# Green light induces transcription of the phycoerythrin operon in the cyanobacterium *Calothrix* 7601

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## ABSTRACT

Phycobilisomes, the major light-harvesting complexes of cyanobacteria are multimolecular structures made up of chromophoric proteins called phycobiliproteins and non chromophoric linker polypeptides. We report here the isolation and nucleotide sequence of the genes, cpeA and cpeB, which in Calothrix PCC 7601 encode the α and β subunits of phycoerythrin, one of the major phycobiliproteins. In Calothrix PCC 7601, modulation of the polypeptide composition of the phycobilisomes occurs in response to changes of the light wavelength, a phenomenon known as complementary chromatic adaptation. Under green illumination, cells synthesize phycoerythrin and its two specifically associated linker polypeptides ($L_R^{35}$ and $L_R^{36}$), while under red illumination none of these proteins are detected. Using specific probes, a single transcript (1450 nucleotide long) corresponding to the cpe genes was detected but only in green-light-grown cells, establishing the occurrence of transcriptional regulation for the expression of this operon in response to light wavelength changes. The size of this transcript excludes the possibility that the phycoerythrin-associated $L_R^{35}$ and $L_R^{36}$ could be cotranscribed with the cpeA and cpeB genes.

## INTRODUCTION

In cyanobacteria, as in rhodophyta, photons are collected and transferred to the reaction centers of photosystem II by organelles, called phycobilisomes. These light-harvesting structures are anchored to the thylakoid membrane, and are formed of two domains: the core and the surrounding rods. Each of these structural elements is built of stacked disks. The most abundant proteins in these complexes are the chromophoric proteins called phycobiliproteins: these are comprised of the allophycocyanins (AP) which are located in the core, as well as the phycocyanins (PC) and the phycoerythrin (PE) both of which being located in the rods (1,2). Each of these chromophoric proteins comprise two different subunits (α and β) which are present in equimolar amounts and assembled into trimeric or hexameric disks. In addition, to insure the complex structure

and optimize photon harvesting and transfer, one finds non-chromophoric linker polypeptides which are specifically associated with each of the phycobiliprotein species (3).

   In photosynthetic organisms, the pigment content is modulated by many environmental factors, such as nutrient availability (e.g., carbon, nitrogen, sulfur, phosphate and iron), temperature, and light, the latter being the best studied factor so far. In both photosynthetic prokaryotes and eukaryotes, an inverse correlation between light intensity and pigment content can be observed. In addition, several cyanobacterial strains exhibit an interesting response to chromatic illumination known as complementary chromatic adaptation (4). When grown in green light, cells contain a high ratio of PE to PC, the absorption maximum of PE being at about 560 nm (green light). When cells are grown in red light they synthesized only PC, the absorption maximum of which is situated at about 620 nm (red light). This phenomenon results in an optimal trapping of the available light energy during cell growth. More precisely, complementary chromatic adaptation affects the rod composition of the phycobilisome. In the filamentous cyanobacterium Calothrix PCC 7601 (also called Fremyella diplosiphon) as well as in some other species (4), the synthesis of both PE and PC is light-wavelength regulated. Growth in green light results in rods composed of three distal disks of PE, linked to the core by a proximal disk of PC-1 ("constitutive" PC). When cells are grown in red light, the rods are composed of three disks composed of subunits of two phycocyanins (PC-1 and PC-2 or "inducible" PC) (5). Physiological and biochemical studies of this adaptation phenomenon suggest that the synthesis of the major phycobiliproteins (PC-2 and PE) and their associated linker polypeptides is controlled at the level of transcription and involves a regulatory system responding antagonistically to green and red irradiations (4,6). Recently the genes encoding the α and β subunits of PC-2 from Calothrix PCC 7601 have been cloned and a preliminary study of their expression has confirmed the transcriptional stimulation of PC-2 mRNA synthesis in response to red light (7). We report here the cloning of the genes encoding the α and β subunits of PE (cpeA, cpeB), the other major light-regulated phycobiliprotein during complementary chromatic adaptation, as well as a comparative study of the expression of these genes in response to the triggering effect of green versus red irradiations in Calothrix PCC 7601.

MATERIALS AND METHODS

Materials

Restriction enzymes, calf intestinal alkaline phosphatase, T4 DNA ligase and T4 polynucleotide kinase were purchased from either Boehringer or Genofit. Klenow fragment of DNA polymerase I, $\alpha^{32}P$ dATP (400 Ci/mmol), $\alpha^{35}S$ dATP (400 Ci/mmol), $\gamma^{32}P$ ATP (3000 Ci/mmol), and nick translation kit were from Amersham. Nucleotides, dideoxynucleotide triphophates, pTZ18R and 19R were purchased from PL-Pharmacia. All enzymes were used according to the manufacturer's instructions. All chemicals were reagent grade.

Culture conditions and cyanobacterial DNA or RNA purifications

The strain Calothrix PCC 7601 (= Fremyella diplosiphon, UTEX 481) was grown in BG 11 medium (8) at 25° C. Cultures were bubbled continuously with 1 % $CO_2$/99 % air and illuminated with cool-white fluorescent tubes (50 $\mu E.m^{-2}.s^{-1}$). Chromatic illumination was provided by red or green plastic filters interposed between the culture vessels and the fluorescent tubes (25 $\mu E.m^{-2}.s^{-1}$). Cells were harvested in early exponential growth phase.

Chromosomal DNA was isolated as follows: cells were taken up in buffer A (50 mM Tris-HCl pH 8.5; 50 mM Na2 EDTA) containing 1 M NaCl (20 ml per g of cells). Sarkosyl (0.2 % final concentration) was added and the suspension incubated for 1 h at 4° C on a gyrotory shaker before being centrifuged at 10,000 g for 20 min. The pellet was washed once in the same volume of buffer A and recentrifuged. Lysis was achieved as follows: the pellet resuspended in 5 ml of buffer A containing 25 % sucrose was incubated for 1 h at room temperature; after addition of egg-white lysozyme to a final concentration of 12 mg.ml$^{-1}$ and an incubation at 37° C for 15 min, the suspension was diluted four-fold with 10 mM Tris-HCl pH 8.5/50 mM Na2 EDTA and incubated for 1 h at 37° C; finally, SDS (1 % final concentration) and proteinase K (100 $\mu g.ml^{-1}$ final concentration) were added and the suspension incubated at 4° C overnight and at 37° C for 2 h. The suspension was centrifuged at 10,000 g for 20 min and the pellet taken up in 20 ml of 10 mM Tris-HCl pH 8.5/1mM Na2 EDTA containing 1M NaCl and extracted twice with an equal volume of chloroform:isoamyl alcohol (24:1). The aqueous phase, to which 2 volumes of cold ethanol were added, was incubated overnight at 4° C, and centrifuged for 20 min at 10,000 g. The pellet was finally dissolved in 10 mM Tris-HCl pH 8/1mM Na2 EDTA and incubated with ribonuclease B (2 ng per $\mu$g of DNA) for 30 min at 37° C.

Total RNA from cells grown under red or green illuminations was isolated by a modification of a procedure previously described (9). Cells were harvested, resuspended in BG11 medium supplemented with $5.10^{-2}$M NaN$_3$ to an OD$_{750nm}$ of 100; 3 ml aliquots were immediately frozen and kept until used in liquid nitrogen. Cell aliquots were thawed on ice in the presence of 3.5 g glass beads (0.1 to 0.11 mm diameter), 0.2 ml of 20 % SDS, 1 ml of 3 % Macaloïd, and 3.5 ml of phenol saturated with 100 mM Tris 1 mM EDTA pH 8. The cells were then disrupted in a Mickle desintegrator three times for 1 min, at 4° C, and the samples were centrifuged at 7000 g for 15 min at 4° C. The aqueous phases were collected, adjusted to 1M NaCl and ethanol precipitated for 12 h at -30° C. After centrifugation at 12,000 g for 15 min at 4° C, the pellets were taken up in 150 µl of sterile distilled water and dialysed against distilled water for 24 h, at 4° C.

Construction of a partial library

Total DNA from Calothrix PCC 7601 was digested with EcoRI and

| Amino acid sequence | : | | 106 Asp | Glu | Trp | Gly | Ile | 111 Ala | |
|---|---|---|---|---|---|---|---|---|---|
| Possible codons | : | 5' | GAU C | GAA G | UGG | GGU C A G | AUU C A | GCU C A G | 3' |

Synthesized oligonucleotide sequences :

| Set A | : 3' | CTA | CTC T | ACC | CCC T | TAA G | CG | 5' |
|---|---|---|---|---|---|---|---|---|
| Set B | : 3' | CTA | CTC T | ACC | CCA | TAA G | CG | 5' |
| Set C | : 3' | CTA | CTC T | ACC | CCG | TAA G | CG | 5' |
| Set D | : 3' | CTG | CTC T | ACC | CCC T | TAA G | CG | 5' |
| Set E | : 3' | CTG | CTC T | ACC | CCA | TAA G | CG | 5' |

Figure 1: Sequences of the heptadecameric oligonucleotide probes. The amino-acid sequence for the α-PE subunit (14) was used to design the probes which were synthesized complementary to the possible codons. Possible combinations involving the AUA codon of Ile were omitted. Set A and D are mixtures of eight sequences synthesized simultaneously while set B, C, E and F are only composed of four sequences. Numbers above the amino-acid residues refer to their position in the polypeptide chain.

fractionated by electrophoresis on a 0.7 % agarose gel. DNA fragments ranging in size between 7-8.5 kbp were eluted by the glass beads method (10) and inserted into the EcoRI site of pUC8.

Subcloning and sequencing

Before being subcloned into pTZ18R or pTZ19R for sequence analysis, plasmid DNAs from the positive clones were digested with convenient restriction enzymes and the inserted DNAs purified as described above. Sequencing was carried out by the dideoxy chain termination method (11) on single stranded DNA, using the M13 reverse primer, according to the protocol provided by Amersham.

Synthesis of oligonucleotides

The heptadecameric synthetic oligonucleotides (17-mers) corresponding to a portion of the amino-acid sequence of the α subunit of PE were obtained from the Unité de Chimie Organique, Institut Pasteur, Paris. They were synthesized by an Applied Biosystems Model 380A automated DNA synthesizer.

Hybridization with $^{32}$P-labelled probes

Nick-translation, as well as Southern and Northern hybridization experiments were performed as described previously (12,13). Hybridizations were done at 42° C with the 17-mer oligonucleotide probes and either at 60°C (Southern) or at 65°C (Northern) with the nick translated probes.


RESULTS AND DISCUSSION

Cloning of the cpeA and cpeB genes

Phycobiliproteins may constitute up to 50 % of the total cellular proteins, and, as phycoerythrin (PE) represents half of this percentage in cells grown under green light, one can expect that in these cells, messenger RNAs specific to phycoerythrin (PE mRNA) constitute a large proportion of the total mRNA. In addition, if, as postulated (4,6), regulation occurs at the transcriptional level, PE mRNAs must be absent from cells grown under red light. The complete amino acid sequence of both the α and β subunits of PE from Calothrix PCC 7601 has recently been determined (14). We thus decided to look for the cpe genes using synthetic oligonucleotides, the sequence of which were deduced from a portion of the known amino acid sequence of the α-PE subunit. The oligonucleotides were synthesized corresponding to the residues 106 to 111 (Fig. 1). If the degenerated third base of the Ala codon was not included, the number of combinations encoding this hexapeptide was 48. However, since among all cyanobacterial genes sequenced so far, the AUA codon for Ile has seldom
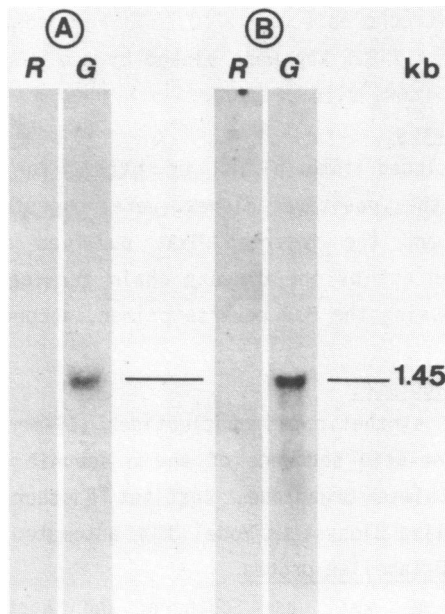
Figure 2: Total RNAs extracted from Calothrix PCC 7601 cells grown either under green (G) or red (R) illumination and hybridized: Panel A, with the synthetic oligonucleotide probe, set B, or Panel B with the HindIII-XbaI DNA fragment 205 base-pairs long, internal to the cpeB gene from Calothrix PCC 7601. kb, kilobases.

been found, none of the combinations involving this sequence were synthesized. Consequently the number of combinations was decreased to 32. In addition, since it is easier to perform hybridization with even a more limited number of oligonucleotides, we decided to synthesize six sets of different sequences covering the 32 possibilities. In order to select the set of oligonucleotides containing the sequence complementary to the α-PE mRNA, comparative Northern hybridization experiments were performed. Each of the six sets was tested with total RNAs extracted from either red-or green-light grown cells. As expected, only one of them gave a strong hybridization signal specific to green-light RNAs and none with red light RNAs (Fig. 2A). Thus, this set of oligonucleotides, set B (Fig. 1), most probably contained, among the four sequences, one sequence complementary to the PE mRNA. We used this oligonucleotide set in turn to probe a Southern blot of total DNA digested with EcoRI. The corresponding autoradiogram (Fig. 3A) showed three hybridizing bands of differing sizes 6.8 kbp, 7.3
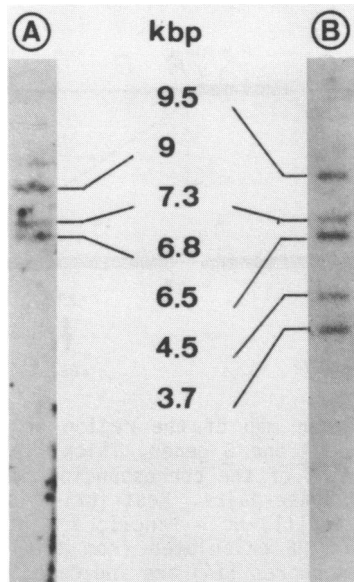
Figure 3: Total DNA extracted from Calothrix PCC 7601 cells digested with EcoRI and hybridized with: Panel A, the synthetic oligonucleotide probe, set B, or Panel B, with an AvaI-BamHI DNA fragment 305 base-pairs long internal to the cpcB gene from Synechococcus PCC 7002 (15). kb, kilobases.

kbp and 9 kbp. These bands could either result from the presence of multiple copies of the gene encoding the α subunit of PE (cpeA gene), or result, for some of them, from non-specific hybridization. To clarify this point, two possibilities were available, either to use a second unique synthetic oligonucleotide (or set of oligonucleotides) corresponding to another part of the amino acid-sequence or to use an heterologous DNA probe carrying another phycobiliprotein gene such as the genes encoding the α and/or β subunits of PC (cpcA and cpcB genes, respectively). Indeed, both biochemical and genetic studies have clearly established that a high degree of homology exists between the amino-acid sequences of the phycobiliprotein subunits (4,15,16). From these results, it appears that the α subunits on one hand and the β subunits on the other hand of PE from Calothrix PCC 7601 and PC from Synechococcus PCC 7002 (Agmenellum quadruplicatum PR-6) are 43 % and 51 % homologous respectively, with a stretch of about 80 % between the amino-acids in position 80 to 120 in the β subunits. Since the cpcA and cpcB genes from Synechococcus PCC 7002 (15) were cloned, we used a fragment
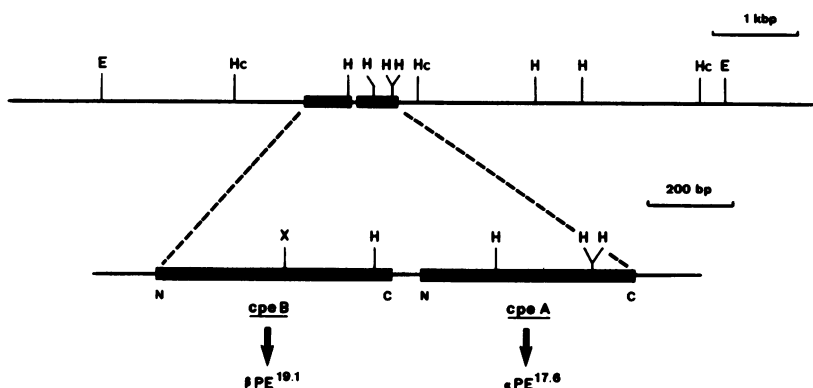
Figure 4: Partial restriction map of the region of the Calothrix PCC 7601 genome which carries the cpeA and B genes. Thick lines show the cpe genes, with the NH$_2$ and COOH termini of the corresponding polypeptides indicated (N and C, respectively). bp, base-pairs. Restriction sites are indicated as follows: E = EcoRI; H = HindIII; Hc = HincII; X = XbaI. The molecular mass of the α and β subunits of PE calculated from their respective amino-acid sequences without the chromophores (14) are indicated as superscripts.

internal to the cpcB gene to probe similar Southern blots of total DNA from Calothrix PCC 7601 (Fig. 3B). Five hybridizing fragments were detected with this probe; one of these (7.3 kbp) appeared to be common to both the oligonucleotides set B and the cpc'B' probe, and thus most probably carried the cpe genes. To clone this fragment we constructed a partial library enriched in DNA fragments of 7 to 8.5 kbp. Recombinant colonies were screened with the two different probes, and 4 of the 200 recombinants tested were found to hybridize to both probes. DNA extracted from these different recombinants showed the same restriction pattern and one of them, pPM40, was analyzed further.

The 7.3 kbp EcoRI insert of pPM40 was mapped with restriction endonucleases; its physical map is shown in Fig. 4. A portion of this EcoRI fragment has been sequenced on both strands. Since the complete amino-acid sequence of both PE subunits has been determined by Sidler et al. (14) from purified polypeptides, we were able to locate the cpe genes on this DNA fragment. The two genes, cpeA and cpeB were found to be separated by 79 base pairs. The sequence of the cpeB gene (encoding the β subunit), of the intercistronic region and of the cpeA gene (encoding the α subunit) is shown in Fig. 5. For both genes, the amino-acid sequence deduced were identical to that determined by Sidler et al. (14), except for residue 70 of the β subunit where we found an asparagine instead of a seryl residue.

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    M  L  D  A  F  S  R  A  V  V  S  A  D  A  S  T  S  T  V  S  D  I  A  A  L  R  A  F  V  A  S  G  N  R  R  L  D  A  V  N
   ATGCTTGATGCTTTTTCTAGAGCTGTAGTTTCAGCAGATGCTAGCACTTCTACCGTATCTGATATTGCTGCTCTGAGAGCCTTTGTTGCTAGTGGTAACAGACGTTTGGATGCTGTAAAT
      .        .        .        50       .        .        .        .        100      .

    A  I  A  S  N  A  S  C  H  V  S  D  A  V  A  G  H  I  C  E  N  Q  G  L  I  Q  A  G  G  N  C  Y  P  N  R  R  H  A  A  C
   GCGATCGCAAGCAACGCTAGCTGCATGGTTTCTGATGCTGTAGCTGGAATGATCTGCGAAAACCAAGGTTTAATCCAAGCTGGTGGTAACTGCTATCCTAACCGTCGTATGGCTGCCTGC
      .        .        150      .        .        .        .        200      .        .        .        .

    L  R  D  A  E  I  V  L  R  Y  Y  V  T  Y  A  L  L  A  G  D  A  S  V  L  D  D  R  C  L  N  G  L  K  E  T  Y  A  A  L  G  V
   TTACGCGATGCAGAAATCGTCTTACGCTATGTAACCTACGCTCTATTAGCTGGTGACGCTTCAGTTCTAGATGATCGTTGCTTAAACGGTTTGAAAGAAACCTACGCTGCTCTAGGCGTA
      .        250      .        .        .        300      .        .        .        .        350      .

    P  T  T  S  T  V  R  A  V  Q  I  H  K  A  Q  A  A  A  H  I  Q  D  T  P  S  E  A  R  A  G  A  K  L  R  K  H  G  T  P  V
   CCCACCACCTCTACAGTACGTGCCGTTCAAATCATGAAGGCTCAAGCTGCTGCTCACATTCAAGACACTCCCAGCGAAGCTCGTGCTGGTGCCAAATTGCGTAAGATGGGAACCCCCGTT
      .        .        400      .        .        .        .        450      .        .        .

    V  E  D  R  C  A  S  L  V  A  F  A  S  S  Y  F  D  R  V  I  S  A  L  S  *
   GTTGAAGATCGTTGCGCTAGCTTAGTTGCTGAAGCTTCTAGCTACTTCGATCGCGTAATCTCTGCTTTGAGCTAATTGATTGCCACTTGCAATTCACTCAACAGATTGACATCCAACACA
      .        .        500      .        .        .        .        550      .        .        .        600

                      M  K  S  V  V  T  T  V  I  A  A  A  D  A  A  G  R  F  P  S  T  S  D  L  E  S  V  Q  G
   GTTTAAATAAAAAGCCTTTTGGGAGATTTAAGAAATGAAATCAGTTGTTACCACCGTAATTGCAGCAGCAGATGCCGCAGGTCGTTTCCCCAGTACCTCTGATTTAGAATCCGTACAAGG
      .        .        .        .        650      .        .        .        .        700      .

    S  I  Q  R  A  A  A  R  L  E  A  A  E  K  L  A  N  N  I  D  A  V  A  T  E  A  Y  N  A  C  I  K  K  Y  P  Y  L  N  N  S
   TTCTATCCAACGTGCCGGCTGCACGTTTAGAAGCTGCTGAAAAGCTAGCTAACAACATTGATGCAGTTGCAACCGAAGCTTACAACGCTTGTATCAAGAAGTATCCTTACTTGAACAATTC
      .        .        750      .        .        .        .        800      .        .

    G  E  A  N  S  T  D  T  F  K  A  K  C  A  R  D  I  K  H  Y  L  R  L  I  Q  Y  S  L  V  V  G  G  T  G  P  L  D  E  W  G
   TGGAGAAGCTAACTCCACCGATACCTTCAAAGCTAAGTGCGCTCGTGACATCAAACACTACTTGCGCCCTCATCCAATACTCCTTGGTTGTTGGTGGTACTGGCCCATTGGATGAGTGGGG
      .        850      .        .        .        .        900      .        .        .        950      .

    I  A  G  Q  R  E  V  Y  R  A  L  G  L  P  T  A  P  Y  V  E  A  L  S  F  A  R  N  R  G  C  A  P  P  D  M  S  A  Q  A  L
   TATTGCTGGACAACGTGAAGTTTATCGCCGCTTTAGGCTTGCCTACTGCTCCTTATGTTGAAGCTTTAAGCTTTGCTCGTAACCGTGGTTGTGCACCTCGTGATATGTCTGCTCAAGCATT
      .        .        .        1000     .        .        .        1050     .        .

    T  E  Y  N  A  L  L  D  Y  A  I  N  S  L  S  *
   GACTGAATACAATGCTTTACTAGACTACGCTATTAACTCTCTCTCCTAG
      .        1100     .        .
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Figure 5: Nucleotide and deduced amino-acid sequence of the DNA fragment carrying the cpeB gene, the intercistronic region and the cpeA gene from Calothrix PCC 7601.
A = alanine; D = aspartic acid; E = glutamic acid; F = phenylalanine; G = glycine; H = histidine; I = isoleucine; K = lysine; L = leucine; M = methionine; N = asparagine; P = proline; Q = glutamine; R = arginine; S = serine; T = threonine; V = valine; Y = tyrosine.

When compared with the deduced amino-acid sequence of the PE α subunit recently determined from another chromatically adapting cyanobacterium, Pseudanabaena PCC 7409, the two α-type polypeptides are 94 % homologous (J.M. Dubbs & D.A. Bryant, unpublished data). A corresponding very high degree of homology, 83 %, is also observed at the nucleotide level. These results indicate that there is a high degree of conservation of both the protein and the nucleotide sequences for the PEs of these taxonomically distinct organisms. Whatever the organism, the phycobiliproteins have about the same size (160 to 184 residues) and share homologies. Until now, homologies of about 75 % have been reported between the α subunits of either allophycocyanin or phycocyanin and around 85 % between the corresponding β subunits, for the various organisms examined so far (14, 15, 16). However, only one chromophore is covalently linked to the α subunits of both AP and PC while two chromophores are attached to the PE α subunits (14). The strikingly high degree of homology observed between the

PE α subunits of Calothrix PCC 7601 and Pseudanabaena PCC 7409 may reflect the additional constraints arising from the presence of a second chromophore linked to this relatively short polypeptide chain and/or a more recent phylogenetic divergence.

## Genomic organization of the cpe genes

Interestingly, cpeB was found upstream from cpeA, in both Calothrix PCC 7601 and Pseudanabaena PCC 7409 (J.M. Dubbs and D.A. Bryant, unpublished data), the intercistronic region being 79 and 74 base pairs long, respectively. A similar gene organization has already been found for the genes encoding the α and β subunits of phycocyanin (cpcA and cpcB genes, respectively) in Synechococcus PCC 7002 (15) and Calothrix PCC 7601 (7). However, the inverse orientation was found for the genes encoding the α and β subunits of allophycocyanin (apcA and apcB genes, respectively); apcB lies downstream from apcA on the Synechococcus PCC 6301 (16) and Synechococcus PCC 7002 genomes (17). This result suggests a more recent divergence between PC and PE than between either PC or PE and AP. In agreement with this hypothesis, amino-acid sequence comparisons show that there are fewer substitutions between PC and PE than between either of these proteins and AP.

## Transcription of the cpe genes

Northern hybridization experiments with total RNAs from both green and red light grown cultures were performed using the HindIII/XbaI fragment internal to the cpeB gene as a probe (Fig. 4). This probe detected a single green light-induced transcript 1.45 kb long, identical to the one detected by hybridization with the cpeA gene specific oligonucleotide present in set B. No hybridization was detected with RNAs from red light-grown cells. Thus, as postulated from metabolic studies involving the use of inhibitors, the regulation of PE synthesis occurs at the level of transcription (4). In Calothrix PCC 7601 it has previously been shown that one of phycocyanin genes is also transcriptionally regulated by light but only expressed during growth in red light (7). In addition, as described previously for other phycobiliprotein genes, the two cpe genes are likely organized in a single operon and cotranscribed (7,15,16). However, in contrast to the results obtained for either the apcA and B genes from Synechococcus PCC 6301 (16) or the genes encoding the α and β subunits of the inducible PC from Calothrix PCC 7601 (7), no transcript of a larger size, able to encode specific linker polypeptides in addition to the α and β PE subunits, was detected. Indeed, the size of the detected transcript, estimated to be of

1.45 kb, is insufficient to encode either of the two PE linker polypeptides ($L_R^{35}$ and $L_R^{36}$, respectively 35 and 36 kDa) in addition to the two PE subunits. This result indicates that these linker polypeptide genes must be transcribed independently and suggests that these genes may not be physically linked to the cpeA and cpeB genes.

From our results, it appears that, as for all the other phycobiliproteins studied so far, the genes encoding the α and β subunits are clustered on the genome and cotranscribed. The gene order of the cpe genes is identical to that of the cpc genes, cpcB and cpeB being upstream from cpcA and cpeA, respectively, but inverse to that of the apc genes, apcB being located downstream from apcA. A rather large intercistronic spacer separates each phycobiliprotein gene pair examined to date. This is similar to the situation observed for other cyanobacterial genes which are co-transcribed, such as nifH and nifD (114 bp) from Anabaena PCC 7120 (18) or between the genes encoding the large (rbcL) and small (rbcS) subunits of ribulose-1,5-biphosphate carboxylase (93 bp) from Synechococcus PCC 6301 (19). Unlike the Synechococcus PCC 7002 cpcBACD gene cluster (20), the Calothrix PCC 7601 cpcB2A2C2 operon (7) and the Synechococcus PCC 6301 apcABC operon (16), no gene encoding linker polypeptides seems to be closely linked with the cpeB and cpeA genes of Calothrix PCC 7601. Since from biochemical data, a coordinated synthesis of both PE subunits and of their associated linker polypeptides, $L_R^{35}$ and $L_R^{36}$, has been postulated (4), the transcription of these linker polypeptides must be under a regulation similar to that of the cpeA and B genes. As proposed by Tandeau de Marsac in her model for complementary chromatic adaptation (4), the expression of the genes implicated in this adaptation would be under the control of a photoreversible pigment acting either directly or indirectly at the level of transcription. The characterization of the regulatory sequences of the genes encoding the components of the Calothrix PCC 7601 phycobilisomes is the first step in the elucidation, at the molecular level, of the mechanism by which the cyanobacterial photoreceptor regulates the expression of these genes. Moreover, although its action maxima are situated at shorter wavelenghts in the visible spectra, the property of photoreversibility of this receptor resembles that of the phytochrome present in higher plants and algae (4). Consequently, in addition to its inherent interest, the study of this regulation process in cyanobacteria could bring new insights in the understanding of the regulations mediated by phytochrome in eukaryotic cells.

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