JOURNAL OF BACTERIOLOGY, Feb. 1993, p. 575–582
0021-9193/93/030575-08$02.00/0

Vol. 175, No. 3

MINIREVIEW

Environmental Effects on the Light-Harvesting Complex of Cyanobacteria†

ARTHUR R. GROSSMAN,* MICHAEL R. SCHAEFER, GISELA G. CHIANG, AND JACKIE L. COLLIER

Department of Plant Biology, The Carnegie Institution of Washington, 290 Panama Street, Stanford, California 94305

INTRODUCTORY REMARKS

While all oxygen-evolving organisms contain chlorophyll *a* in their photosynthetic reaction centers, the pigments of the antenna complexes that harvest light energy may vary markedly. In the prokaryotic cyanobacteria and eukaryotic red algae, phycobiliproteins are the most prominent light-harvesting polypeptides of the cell. Phycobiliproteins are brilliantly pigmented, water-soluble proteins that may constitute up to half of the soluble proteins of the cell. The major phycobiliproteins are phycoerythrin (PE) (maximum absorbance [A_{max}] ≅ 565 nm), phycocyanin (PC) (A_{max} ≅ 620 nm), and allophycocyanin (AP) (A_{max} ≅ 650 nm). These proteins become constituents of a macromolecular complex called the phycobilisome (PBS) (7, 22, 24, 25, 59), which is peripherally associated with the cytoplasmic sides of the photosynthetic membranes. In this minireview, we focus on how environmental factors such as light quality and nutrient availability can dramatically affect PBS biosynthesis.

PBS STRUCTURE

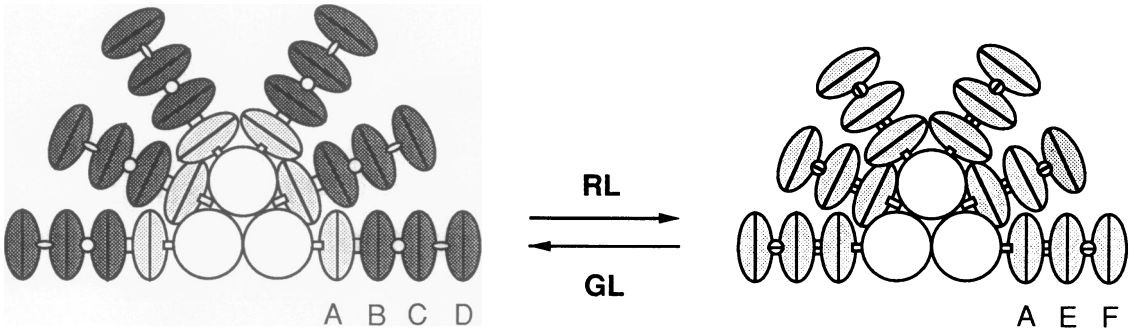
In red algae and cyanobacteria, the outer surfaces of the thylakoid or photosynthetic membranes are covered by rows of closely spaced granules, as seen with the electron microscope. These granules are light-harvesting PBS complexes. Phycobiliproteins make up approximately 85% of the PBS complex. Together, these proteins can absorb visible light in the range of 450 to 660 nm. The excitation energy absorbed by PE is transferred sequentially to PC, to AP, and then to the chlorophyll molecules associated with the reaction centers of photosynthesis. Each of the phycobiliproteins is composed of two different subunits termed α and β, with molecular masses of between 15 and 22 kDa. The chromophores, or light-absorbing molecules, attached to the phycobiliproteins are linear tetrapyrroles that are isomers of each other. The chromophores are bonded to the apoprotein via thioether linkages. The numbers and types of chromophores associated with a particular phycobiliprotein subunit are usually invariant (with some exceptions) (see reference 25). The absorbance of the chromophore is strongly influenced by its conformation and interactions with amino acid residues of the protein moiety of the molecule. The phycobilin chromophores are very similar to the chromophore associated with the photoreceptor phytochrome, which is crucial in the development and differentiation of higher plants.

Also integral to PBS structures are linker polypeptides, most of which are nonpigmented. These polypeptides range in molecular mass from 8 to 120 kDa. The linker polypeptides may have more than one function in the PBS. These polypeptides help stabilize the PBS structure, establish the specific positions of the phycobiliproteins in the complex, facilitate assembly of phycobiliprotein-containing substructures, modulate the absorption characteristics of the phycobiliproteins to promote unidirectional transfer of energy within the PBS and from the PBS to the chlorophyll molecules of the photosynthetic reaction centers, and physically link the entire complex to the photosynthetic membranes.

A synthesis of the information generated over 20 years from electron microscopy, biochemical and biophysical studies, X-ray crystallography, and molecular analysis of genes encoding PBS polypeptides has led to the development of a detailed model of PBS structure (7, 9, 22, 25, 26). The structure of a PBS from the filamentous cyanobacterium *Fremyella diplosiphon* (similar to *Calothrix* sp. strain PCC 7601 and used synonymously in this minireview) and the constituents that make up each of the two PBS domains, the rods and the core, are presented diagramatically in Fig. 1. This structure is fundamentally similar to the PBS structures present in most cyanobacteria. As shown in Fig. 1, the composition of the *F. diplosiphon* PBS is different in cells grown under red light (RL) and green light (GL). This phenomenon is discussed in the next section. The complex is fan-like in appearance, containing a tricylindrical core structure and a set of six cylindrical rods that radiate from the core. The phycobiliproteins assemble into hexamers, or double discs, that are stacked upon each other to form the cylinders that make up both the rod and core substructures. The rods contain PE and PC hexamers associated with specific linker (L) polypeptides. The core is composed primarily of AP subunits. A channel of approximately 35 Å (3.5 nm) in the center of the hexamers is the site of linker polypeptide association. The core substructure is in direct contact with thylakoid membranes and mediates the transfer of excitation energy from the PBS to the photosynthetic reaction centers.

Essentially all of the genes encoding structural components of the PBS have been isolated and characterized from many different organisms (summarized in references 7, 30, and 52). This work has added enormously to our knowledge of PBS structure and the functions of the individual components that make up the complex, our ideas concerning the evolution of the genes encoding PBS polypeptides, and our

* Corresponding author.
† Publication 1157 of The Carnegie Institution of Washington.



(A) $(\alpha^{PC}\beta^{PC})_6\ L^{27}_{RC}$
(B) $(\alpha^{PE}\beta^{PE})_6\ L^{31.8}_{R}$
(C) $(\alpha^{PE}\beta^{PE})_6\ L^{27.9}_{R}$
(D) $(\alpha^{PE}\beta^{PE})_6\ L^{27.6}_{R}$

(A) $(\alpha^{PC}\beta^{PC})_6\ L^{27}_{RC}$
(E) $(\alpha^{PC}\beta^{PC})_6\ L^{30.5}_{R}$
(F) $(\alpha^{PC}\beta^{PC})_6\ L^{32.5}_{R}\ L^{9.7}_{R}$

FIG. 1. PBS of *F. diplosiphon* grown under RL and GL. The dark speckled double discs represent hexamers of PE [$(\alpha^{PE}\beta^{PE})_6$], while the light speckled double discs represent hexamers of PC [$(\alpha^{PC}\beta^{PC})_6$]. The linker polypeptides are indicated by L. The subscript to L denotes the position of the linker in the PBS substructure (R for rod substructure; RC for rod-core interface). The superscript to L is the molecular mass (in kilodaltons) of the polypeptide. The molecular masses used were determined from the gene sequences. The cylinders of the PBS core are depicted as white circles that are unspeckled. The core substructure of the PBS is invariant. For a more detailed discussion of core structure, see reference 25. The composition of the rod substructures varies dramatically between organisms grown under RL and GL. The composition of each of the double discs of the PBS in GL-grown cells (discs A, B, C, and D) and RL-grown cells (discs A, E, and F) is given below the PBS structures.

understanding of the ways in which the levels of the different phycobiliprotein components are modulated. Table 1 enumerates the different polypeptide constituents of the PBS, provides their gene designations, and offers a brief description of the function of each of the polypeptides.

Different environmental conditions alter the composition or abundance of PBS. Low light intensities stimulate the synthesis of PBS, and the rod substructure may increase in size (34, 45, 46). Growing cyanobacteria with light that is not efficiently harvested by the PBS (long-wavelength RL) may cause increased phycobiliprotein accumulation in the cell (35, 36, 41). Macronutrient limitation results in extensive PBS degradation and chlorosis, or a bleaching response (3, 14, 58). Finally, light quality can cause a dramatic change in PBS composition (5, 28, 51).

CHROMATIC ADAPTATION

Initial observations. The process by which cyanobacteria alter the composition of their PBS structures in response to different wavelengths of light (5, 8, 28, 51, 53) is termed chromatic adaptation. When the levels of both the PE and PC pigments in the PBS are modulated in opposite directions to each other, the process in termed complementary chromatic adaptation. Complementary chromatic adaptation is most clearly demonstrated when the adapting organisms are compared after growth under RL and GL. Under RL, *F. diplosiphon* accumulates high levels of the blue pigment PC and very little of the red pigment PE. Conversely, under GL, the organism has low levels of PC and high levels of PE. This change in the light-harvesting complex affords the cells an adaptive advantage, since PC effectively absorbs RL and PE effectively absorbs GL. The consequence of chromatic adaptation on PBS composition is shown in Fig. 1.

The differences in the composition of the PBS structures observed in cells grown in different light qualities are a consequence of altered gene expression. The initial events that trigger changes in phycobiliprotein gene expression during chromatic adaptation probably involve absorption of light by one or more photoreversible photoreceptor molecule(s) that absorb both RL and GL. The action maximum for PE synthesis in both *F. diplosiphon* (31, 54) and *Tolypothrix tenuis* (18) is between 540 and 550 nm, while the maximum for PC synthesis is between 650 and 660 nm. These results indicate that the photoreceptor involved in chromatic adaptation is most likely a phycobiliprotein which is similar to phytochrome, the photoreceptor that controls many physiological processes in higher plants. Early biochemical attempts to isolate the photoreceptor involved in controlling chromatic adaptation were plagued by various artifacts. With the isolation of genes encoding both phycobiliproteins and linker polypeptides, the utilization of mutants that exhibit aberrant light responsiveness, and the development of gene transfer technology, the processes controlling chromatic adaptation have now become amenable to genetic analysis.

Gene expression. In cyanobacteria, the α and β genes of a given phycobiliprotein are always contiguous and cotranscribed. A number of cyanobacteria also have multiple sets of genes encoding the α and β subunits of PC (gene designation *cpcBA*). In *F. diplosiphon*, there are three PC gene sets (15, 16, 39, 40), two of which are important to our discussion of chromatic adaptation. The mRNA from one of these PC gene sets accumulates constitutively, while the mRNA from the second accumulates only in cells maintained under RL (15). The former gene set, *cpcB1A1*, encodes constitutively expressed PC (PC_c), while the latter gene set, *cpcB2A2*,

TABLE 1. Structural components of a PBS

Protein designation[a]	Gene designation	Position and function
α^{PC}, β^{PC}, α^{PE}, β^{PE}	*cpcA*, *cpcB*, *cpeA*, *cpeB*	Phycobiliprotein subunits that form the hexameric building blocks of the rod substructure[b]
L_{RC}	*cpcG*	Linker polypeptide that serves as an interface between the rods and the PBS core
L_R (for PC hexamers)	*cpcCD*, *cpcHI*	Different linker polypeptides associated with PC hexamers of the rods
L_R (for PE hexamers)	*cpeCDE*	Linker polypeptides associated with PE hexamers in the rods
α^{AP}, β^{AP}	*apcA*, *apcB*	Phycobiliproteins that form the building blocks of the PBS core
α^{APB}	*apcD*	Subunit that associates with β^{AP} as part of a terminal energy acceptor in the core
$\beta^{18.5}$	*apcF*	Subunit resembling β^{AP} that associates with L_{CM} as part of a terminal energy acceptor in the core
L_C	*apcC*	Small linker polypeptide that may stabilize the core substructure
L_{CM}	*apcE*	Terminal energy acceptor in the core; may help stabilize the core and/or nucleate its formation; may also establish a physical association between the PBS and thylakoid membranes

[a] To designate the α and β subunits of the different phycobiliproteins, the phycobiliprotein class (PC, PE, or AP) is given as a superscript to the specific subunit. For the linker polypeptides (L), the subscript indicates the position within the PBS (R for rod, RC for rod-core interface, C for core, and CM for core-thylakoid membrane interface). More thorough discussions of each of the components of the PBS are given by Bryant (7) and Grossman et al. (30).

[b] Not all cyanobacteria have PBS structures containing PE or PE linker polypeptides.

encodes RL-inducible PC (PC_i). Transcripts from the PE gene set (gene designation *cpeBA*) accumulate to high levels only under GL (38, 42). The abundance of the mRNAs from *cpcB1A1*, *cpcB2A2*, and *cpeBA* reflects the polypeptide composition of the PBS structures under a given light condition. Furthermore, the half-lives of mRNAs encoding PC_i, PC_c, and PE are not changed by altering the light condition under which the cells are grown (42). These observations implied that PBS biosynthesis under different light conditions is, to a great extent, a consequence of the differential transcription of the *cpcB2A2* and *cpeBA* operons. The expression of specific linker polypeptides associated with PC_i and PE must also be appropriately regulated in the different light conditions. The genes encoding linker polypeptides associated with PC_i are located downstream of *cpcB2A2* (33). This region of the genome exhibits overlapping transcripts. A 1.6-kb transcript encodes the α and β subunits of PC_i, while a 3.7-kb transcript encodes both the α and β subunits of PC_i and the three linker polypeptides that are required for assembly of PC_i onto the rod substructure. These linker polypeptides, $L_R^{30.5}$, $L_R^{32.5}$, and $L_R^{9.7}$, are encoded by the *cpcH2*, *cpcI2*, and *cpcD2* genes, respectively, of the *cpcB2A2H2I2D2* operon. The ratio of the 1.6- and 3.7-kb mRNAs from this operon approximately reflects the ratio of the PC_i subunits and the linker polypeptides in the PBS; the level of the 3.7-kb species is about 10% that of the 1.6-kb species. The larger mRNA may be generated by transcriptional read-through approximately 10% of the time. Alternatively, the 3.7-kb species may be a primary transcript that is rapidly degraded to the more stable 1.6-kb transcript by a 3′ exonuclease or a 5′ exonuclease aided by endonuclease activity. The potential large hairpin-loop structure at the 3′ end of the 1.6-kb transcript (16) may serve as a transcription termination signal, a recognition site for RNA processing, or a structure that prevents further 3′ exonuclease activity (see reference 28).

The *cpeBA* genes, which encode the α and β subunits of PE, and the *cpeCDE* genes, which encode the linker polypeptides associated with the PE hexamers, have also been characterized for *F. diplosiphon*. The former gene set is transcribed as a 1.5-kb mRNA (29, 38). The *cpeCDE* operon is not contiguous to *cpeBA* (in contrast to the PC_i linker polypeptide genes *cpcH2I2D2*, which are immediately downstream of *cpcB2A2*). The linker proteins encoded by *cpeCDE* are $L_R^{31.8}$, $L_R^{27.9}$, and $L_R^{27.6}$. Transcripts from *cpeCDE* are 2.1 and 3.2 kb long. The former transcript covers *cpeCD* (19), while the latter covers *cpeCDE* (20). As expected, these transcripts accumulate in coordination with *cpeBA* mRNA under GL (19).

Photobiology. Examination of the photobiology of chromatic adaptation has provided us with some clues about the nature of the photoreceptor and the signal transduction pathway involved in the process (42–44). (i) A pulse of inductive light triggers transcription from both *cpcB2A2* and *cpeBA*. For both gene sets, the transcript population immediately increases after the light pulse and reaches a maximum by approximately 2 h at 25°C. (ii) The fluences required for altered transcription from the two gene sets are different. The response for both the increase and decrease in transcription from *cpcB2A2* is saturated at a fluence of 3×10^3 μmol m^{-2}. The response for altering transcription from *cpeBA* is saturated at a fluence of 6×10^3 μmol m^{-2}. These data suggest that two distinct photoreceptors may regulate expression from the two different phycobiliprotein gene sets or that a complex signal transduction chain links photoperception to transcriptional control. (iii) When cells are transferred from inductive to noninductive light, there is a difference in the kinetics of change in the rate of transcription from *cpcB2A2* and *cpeBA*. Transcription from *cpcB2A2* declines rapidly upon transfer to noninductive light and is barely detectable after 2 h, while transcription from *cpeBA* is still high even 10 h after the shift to noninductive light and appears to decrease in concert with cell division. This phenomenon is evidence that a factor involved in positive regulation of the gene set is becoming progressively more dilute during cell growth. The rapid decline in transcriptional activity of *cpcB2A2* upon shifting from inductive to noninductive light suggests that this gene set is controlled by either a positive regulatory element that is unstable under noninductive light conditions or a negative regulatory element that is either synthesized de novo or activated under noninductive light conditions.

Despite extensive characterizations at the level of phycobiliprotein gene expression, there is still little information concerning the photosensory and signal transfer components that govern chromatic adaptation. The photobiological data

suggest that the state of the photoreceptor is linked to the activity of gene-specific transcriptional regulators. Since some cyanobacteria are amenable to standard prokaryotic genetic manipulation, they can be used as a relatively simple model to investigate the molecular mechanisms of the photoresponses. Especially beneficial for this analysis are the isolation and characterization of mutants that display aberrant chromatic adaptation.

Regulatory mutants. Many *F. diplosiphon* mutants that exhibit aberrations in chromatic adaptation have been isolated (6, 12, 51). These mutants can be visually identified and may arise spontaneously, although UV light, chemical mutagens, or electric shock significantly increases their frequencies. Cobley and Miranda (12) isolated three mutant classes after UV irradiation of cells. In green mutants, the synthesis of PE did not occur under either GL or RL, while PC synthesis was normal. In blue mutants, both photoinduction of PE and photorepression of PC synthesis were impaired. In a black mutant, PE was partially induced and PC was partially repressed under RL. We have also isolated numerous mutants in chromatic adaptation and have focused on characterizing those that were likely to be in the regulatory machinery. The mutants were generated by electric shocks, which probably stimulate the activities of mobile genetic elements in the *F. diplosiphon* genome. Three mutant classes were generated (red, blue, and green) and characterized (6). Red mutants (designated FdR) could not acclimate to RL. These strains have high levels of PE, normal levels of PC_c, and no detectable PC_i under conditions of either RL or GL. Blue mutants (designated FdB) exhibit normal regulation of *cpeBA* and elevated transcription from *cpcB2A2* under both RL and GL. Green mutants (designated FdG) do not transcribe *cpeBA* but exhibit normal regulation of *cpcB2A2*. In all of these mutants, the levels of the individual phycobiliproteins are reflected in the levels of mRNA encoding these proteins. Information obtained from the characterizations of these mutants has established the following two conclusions. (i) Single lesions can alter the regulated transcription from both *cpcB2A2* and *cpeBA* (FdR strains). This result indicates that there are common elements that directly or indirectly govern transcription of *cpcB2A2* and *cpeBA*. (ii) There are elements that specifically control expression of GL- or RL-activated genes (FdG and FdB strains).

Approaches for isolating regulatory elements. Recently, at least one of the mutant strains has been complemented in *trans* with a library of wild-type *F. diplosiphon* DNA. We have developed a gene transfer system, initially established by John Cobley (13), for the introduction of DNA into *F. diplosiphon*. This system makes use of a shuttle vector that can replicate both in *Escherichia coli* and *F. diplosiphon* (11). For complementation experiments, random *Sau*3A fragments of the wild-type genomic DNA were ligated into the shuttle vector and the recombinant molecules were introduced into *F. diplosiphon* by electroporation or conjugation. Dramatic pigmentation differences between the wild-type and PBS regulatory mutant strains provide a clear visual screen for complementation. For example, after introduction of the genomic DNA into an FdR strain and growth of transformed cells under RL, only the cells in which the lesion in the FdR mutant had been complemented would appear blue-green. These cells would be readily identified in a background of red colonies containing cells that were transformed and therefore resistant to kanamycin, but not complemented. Similarly, complemented FdG mutants should be identifiable as red colonies among noncomplemented green cells when grown under GL.

A gene involved in regulation of chromatic adaptation was isolated after complementation of a member of the FdR mutant class (10, 11). This gene, designated *rcaC*, encodes a protein with strong sequence identity to the *Bacillus subtilis* PhoP protein (47), a member of the superclass of response regulator proteins associated with bacterial two-component regulatory systems (for reviews, see references 1 and 50). Most response regulators have an N-terminal domain with a conserved aspartate residue that becomes phosphorylated. RcaC is unusual in that it has this regulatory domain at both the N and C termini. Southern blot analysis of genomic DNA isolated from two red mutants revealed gross, nonidentical structural aberrations at the *rcaC* locus, suggesting that the initial lesions were due to independent events involving mobile genetic elements and that electroporation activated the movement of these elements. Recently, *F. diplosiphon* has been shown to harbor at least two different types of mobile genetic elements (37).

Further insight into the signal transduction mechanism governing chromatic adaptation can be achieved by studying the nature and activity of the regulatory proteins that directly interact with the region of the *cpeBA* and *cpcB2A2* genes upstream of the transcription initiation site. Gel retardation studies using DNA fragments from the vicinity of the transcription initiation site for *cpeBA* demonstrated the binding of specific proteins, present only in extracts from GL-grown cells (19a). No binding to the same region was observed by proteins extracted from RL-grown cells. These data suggest that a *trans*-acting element that binds to and may facilitate transcription from *cpeBA* is present or active only in cells cultured under GL. Additional evidence for regulatory factors that interact with regions that precede the transcription initiation sites of *cpcB2A2* and *cpeBA* comes from recent studies by J. Houmard (31a) and E. Casey and A. R. Grossman (9a). J. Houmard found that proteins from extracts of GL-grown cells were bound to DNA upstream of the transcription initiation site of *cpeBA* at two different locations. These proteins have been named RcaA and RcaB. One of the proteins loses its binding activity when it is treated with alkaline phosphatase (31a). Hence, multiple *trans*-acting elements may interact with DNA sequences upstream of the transcription initiation site of *cpeBA* and alter transcription from that gene set. These regulatory proteins may be controlled by phosphorylation events triggered by changes in the light environment. Proteins have also been shown to bind DNA sequences associated with *cpcB2A2* (9a, 31b). We find one strong binding site that is 30 nucleotides long and positioned approximately 120 to 150 nucleotides upstream of the *cpcB2A2* transcription start site. This region binds proteins from cells grown under GL and RL. In vivo analysis of chimeric genes in which regulated promoters are fused to reporter genes (GUS and luciferase) will help establish the significance of both *cis*-acting DNA sequences and *trans*-acting proteins in the regulated expression of phycobiliprotein genes.

The information gleaned from the study of the expression of the phycobiliprotein genes, the photobiological experiments, and the characterization of the regulatory mutants has allowed us to develop a model describing regulation during chromatic adaptation. In this model, a phosphorylation cascade is proposed to regulate the activity of an activator of the *cpeBA* gene set and a repressor of the *cpcB2A2* gene set. The phosphorylated activator and repressor promote transcription of *cpeBA* and repress transcription

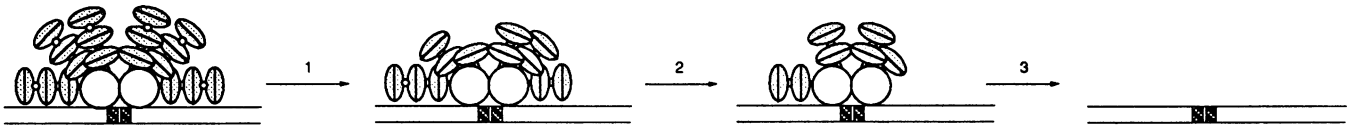


FIG. 2. Breakdown of PBS structures of *Synechococcus* sp. strain PCC 7942 during nitrogen or sulfur deprivation. The PBS is shown attached to the thylakoid membranes and associated with a photosynthetic reaction center. The time at which each of the events depicted in the figure occurs varies, depending on the culture conditions and the specific nutrient that is removed from the growth medium. Generally, nitrogen deprivation is most severe and a loss of the terminal hexamer of the rod substructure is observed within 12 h (step 1). By 24 h, there is increased degradation of the rod substructure and some loss of complete rods (step 2). After 36 h, the entire rod and core substructures have been degraded (step 3).

of *cpcB2A2*, respectively. Under GL, this cascade is maximally active. RL changes the conformation of the photoreceptor, which in turn inhibits the phosphorylation cascade. Under these latter conditions, the *cpeBA* gene set is no longer transcriptionally active and the *cpcB2A2* gene set is relieved from repression. Details of the model will be discussed elsewhere (30).

PBS DEGRADATION AND THE RESPONSE OF CYANOBACTERIA TO NUTRIENT LIMITATION

The suite of responses. Cyanobacteria exhibit a suite of responses during nutrient-limited growth. Some of these are specific responses triggered by the depletion of a single nutrient. Others are general responses and are manifested in medium lacking any of a number of different nutrients (2, 3, 14, 49). The former category includes the increased synthesis of specific transport systems (e.g., sulfate transport during sulfur-limited growth [27, 32]) and periplasmic enzymes that may help generate substrates that can be readily transported into the cell (e.g., periplasmic alkaline phosphatase during phosphate-limited growth [4]). The latter category includes changes in both cellular morphology and physiological processes.

Dramatic alterations in the ultrastructure of cyanobacterial cells grown under adverse nutrient conditions have been observed with the electron microscope. Iron-, nitrogen-, and sulfur-deficient cells contain less than half of the normal complement of thylakoid membranes (48, 55). The remaining membranes are disorganized and interspersed with large deposits of glycogen. A dramatic response of cyanobacteria to nutrient limitation is a decrease in the abundance of pigment molecules in the cell. Cultures deprived of nitrogen for 30 h exhibit no detectable PC (3). Sulfur-deprived cultures show a similar rapid decline in the absolute levels of PC and AP (14, 55).

During sulfur and nitrogen starvation, there is a rapid and near complete degradation of the PBS. PBS degradation could provide nitrogen-limited cells with amino acids used for the synthesis of proteins important for the acclimation process. It is more difficult to understand why PBS would be degraded in cells limited for other macronutrients, such as sulfur, since phycobiliproteins are a poor source of sulfur amino acids. However, the PBS is degraded in the same ordered manner in both sulfur- and nitrogen-starved cells (14, 58). In contrast, cells deprived of phosphorus show only very limited degradation of the PBS (14), as might be expected since the PBS contains no phosphorus. The diminished PBS levels that are observed in phosphorus-limited cells are the consequence of decreased PBS biosynthesis relative to the rate of cell division.

PBS degradation. The degradation of the PBS during nitrogen and sulfur starvation is an ordered process. In cultures of *Synechococcus* sp. strains PCC 6301 and PCC 7942, the elimination of nitrogen from the growth medium initially provokes the rapid degradation of the terminal hexamer of the PBS rods and its associated 30-kDa linker polypeptide (14, 58). This degradation is followed by degradation of the next PC hexamer and its associated 33-kDa linker polypeptide and a decrease in the number of rods associated with the PBS core. The loss of these components results in a decrease in the PBS sedimentation coefficient and a reduction in the ratio of PC to AP. The smaller PBS can still harvest light energy. After the loss of the rods, the entire complex is destroyed. A diagram that depicts the different stages in PBS degradation is shown in Fig. 2. Upon adding the limiting nutrient back to a deprived culture, new PBS complexes are rapidly synthesized.

The levels of mRNAs encoding the phycobiliproteins have also been reported to decline during nutrient-limited growth. de Lorimier et al. (17) reported that the level of PC mRNA decreased to nearly zero in nitrogen-starved *Synechococcus* sp. strain PCC 7002. Further analyses have shown that 3 to 5 h after the initiation of nitrogen deprivation, the transcripts from the *cpcBA* operon were essentially undetectable (7). Data obtained by Gasparich et al. (23) using a *cpcB-lacZ* fusion suggest that nitrogen deprivation results in a marked decrease in the transcription of *cpcBA*. In *Synechococcus* sp. strain PCC 7942, the mRNAs encoding both PC and AP declined rapidly during nitrogen or sulfur limitation and less rapidly during phosphorus limitation. However, levels of all of these mRNA species remained at 5 to 10% that of nutrient-replete cells (14), even 48 h after the cells were transferred to medium lacking nitrogen or sulfur. This change in the steady-state levels of phycobiliprotein mRNAs may be a consequence of both altered rates of transcription and mRNA turnover. While the PC and AP mRNA levels did not fall below 5% of the levels measured in nutrient-replete cells, the translation of phycobiliproteins could not be detected (3a). This result suggests that the production of phycobiliproteins during nutrient-limited growth is blocked by both transcriptional and posttranscriptional events.

The analysis of PBS synthesis and degradation under conditions in which the nutrient status of the medium is modulated offers the opportunity to examine the processes important for the biogenesis of this abundant macromolecular complex. In early biochemical studies of PBS degradation in *Anabaena* sp., it was suggested that a specific protease increased in nitrogen-limited cells and that this increase was responsible for the degradation of the complex (21, 56, 57). To address this question by using a different approach, we mutated *Synechococcus* sp. strain PCC 7942 and screened for organisms that did not bleach when deprived of sulfur (14a). These nonbleaching mutants did not degrade their PBS structures during either sulfur or nitrogen starvation, although no new PBS complexes were synthe-

sized during the stress treatment. The gene responsible for complementing the nonbleaching phenotype was named *nblA*. This gene contains an open reading frame of 59 amino acids. A small transcript covering this gene accumulates at high levels only in cells starved for either nitrogen or sulfur. Insertional inactivation of *nblA* results in a nonbleaching phenotype, confirming the role of *nblA* in the nutrient stress response. The strain initially isolated as a nonbleaching mutant contained a single-base change in *nblA* that changed a serine codon into a phenylalanine codon. When the *nblA* gene is placed on a multicopy plasmid and transformed into *Synechococcus* sp. strain PCC 7942, the cells bleach to some extent in nutrient-replete medium and break down the PBS much more quickly than wild-type cells during nutrient-limited growth. The transformed cells also degrade the PBS structures during phosphate stress if *nblA* is placed under the control of the derepressible alkaline phosphatase promoter. Hence, any conditions that favor increased expression of *nblA* trigger the degradative process. No similarity was observed between the amino acid sequence encoded by *nblA* and any sequence in the GenBank data base. A number of interesting ideas can be gleaned from these studies. First, it is likely that the bleaching response provoked by the NblA protein is a direct consequence of elevated levels of this protein in the cell. Second, the small size of this protein and the lack of similarity to any known protease suggest that it is not a protease itself. NblA may function to activate a protease, such as the one studied by Wood and Haselkorn (57), that subsequently degrades the PBS. Alternatively, the small protein may trigger PBS degradation by interacting with the constituents of the complex and altering their susceptibility to proteolysis. Such interactions may involve covalent attachment, similar to the binding of ubiquitin to proteins in eukaryotes, which marks the PBS for degradation, or disruption of hydrophobic and/or ionic interactions among various constituents of the PBS, rendering them protease susceptible. Finally, the *nblA* gene product may be involved in activating or derepressing other genes that are directly responsible for causing PBS degradation. These findings may be of general importance in understanding the degradation of macromolecular complexes in prokaryotic organisms.

CONCLUDING REMARKS

The structure and number of PBS in cyanobacteria can be exquisitely sensitive to the environmental milieu in which the organism is grown. The response of certain cyanobacteria to different light conditions may provide us with a broader understanding of photoperception in photosynthetic microbes and may also yield information on proteins ancestral to phytochrome, the ubiquitous photoreceptor of higher plants. Studies concerning environmentally regulated degradation of the PBS will yield insights into processes involved in the targeting of macromolecular complexes for degradation and the machinery that implements this degradation. There is still a relative dearth of information available on factors that control PBS biosynthesis, and this field remains a fertile area for future investigation.

ACKNOWLEDGMENTS

We are grateful to Jean Houmard for sharing unpublished data and to Kirk Apt, Elena Casey, and John Davies for helpful discussions. Jane Edwards was invaluable in typing and formatting the manuscript, and Glenn Ford was always available to aid us with computer-assisted analyses of the PBS polypeptides and genes.

We are also very grateful to the National Institutes of Health, National Science Foundation, and The Carnegie Institution of Washington, agencies that have supported our work for the last 10 years.

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