C, and T) used to size the S1 nuclease and primer extension products. See text for a description of the experiments. The putative transcription initiation site is marked by asterisks in the listed nucleotide sequence.

light-regulated accumulation of PE mRNA in the PE assembly mutant UV16-40 differed from that in the WT (Fig. 4b) and is discussed below.

Genes that are regulated at the level of transcription by similar environmental parameters often show common sequences upstream of transcription initiation sites (12, 19, 23). The 5′ end of the *Synechocystis* strain 6701 PE transcript was mapped by both S1 nuclease and primer extension experiments. A single fragment protected from S1 nuclease digestion corresponded to the major signal from the primer extension experiment with RNA from green light-grown cells (Fig. 5). A weak signal was detected at the same position if an equivalent amount of RNA from red light-grown cells was used for the reaction. The primer extension reactions also yielded a second, less intense signal about 20 bp 3′ of the major signal (Fig. 5). Since the second site was not observed in the S1 nuclease experiments, it is likely to be an artifact of primer extension. The position of the 5′ end of the transcript, indicated in Fig. 2, was 60 bp upstream from the initiation codon of *cpeB*. The 5′ end of the *F. diplosiphon* PE transcript has also been mapped (17) and is located between 60 and 70 bp upstream from the translation start site. If the 5′ ends of PE transcripts from *Synechocystis* strain 6701 and *F. diplosiphon* represent the sites of transcription initiation, then DNA regions upstream of these sites should include regulatory elements involved in the response of these genes to light wavelength. While there were few similarities between the untranslated leader sequences of the two PE mRNAs, some significant homologies upstream of the putative transcription initiation sites were evident (Fig. 6). Homologies at the −10 and −35 regions could represent RNA polymerase-binding sites. A region upstream of the −35 region includes a direct repeat, 5′-TGTTA-3′, that occurs twice in the *F. diplosiphon* sequence and three times in the *Synechocystis* strain 6701 sequence. The extent of homology in the 5′ regions of the two gene sets may define the boundaries of the upstream regulatory elements involved in light regulation.

The data presented above indicate that *F. diplosiphon* and *Synechocystis* strain 6701 have similar patterns of light-regulated PE mRNA accumulation and share homologies in potential regulatory regions of the *cpeBA* operon. However, their patterns of PE incorporation into phycobilisomes are different. While the transfer from red to green light growth conditions causes a 20-fold increase in PE transcript levels in both organisms, only *F. diplosiphon* shows a corresponding large increase in phycobilisome PE content (a minimum 20-fold increase [24]). While *Synechocystis* strain 6701 has approximately 20-fold more PE mRNA in green light than it does in red light, there is only a 4-fold difference in phycobilisome PE content (red light phycobilisomes have 25% of the PE content of green light phycobilisomes; data not shown). There is some variability in the PE content of *Synechocystis* strain 6701 grown in red and green light; the PE content of red light phycobilisomes can range from 12 to 40% of that of green light phycobilisomes (3, 8, 14, 43). In contrast, measurements of the relative levels of PE in red and green light-grown *F. diplosiphon* are consistent among different laboratories (7, 24, 27, 35). Red light phycobilisomes contain, at most, 5% of the PE present in green light phycobilisomes. This suggests that the chromatic adaptation response in *Synechocystis* strain 6701 may be more sensitive than *F. diplosiphon* to environmental factors such as light intensity or nutrient availability (14). The variability of PE incorporation in *Synechocystis* strain 6701 phycobilisomes during chromatic adaptation may be related to the differential expression of the two PE-associated linkers in this organism (3, 14). The presence of the terminal PE linker is acutely dependent on light wavelength and is not detected in red light-grown cells, while the level of the internal PE linker is directly proportional to the PE content of the phycobilisomes. This contrasts with *F. diplosiphon* phycobilisomes, in which the PE linkers are coordinately regulated; they are both present at extremely low levels in red light (N. Federspiel and A. R. Grossman, manuscript in preparation).

F. diplosiphon shows a good correlation between the levels of PE protein and mRNA, while *Synechocystis* strain 6701 does not. This observation suggests that posttranscriptional processes may be important in establishing PE levels in *Synechocystis* strain 6701 in response to various environ-

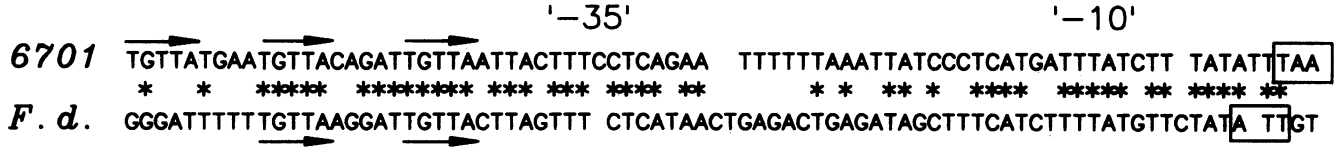

FIG. 6. Comparison of structural elements upstream of the putative transcription initiation sites for *cpeBA* of *Synechocystis* strain 6701 (*6701*) and *F. diplosiphon* (*F.d.*). The transcription start sites are boxed. Two regions of homology are marked −10 and −35 for their approximate positions upstream of the transcription initiation sites. The arrows mark the 5-base direct repeat, TGTTA, which is seen twice in the *F. diplosiphon* sequence and three times in the *Synechocystis* strain 6701 sequence.

mental cues and demonstrates an interesting difference in the responses of these organisms to light wavelength.

Analysis of PE genes in a phycoerythrin assembly mutant. One approach to studying phycobilisome structure and assembly has involved the analysis of UV-induced phycobilisome assembly mutants in *Synechocystis* strain 6701 (2–4). The UV16 mutant is defective in PC assembly, but it also exhibits other aberrant characteristics such as decreased levels of PE (55% that of the WT) and an abnormal response to red light during chromatic adaptation. UV mutagenesis of UV16 produced the mutant UV16-40, which was selected on the basis of its extremely low PE content (about 13% that of the WT). Biochemical analysis of UV16-40 PE showed that most of the β subunits were missing a specific one of the three phycoerythrobilin chromophores normally associated with this protein, while PE assembly was blocked at the hexameric stage (4).

We cloned and sequenced the UV16-40 PE genes to determine whether a mutation in either of the PE subunits was responsible for the missing β chromophore. The UV16-40 PE genes were amplified in two fragments from chromosomal DNA by the PCR method (see Materials and Methods). Primers 1A and 1B and primers 2A and 2B (shown in Fig. 2) were used to generate, respectively, the PCR-1 and PCR-2 fragments shown in Fig. 1b. The sequence of the UV16-40 PE gene set was identical to that of WT, proving that the missing PE β-subunit chromophore in UV16-40 is not due to a mutation in either *cpeB* or *cpeA*. This leaves at least two possible explanations for the UV16-40 phenotype. A lesion in one of the PE linker polypeptides could result in abnormal protein interactions that disrupt the environment of a chromophore, making it susceptible to enzymatic removal. Alternatively, there may be a mutation in a protein that catalyzes chromophore attachment.

Sequence analysis of the PE gene set allowed us to predict the site in the PE β subunit that is not chromophorylated in the UV16-40 strain. Previous characterization of the PE β subunit included fractionation of CNBr-cleaved peptides from WT and UV16-40 by reverse-phase chromatography (4). Inspection of the deduced amino acid sequence from *cpeB* predicts that three chromophoric peptides would be generated by CNBr cleavage of the PE β subunit at methionine residues (Fig. 7). The largest chromopeptide is actually two peptides bridged by the doubly linked chromophore at Cys-50 and Cys-61 (20). The large mass differences between these three predicted chromopeptides (7,508, 5,856, and 2,943 daltons) show good correlation with three discrete chromophoric peaks in the reverse-phase elution profile of CNBr fragments of the PE β subunit from the WT (4). The elution profile of UV16-40 PE β-subunit fragments showed only two chromophoric peaks, the first and last, with a nonchromophoric intermediate peak that shifted to an earlier elution time because of the loss of the hydrophobic chromophore. Since each of the chromopeptides have the same level of hydrophobic residues (about 50%), their resolution in reverse-phase chromotography is primarily a function of peptide length. Therefore, the nonchromophoric CNBr fragment from UV16-40 would probably correspond to the 5,856-dalton PE β-subunit peptide that contains the Cys-82 chromophore site (Fig. 7).

The Cys-82 chromophore of the PE β subunit from *Synechocystis* strain 6701 corresponds to the Cys-84 chromophore of the PC β subunit from *Mastigocladus laminosus* and *Agmenellum quadruplicatum*. PC trimers and hexamers from these latter two organisms have been crystallized and their structures have been determined at a resolution of 0.21

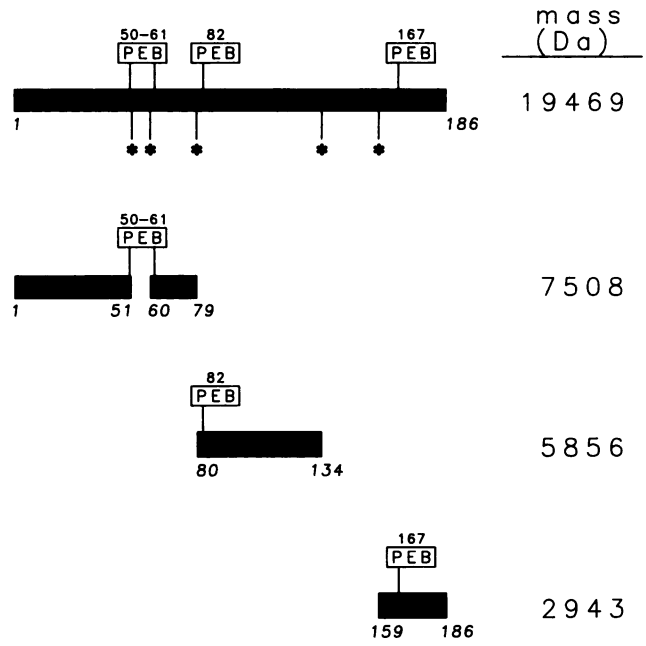

FIG. 7. Predicted masses of chromopeptides from CNBr-cleaved PE β. Sites of cleavage by CNBr (Met residues) are indicated on the complete β subunit by asterisks. The phycoerythrobilin chromophores (PEB) are marked by the number of the Cys residues to which they are attached. The chromopeptides and their predicted masses are indicated below the complete β subunit. All masses are calculated without the inclusion of the chromophore mass (approx. 600 daltons [Da]).

nm (36–38). Given the significant homologies between PE and PC subunits (17, 44) and that both subunits assemble into hexameric aggregates, it is likely that they have similar structures. This would place the PE β-subunit Cys-82 chromophore in the 3.0- to 3.5-nm central channel of the hexamer, with the other chromophores being at the periphery (36, 38, 42). The absence of the β-subunit Cys-82 chromophores could affect PE assembly in a manner consistent with that observed in UV16-40, in which PE assembly does not proceed beyond the hexamer (4). The formation of the rod substructure from hexamers is mediated by the linker proteins that may occupy the central channel of the hexamer. This assessment of linker location is based on data that include proteolysis protection experiments (48) and the demonstration that linker interactions with PC hexamers affect the spectral properties of the PC β-subunit Cys-84 chromophore (25, 29). A missing β-subunit Cys-82 chromophore in PE hexamers could eliminate contact points for linker interaction and disrupt subsequent assembly steps.

PE mRNA accumulation in phycobilisome assembly mutants. A block at a specific stage of phycobilisome assembly may have pleiotropic effects on expression and assembly of other phycobilisome components. UV16, the parent of UV16-40, cannot assemble PC hexamers, and therefore, the phycobilisome has no attachment sites for PE hexamer-linker complexes. The unassembled PE aggregates into large insoluble granules that can be seen in the fluorescence microscope (3). The PC assembly block may also affect expression of genes for PE and PE-associated linkers. UV16 has 55% of the PE content of WT and the 30.5-kilodalton linker, which is normally present only in cells grown in green light, becomes constitutively expressed (3). The UV16-40 mutant has the same genetic background as UV16 plus a

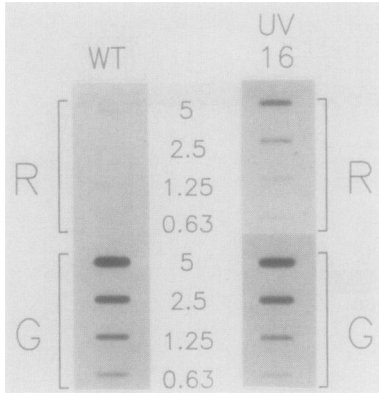

FIG. 8. Slot blot analysis of PE mRNA from red and green light-grown cultures of WT and UV16. RNA was from red (R) or green (G) light-grown cells. The numbers between the columns of slots indicate the amount of total RNA (in micrograms) placed in each slot (twofold dilution series).

lesion that produces PE β subunits that are missing a specific chromophore. This mutant also exhibits a decline in PE content to 13% of that in WT. The change in PE content in both UV16 and UV16-40 could be caused by multiple factors, including decreased rates of transcription, elevated RNase activities, and reduced translational efficiency or protein stability.

As an initial step in relating the mutant phenotypes to biochemical events in the cell, we quantitated the accumulation of PE transcripts in both WT and mutant organisms. Northern hybridizations showed that PE mRNA in UV16-40 is higher when the organism is grown in green light than when it is grown in red light (Fig. 4a). However, compared with the WT, UV16-40 has more PE mRNA when it is grown in red light and less PE mRNA when it is grown in green light. The very low level of PE in UV16-40 is not related to the level of its mRNA, since similar amounts of this mRNA are present in UV16 under both light conditions (data not shown). The lower PE content in UV16-40 may reflect a rapid degradation of abnormal, unassembled subunits. This is supported by the previous observation that approximately 30% of the PE α subunit in UV16-40 is not associated with the PE β subunit (3), suggesting a preferential degradation of the β subunit. Proteolytic degradation of unassembled components has been observed for other complexes involved in photosynthesis (6, 39).

The level of PE transcript in the PC assembly mutant UV16 was fivefold higher than that in the WT when UV16 was grown in red light and 80% of the WT level when UV16 was grown in green light (Fig. 8). The PE mRNA level of UV16 cultured in green light was 3-fold more than that observed for UV16 cultured in red light and contrasts with the 20-fold increase in PE mRNA seen in green versus red light-grown WT cells. These findings imply that mechanisms for controlling PE expression are impaired in this mutant. Since genes for PE and PE-associated linkers are coordinately expressed, the constitutive expression of the 30.5-kilodalton linker in UV16 (3) supports this hypothesis.

The complex phenotypes of UV16 and UV16-40 may be a product of multiple mutations introduced by mutagenesis with UV irradiation. However, all of the phenotypic differences between UV16 and the WT involve components of the phycobilisome rod substructure. Because of its central position in the phycobilisome assembly pathway, it is feasible that a lesion that blocks PC assembly affects the expression and assembly of the other rod components. It is also reasonable that elimination of one of the chromophores on the PE β subunit of UV16-40 would result in a more rapid degradation of that subunit. Unraveling of the complexity of the UV16 and UV16-40 phenotypes might reveal many events that are involved in the assembly of phycobilisome rod components and the ways in which the levels of the different components are controlled.

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