Nucleotide Sequence and Expression of the Genes for the \( \alpha \) and \( \beta \) Subunits of Phycocyanin in Cyanidium caldarium

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The nucleotide sequence of the plastid-encoded operon containing genes for the \( \alpha \) (cpcA) and \( \beta \) (cpcB) subunits of phycocyanin in the unicellular red alga Cyanidium caldarium is described. cpcB is located 5' to cpcA and the two genes are separated by a 102-bp spacer region. The transcription start site of the unicellular red alga transcription initiation site. Northern blotting revealed an abundant

expression and phycocyanin synthesis was similar. These

results provide strong evidence that light induction of the cpcBA operon is dependent on synthesis of heme.

PBPs are bile pigment-apoprotein complexes that constitute the major light-harvesting antennae for oxygenic photosynthesis in cyanobacteria, red algae, and cryptomonads (Gantt, 1981, 1990; MacColl and Guard-Friar, 1987; Glazer, 1989). The principal PBPs are APC, PC, and PE, which comprise \( \alpha \) and \( \beta \) subunit polypeptides, each containing one or more covalently attached bile pigment (phycocyanobilin and phycoerythrobilin) chromophores. Subunits associate into \( \alpha - \beta \) monomers, which aggregate into disc-shaped \( \alpha\beta\_s \) hexamers that are the building blocks of macromolecular light-harvesting complexes, called PBsomes, found on the stroma surfaces of thylakoid membranes in cyanobacteria and red algae (Glazer, 1989; Gantt, 1990; Bryant, 1991). PBsomes consist of a core comprising two to three cylinders, each containing two APC hexameric discs. The core is surrounded by five to six rods made up of hexameric discs of PC and sometimes PE. Linker proteins constitute about 15% of the PBsome mass (Tandeau de Marsac and Cohen-Bazire, 1977). These proteins stabilize interactions between APC discs in the core, interactions between the rod and core, and interactions between PC hexamers and PE hexamers in rods (Glazer, 1989; Bryant, 1991).

Synthesis and assembly of PBsomes require exquisite regulation of the genes for PBPs and linker proteins as well as coordinate regulation of the heme biosynthetic pathway to provide phycocyanobilin and phycoerythrobilin chromophores needed for synthesis of \( \alpha \) and \( \beta \) subunits of PBPs (Troxler, 1986). In cyanobacteria, this is achieved in part by organization of genes for PBPs and associated linker proteins into polycistronic transcription units that are differentially expressed and processed such that the abundance of transcripts for components approximates their composition in PBsomes (Grossman et al., 1988; Bryant, 1991). Earlier work on red algae showed that mRNAs for PBPs are not polyadenylated, suggesting that genes for PBPs are encoded in the plastid genome (Belford et al., 1983; Steinmuller et al., 1983; Steinmuller and Zetsche, 1984). In three species of red algae, pulse-labeling studies in the presence of inhibitors of protein synthesis indicated that PB mRNAs are translated on 70S ribosomes and linker protein mRNAs are translated on 80S ribosomes, suggesting that the genes for PBPs are plastid encoded and the genes for linker proteins are nuclear encoded (Egelhoff and Grossman, 1983; Grossman et al., 1988). This implied a need for information trafficking between plastids and the nucleus to ensure expression of genes for synthesis of PBPs and linker proteins in the correct stoichiometry. More recently, genes for many PC- and APC-associated linker proteins have been found in the plastid genome of red algae (Valentin et al., 1992; Apt and Grossman, 1993a; Reith and Munhold, 1993). Even if genes for all linker proteins turn out to be plastid encoded, inter-organellar information exchange may still be necessary because genes for the three enzymes required for ALA biosynthesis are encoded in the nucleus (Beale and Weinstein, 1990).

In the unicellular rhodophyte Cyanidium caldarium, plastid genes for PC (cpcA, cpcB), APC (apcA, apcB), and several linker proteins are "photogenes," defined as genes whose transcript abundance increases significantly in light (Lin, 1991; Lin et al., 1990; Valentin et al., 1992). C. caldarium does not contain genes for PE. Other plastid photogenes such as psaA, psbA, rbcL, and rbcS are not expressed at detectable levels in dark-grown cells but are expressed at high levels

Abbreviations: ALA, \( \delta \)-aminolevulinic acid; APC, allophycocyanin; PBP, phycobiliprotein; PBsome, phycobilisome; PC, phycocyanin; PE, phycoerythrin; RIF, rifampicin; RT, reverse transcriptase.

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when dark-grown cells are illuminated (Troxler et al., 1989). Slot-blot experiments showed that in cells incubated with ALA or heme in the dark APC and PC transcript levels were comparable to those in illuminated cells, although heme has no effect on the transcript levels of psaA, psbA, rbcL, and rbcS (Troxler et al., 1989). This suggests that heme may selectively bind to protein factors that positively (transcription factors) or negatively (repressor proteins) regulate expression of genes for APC and PC. The finding that transcript levels of some plastid genes are heme regulated and some are not shows that there are at least two general mechanisms for control of plastid gene expression in C. caldarium.

In this paper we describe the organization, nucleotide sequence, and regulation of cpcBA encoding the genes for the α and β subunit polypeptides of PC in C. caldarium.

**MATERIALS AND METHODS**

**Culture Conditions**

*Cyanidium caldarium*, strain III-D-2, was used in the present work (Nichols and Bogorad, 1962). This strain differs substantially from other *Cyanidium* strains and it has been suggested that it should be renamed *Galdieria sulphuraria* (Seckbach, 1991). Cells of this strain grown in the dark do not produce Chl α, APC, or PC, but when illuminated produce more photosynthetic pigments per cell than does the wild type (Troxler, 1972). Cells were grown from a small inoculum in the dark in 1 L of pH 2.0 Allen medium (Allen, 1952) plus 1% Glc in 2-L Erlenmeyer flasks for 5 to 7 d on a rotary shaker at 37°C. Cells were then collected by centrifugation, resuspended in the same medium plus sulfuric acid, and ligated into EcoRI-cut arms of A Zap Blue (Stratagene) according to the manufacturer's instructions. The resulting library contained approximately 10⁶ plaque-forming units. Plaque DNA was transferred to nitrocellulose filters and hybridized with the β subunit PCR probe labeled with [α-32P]dCTP by the random oligonucleotide-directed terminal transferase reaction of Klenow (Stratagene) using a Perkin-Elmer model 553 recording spectrophotometer and both PC and Chl α concentrations were determined as described by Troxler et al. (1989). In other experiments, cells that had been illuminated for 24 h were incubated for an additional 1 to 3 h in the light or dark with RIF (2 μg/mL) or in the light with RIF (2 μg/mL) and 10⁻⁶ M DCMU. Cells were collected by centrifugation, washed with distilled water, and frozen at −80°C until used.

**Nucleic Acid Isolation**

Nucleic acids were isolated by a modification of the procedure of Chomczynski and Sacchi (1987). Cells (1 mL) were suspended in 10 mL of 4 M guanidium isothiocyanate, 25 mM sodium citrate, pH 7.0, 0.5% sarcosyl, and 0.1 M β-mercaptoethanol and disrupted by passage through a French pressure cell at 15,000 p.s.i. The pressate was extracted three times with phenol:chloroform:isoamyl alcohol (50:49:1), an equal volume of isopropanol was added to the final aqueous phase, and the resulting precipitate was dissolved in 10 mM Tris-HCl, pH 8.0, 1 mM EDTA. Carbohydrate was removed by precipitation with 4% hexadecyltrimethyl ammonium bromide as described by Loppes et al. (1985). For DNA isolation, total nucleic acids were treated with RNase (Sigma) at 37°C for 30 min and, after phenol-chloroform extraction, DNA was recovered by ethanol precipitation. For RNA isolation, total nucleic acids were treated with RQI DNase (Promega) for 10 min at room temperature and, after phenol-chloroform extraction, RNA was recovered by ethanol precipitation.

**Isolation of a β Subunit Hybridization Probe**

A hybridization probe for screening a genomic DNA library and for northern and Southern hybridization was prepared by a PCR strategy. Primers were designed by selecting regions where the amino acid sequence of the β subunit of C. caldarium PC (Troxler et al., 1981) was identical with the deduced amino acid sequence of the β subunit of PC from *A. quadruplicatum* (de Lorimier et al., 1984). The 5' (forward/sense) primer (5'-GCTGATGCCAGAGGTGAGTTC-3'; subsequently called P-1) contained bases coding for residues 12 to 18, and the 3' (reverse/antisense) primer (5'-CATGAGAGCGCTGCAGTCACC-3'; subsequently called P-2) contained bases complementary to those coding for residues 151 to 157 of the β subunit of PC from *A. quadruplicatum*. PCR was carried out for 35 cycles under standard conditions (Delidow et al., 1993) using 1 μg of C. caldarium genomic DNA as a template.

**Library Preparation and Screening**

Genomic DNA was digested with EcoRI, passed through a spin column to remove fragments of less than 400 bp, and ligated into EcoRI-cut arms of λ Zap II (Stratagene). Phage were packaged and plated on *Escherichia coli* XL1-Blue (Stratagene) according to the manufacturer's instructions. The resulting library contained approximately 10⁶ plaque-forming units. Plaque DNA was transferred to nitrocellulose filters and hybridized with the β subunit PCR probe labeled with [α-32P]dCTP by the random oligonucleotide-directed terminal transferase reaction of Klenow (Stratagene) using a Perkin-Elmer model 553 recording spectrophotometer and both PC and Chl α concentrations were determined as described by Troxler et al. (1989). In other experiments, cells that had been illuminated for 24 h were incubated for an additional 1 to 3 h in the light or dark with RIF (2 μg/mL) or in the light with RIF (2 μg/mL) and 10⁻⁶ M DCMU. Cells were collected by centrifugation, resuspended in 10 mL of pH 2.0 Allen medium plus 1% Glc, and incubated in 50-mL Erlenmeyer flasks on a rotary shaker in the dark at 37°C for 24 h. Heme is totally insoluble at pH 2 and is trapped inside of the cells under these conditions. After incubation with heme, cells were collected by centrifugation, washed with distilled water, and frozen at −80°C until used.
otide-priming method (Feinberg and Vogelstein, 1984). Filters were prehybridized in 50% formamide, 25 mM potassium phosphate buffer, pH 7.4, 5X SSC (1X SSC = 0.3 M NaCl, 0.03 M sodium citrate), 5X Denhardt’s solution (1X Denhardt’s = 0.02% Ficoll, 0.02% PVP, 0.02% BSA), 0.5% SDS, and denatured calf thymus DNA (100 μg/mL) for 3 h at 42°C and hybridized in the same solution containing 10% dextran sulfate for 16 h at 42°C. Final wash conditions were 0.1X SSC, 0.1% SDS at 42°C. Filters were exposed to Kodak XAR film for 24 to 72 h. More than 25 positive clones were rescued in E. coli XL1-Blue (Short et al., 1988) and sequenced on both strands by the dideoxy chain termination method (Sanger et al., 1977) using Sequenase, version 2.0 (United States Biochemical) with P-1, P-2, and oligonucleotide primers designed on the basis of determined sequences.

**Gel Electrophoresis and Southern and Northern Hybridization**

DNA (5 μg) was digested with restriction enzymes, electrophoresed on 0.8% agarose gels, and blotted by capillary action onto nylon membranes (Hybond N+, Amersham) in 10X SSC. Blots were prehybridized, hybridized with the labeled β subunit PCR probe, and washed as described above. RNA (10 μg) was electrophoresed on 1% agarose formaldehyde denaturing gels in 20 mM Mops, pH 6.0, and transferred to nylon membranes with 10X SSC, and blots were prehybridized, hybridized, and washed under the conditions described above.

**Primer Extension**

A 15-base oligonucleotide (5'-AGCATCAAGTTGGGT-3') complementary to bases +64 to +78 relative to the ATG initiation codon of cpcB was used to map the 5’ end of the cpcBA transcript by primer extension (Ausubel et al., 1992). The oligonucleotide (10 pmol) was labeled with [γ-32P]ATP and T4 kinase for 30 min at 37°C. The labeled primer (50,000 cpm) was hybridized to RNA (10 μg) from illuminated cells and the primer-annealed RNA was recovered by ethanol precipitation. cDNA was synthesized in a 25-μL reaction containing 50 mM Tris-HCl, pH 8.3, 40 mM KCl, 7 mM MgCl2, 10 mM DTT, primer-annealed RNA, deoxynucleoside triphosphates (0.6 mM each), RNAsin (40 units, Promega), and Moloney murine leukemia virus RT (40 units, Promega) for 90 min at 42°C. Primer extension products were analyzed on 6% polyacrylamide denaturing gels by reference to a sequencing ladder generated with pCpcF9 as the template and the same oligonucleotide as the sequencing primer.

**RT-PCR**

RNA (2 μg) from cells grown in the dark and from cells illuminated for 24 h was annealed with P-2 (10 pmol) and incubated in 20-μL reaction mixtures, with (+RT) or without (−RT) RT, for 60 min at 42°C as described above, P-1 (50 pmol) and P-2 (40 pmol) were added to the +RT and −RT reactions, followed by amplification with Taq polymerase in a standard 100-μL PCR reaction for 35 cycles (Delidow et al., 1993). An aliquot (20 μL) of each reaction was electrophoresed on a 1% agarose-ethidium bromide gel in 1X buffer (0.09 M Tris-borate, pH 8.0, 0.002 M EDTA) and the gel was photographed.

**RESULTS**

**Nucleotide Sequence of the cpcB PCR Probe**

A 465-bp DNA fragment was amplified by PCR from genomic DNA using primers designed from regions of similarity between the β subunits of C. caldarium and A. quadruplicatum PC (de Lorimier et al., 1984). This DNA product was ligated into pUC19 digested with Smal and used to transform E. coli JM109. Plasmids were isolated from bacterial cells and the insert was sequenced from both ends with universal primers (data not shown) and found to contain an open reading frame of 465 bp coding for residues 12 to 157 of the β subunit of C. caldarium PC (Troxler et al., 1981). This insert, designated cpcB12-157, was used to screen a C. caldarium genomic library and as a probe for northern and Southern hybridizations.

**Isolation and Sequence of cpcBA cDNA Clones**

One of the 25 λ clones that hybridized to cpcB12-157 was isolated from the genomic library and plaque purified. This clone was recovered in pBluescript SK− (referred to as pCpcF9) and mapped by analysis of restriction fragments that hybridized to cpcB12-157. A map of the 5.0-kb insert in this clone is shown in Figure 1 and the nucleotide sequence of a 1.6-kb internal segment is shown in Figure 2. This region contains two open reading frames, one of 516 bp (cpcB) and a second of 486 bp (cpcA) that code for the β and α subunits, respectively, of PC, with cpcB located 5' to cpcA and the two genes separated by a 102-nucleotide AT-rich spacer region. The deduced amino acid sequences of the proteins encoded in cpcB and cpcA are in agreement with the experimentally determined amino acid sequences of the β and α subunits (Offner et al., 1981; Troxler et al., 1981). Cys's were predicted at residues 82 and 153 of the β subunit.
unit and at residue 84 of the α subunit, which is in agreement with our earlier results showing phycocyanobilin chromophores attached at these positions by cysteinylthioether linkages (Brown et al., 1979). The sequence AGGAGT located 9 nucleotides 5' to the ATG start codon of *cpcA* is in agreement with our earlier results showing phycocyanobilin chromophores attached at these positions by cysteinylthioether linkages (Brown et al., 1979). The sequence AGGAGT located 9 nucleotides 5' to the ATG start codon of *cpcA* is in agreement with our earlier results showing phycocyanobilin chromophores attached at these positions by cysteinylthioether linkages (Brown et al., 1979).

Blots of *C. caldarium* RNA were hybridized with *cpcB* and *cpcA* cDNA probes. The results are in agreement with our earlier results showing phycocyanobilin chromophores attached at these positions by cysteinylthioether linkages (Brown et al., 1979). The sequence AGGAGT located 9 nucleotides 5' to the ATG start codon of *cpcA* is in agreement with our earlier results showing phycocyanobilin chromophores attached at these positions by cysteinylthioether linkages (Brown et al., 1979).

Expression of *cpcBA*

We found previously that RNA from dark-grown *C. caldarium* cells incubated with 10⁻⁶ M heme in the dark for...
Table I. Codon usage of genes in the cpcBA and apcAB* operons in the plastid genome of C. caldarium

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<td>GAG</td>
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* Data from Liu and Troxler (1993, 1994).

24 h contained transcripts that hybridized on slot blots to a heterologous cyanobacterial PC probe (Troxler et al., 1989). Since these studies did not provide information concerning the size and number of heme-induced transcripts, the effect of heme treatment on expression of cpcBA was reexamined. RNA was isolated from dark-grown cells incubated with or without 10⁻⁶ M heme in the dark for 24 h or from cells illuminated for 24 h. RNA samples were electrophoresed and blotted onto nylon membrane, and blots were probed with cpcB₁₂–₁₅₇. Illuminated cells contained a 1.3-kb cpcBA transcript (Fig. 4, lane 1). Dark-grown cells did not contain detectable levels of cpcBA transcripts (Fig. 4, lane 2), whereas dark-grown cells incubated with heme contained a 1.3-kb cpcBA transcript (Fig. 4, lane 3) comparable in size and abundance to that in illuminated cells. These results suggest that in the dark cpcBA expression is positively regulated by heme.

Previously we reported that the size of the cpcBA transcript in C. caldarium was 3 kb (Troxler et al., 1989). The heterologous PC hybridization probe used in these studies was a 1.4-kb Smal/XhoI fragment of a 3.0-kb HindIII genomic clone containing cpcA, cpcB, and cpcC (the 33-kD PC-associated linker polypeptide) from the cyanobacterium A. quadruplicatum (de Lorimier et al., 1984). When the blot shown in Figure 4 was stripped and rehybridized with the heterologous probe, the size of the C. caldarium cpcBA transcript in lanes 1 and 3 was 1.3 kb, the same as that detected with the homologous PC probe (data not shown). Thus, the size of the cpcBA transcript in C. caldarium reported initially was in error.

The hypothesis that heme positively regulates expression of cpcBA was tested directly in studies in which dark-grown cells were illuminated in the presence of gabaculine (an inhibitor of ALA synthesis), levulinic acid (a competitive inhibitor of ALA dehydrase), or N-methyl-mesoporphyrin IX (an inhibitor of ferrochelatase). A series of experiments was carried out to determine the minimum concentration of each inhibitor required to produce maximum inhibition of pigment synthesis. In cells illuminated with 150 μM gabaculine or 10 μM levulinic acid, inhibition of PC and Chl a was nearly complete, whereas in cells illuminated with 200 μM N-methyl-mesoporphyrin IX, inhibition of PC synthesis was 30% and Chl a synthesis was not affected (Fig. 5A). After incubation with inhibitors, RNA was isolated from cells and examined on northern blots probed with cpcB₁₂–₁₅₇. Transcripts of cpcBA were
Figure 3. Identification of the 5' end of the C. caldarium cpcBA transcript by primer extension. An oligonucleotide complementary to nucleotides +64 to +78 was annealed to 10 μg of RNA from cells illuminated for 24 h and extended with RT. The sequencing ladder was generated with pCpcF9 using the same primer. Lanes 1 to 4 show the A, C, G, and T sequencing reactions, respectively, and lane 5 shows the primer extension product. In the expanded sequence at the right, the nucleotide corresponding to the first base (+1) of the primer extension product is indicated by a filled circle.

undetectable in dark-grown cells or in cells incubated with gabaculine or levulinic acid; in cells incubated with N-methyl-mesoporphyrin IX the level of cpcBA transcripts was reduced approximately 30% (Fig. 5B). A separate series of experiments was performed to determine the effect of gabaculine, levulinic acid, and N-methyl-mesoporphyrin IX on cell viability. Cells were incubated for 24 h with inhibitors at the concentrations used in the experiment shown in Figure 5, washed three times with pH 2 Allen medium minus Glc, and illuminated in the same medium for 24 h. Cells treated in this way synthesized PC and Chl a in quantities comparable to cells illuminated for 24 h without inhibitors (data not given). This shows that inhibition of cpcBA expression in cells treated with inhibitors was not due to cell death.

The inability to detect cpcBA transcripts on northern blots of RNA isolated from dark-grown cells could be due to the fact that transcripts are absent or are present at levels below the detection limits of northern hybridization. Therefore, a more sensitive technique (RT-PCR) was used to determine whether transcripts of cpcBA could be detected in the dark. RNA from dark-grown cells was reverse transcribed with P-2 (the 3' reverse/antisense oligonucleotide used to generate cpcB12-157) as primer, and cDNA was amplified with Taq polymerase for 35 cycles in a standard PCR reaction with P-1 and P-2 as primers. This procedure amplified cpcBA12-157 from the cDNA sample derived from RNA of illuminated cells and a smaller quantity of a DNA product of comparable size from the cDNA sample derived from RNA of dark-grown cells (Fig. 6, lanes 2 and 4, +RT).
This shows that transcripts of cpcBA occur in dark-grown cells even though they cannot be detected on northern blots. No PCR product was observed when enzyme (RT) was omitted from RT reactions (Fig. 6, lanes 3 and 5, -RT), which shows that in complete reactions the amplified DNA product was derived from cDNA and not from residual genomic DNA contaminant in the RNA samples.

Cells (10 mL of a culture containing 10⁵ cells/mL) were placed in 1 L of pH 2.0 Allen medium plus 1% Glc and incubated in the dark for 10 d. Measurement of cell number during this time showed that after several days cell division commenced and went through a lag phase lasting about 24 h, an exponential growth phase lasting about 48 h, and a stationary phase in which cell division ceased (Fig. 7). Dark-grown cells contain a small proplastid-like organelle lacking photosynthetic pigments (Troxler, 1994). When dark-grown cells are illuminated in pH 2.0 Allen medium minus Glc, cell division stops and Pbsome-associated APC and PC and thylakoid membrane-associated Chl a accumulate as the proplastid-like organelle develops into a large cup-shaped chloroplast during a 24-h period (Fig. 7). Previously, we measured cpcBA transcripts only in dark-grown cells and in cells illuminated for 24 h (Fig. 4; Troxler et al., 1989) and it was of interest to compare the temporal relationship between accumulation of cpcBA transcripts and accumulation of PC in cells in the light. Therefore, cells grown in the dark were illuminated for various times, and RNA was isolated and examined on blots probed with cpcB₁₂₋₁₅₇. Transcripts were not detectable in dark-grown cells, were first detectable after illumination for 1 h, and increased to a maximum level after illumination for 24 h (Fig. 8A). The kinetics of transcript accumulation was qualitatively similar to that of PC accumulation in cells (Fig. 7). Transcripts were not observed when cells illuminated for 24 h were returned to the dark for 12 h (data not given). This shows that maintenance of the mRNA level requires continuous illumination.

When cells illuminated for 24 h were incubated with RIF (2 μg/mL) in the light, the concentration of cpcBA transcripts declined slightly after 1 h and significantly after 2 h and were no longer detectable after 3 h (Fig. 8A). PC levels...
were unchanged in cells incubated with RIF (data not shown). C. caldarium chloroplast RNA polymerase is inhibited 100% by RIF at 2 μg/mL (R.F. Troxler, unpublished data) and PC, APC, and Chl α synthesis is inhibited 100% by RIF at 5 μg/mL (Troxler et al., 1989). The disappearance of cpcBA transcripts in cells incubated with RIF in the light (Fig. 8A) most likely can be explained by RNA turnover in the absence of transcription. Thus, the rate of cpcBA transcript disappearance in RIF-treated cells (Fig. 8A) is a direct measurement of mRNA turnover.

To examine this process further, cells were grown in the dark or illuminated for 24 h and subsequently incubated with RIF and DCMU in the light or with RIF alone in the dark for an additional 1 to 3 h. RNA was isolated from the variously treated cells and examined on northern blots probed with cpcB1–157. As noted above, transcripts were undetectable in dark-grown cells, abundant in cells illuminated for 24 h, and undetectable in cells illuminated for 24 h and subsequently incubated with RIF in the absence or presence of DCMU (Fig. 8B). This suggests that the machinery for mRNA turnover in chloroplasts is not dependent on products derived directly from photosynthesis. Interestingly, cpcBA transcript levels did not change significantly in cells illuminated for 24 h and incubated with RIF in the dark (Fig. 8B). This implies that the plastid RNases that degrade mRNAs require light to be active but that the light requirement is distinct from the need for an energy source derived from photosynthesis. The cpcBA transcript levels were slightly reduced in cells illuminated for 27 h (Fig. 8B).

**DISCUSSION**

The present investigation describes the nucleotide sequence of the cpcBA operon encoding the genes for the β and α subunits of PC in the unicellular rhodophyte C. caldarium. The cpcBA operon has been found in the small single-copy region of the plastid genome in two red algae (Shivji, 1991; Reith and Munholland, 1993) and in the cyanelle genome of the eukaryotic alga Cyanophora paradoxa (Lemaux and Grossman, 1984). All but 3 kb of the 16-kb small single-copy region in C. caldarium has been sequenced (Valentin et al., 1992) and psbA, rpl21, and a gene cluster containing open reading frames for a β-like APC subunit, an APC-associated linker protein, and a PC-associated linker protein were identified in the sequenced region. The 3-kb region not sequenced is located next to the small single-copy region in two red algae (Shivji, 1991; Reith and Munholland, 1993) and in the cyanobacterium Anabaena sp. PCC 7120 (Troxler et al., 1994; R.F. Troxler, unpublished data). The Anabaena α factor displays a high degree of homology to σ32 in E. coli and σ3 in Bacillus subtilis and contains the conserved domains in bacterial σ factors that recognize −10 and −35 promoter elements (Brahamsha and Haselkorn, 1991). Therefore, it seems likely that the putative promoter-like elements found upstream of cpcBA and the other operons containing genes for PBsomes proteins in C. caldarium are functionally relevant.

Transcripts of cpcBA are not detectable in northern hybridization in cells grown in the dark (Fig. 4), although RT-PCR showed that low levels of cpcBA transcripts are present (Fig. 6). Although no attempt was made to quantify the number of cpcBA transcripts in dark-grown cells, the fact that they are there, albeit in small numbers, means that transcription of cpcBA is not completely “off.” If we assume that the steady-state level of a low-abundance transcript is maintained by equal rates of transcription and RNA turnover, the small number of cpcBA transcripts in the dark could be explained by the existence of a repressor protein that almost completely blocks transcription. However, because of the equilibrium between free and bound repressor, an RNA polymerase molecule could occasionally bind to the cpcBA promoter and initiate transcription. Since administered heme alleviates repression of the cpcBA operon in the dark, an attractive model to explain transcript accumulation in light might be that light promotes heme synthesis and free heme binds to the repressor, resulting in release of the heme-repressor complex leading to an increase in the rate of transcription. Alternatively, the limited number of cpcBA transcripts in the dark could result from limiting for the rod or rod-core linker proteins were not found in the region 200 bp upstream of cpcB or 500 bp downstream of cpcA in C. caldarium, suggesting that genes for PC-associated linker proteins occur in distinct but coordinately regulated operons.

A single 1.3-kb cpcBA transcript was detected in C. caldarium by northern analysis (Fig. 4) and no larger transcripts were seen even after prolonged exposure to film. Multiple transcripts containing cpcBA with or without one or more PC-associated linker polypeptides have been found in cyanobacteria (Grossman et al., 1988; Bryant, 1991). The occurrence of a single cpcBA transcript in C. caldarium provides further evidence that PC-associated linkers are encoded in separate operons.

Genes for PBPs and most linker proteins have been identified in the plastid genome of C. caldarium (present study; Valentin et al., 1992; Liu and Troxler, 1993, 1994). These genes are organized into the following mono-, di-, and tristrictonic operons: cpcG, cpcBA, apcCD, and apcEAB. All of these operons have promoter-like elements in the 5′-flanking regions that are reasonably good matches to the −10 and −35 promoter elements recognized by the σ20 family of σ factors found in bacterial DNA polymerases (Helmann and Chamberlin, 1988; Lonetto et al., 1992). C. caldarium RNA polymerase species contain 32-, 55-, and 120-kD proteins that cross-react with antibodies against the principal σ subunit of RNA polymerase in vegetative cells of the cyanobacterium Anabaena sp. PCC 7120 (Troxler et al., 1994; R.F. Troxler, unpublished data). The Anabaena σ factor displays a high degree of homology to σ32 in E. coli and σ3 in Bacillus subtilis and contains the conserved domains in bacterial σ factors that recognize −10 and −35 promoter elements (Brahamsha and Haselkorn, 1991). Therefore, it seems likely that the putative promoter-like elements found upstream of cpcBA and the other operons containing genes for PBsomes proteins in C. caldarium are functionally relevant.
quantities of a heme-regulated plastid transcription factor, a heme-regulated σ factor, or heme-dependent protein factors that alter RNA stability.

A critical test for the role of heme in expression of cpcBA is shown in Figure 5. Synthesis of PC and Chl a and accumulation of cpcBA transcripts in illuminated cells were blocked by both gabaculine and levulinic acid. The effect of these inhibitors on pigment synthesis would be predicted because gabaculine inhibits ALA synthesis and levulinic acid inhibits ALA utilization, resulting in inability to synthesize phycocyanobilin (the PC chromophore) and Chl a, both of which are derived from ALA. The absence of detectable cpcBA transcripts in gabaculine- or levulinic acid-treated cells implicates ALA or a derived tetrapyrrole in regulation of the cpcBA operon. Administration of N-methylmesoporphyrin IX, an inhibitor of ferrochelatase, to illuminated cells resulted in a 30% reduction in cpcBA expression and a 30% reduction in PC synthesis but had no effect on synthesis of Chl a (Fig. 5). This provides strong evidence that light induction of cpcBA is dependent on heme synthesis.

Light positively modulates cpcBA transcript levels when dark-grown C. caldarium cells are illuminated in Glc-free medium and thus, by definition, cpcB and cpcA are photosynthetic genes. Although there is a strong inhibition of PBP accumulation when dark-grown cells are illuminated in medium containing Glc (Doemel and Brock, 1971), the molecular basis of this “Glc effect” is not known. In C. caldarium, the action spectrum for ALA and PC synthesis in a Chl-less mutant resembles the absorption spectrum of a hemoprotein, and the action spectrum for ALA and Chl a synthesis in a PBP-less mutant resembles the absorption spectrum of Pchlide-holochrome (Schneider and Bogorad, 1979). This suggests that distinct photoreceptors control PBP and Chl a synthesis and is consistent with the selective effect of heme on accumulation of cpcBA (Fig. 5) and apcAB (Troxler et al., 1989; Lin et al., 1990) transcripts in C. caldarium.

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Phycocyanin Genes in Cyanidium caldarium


