MICROBIOLOGICAL REVIEWS, Sept. 1993, p. 725–749
0146-0749/93/030725-25$02.00/0
Copyright © 1993, American Society for Microbiology
Vol. 57, No. 3

The Phycobilisome, a Light-Harvesting Complex Responsive to Environmental Conditions†

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 725
PBS STRUCTURE 725
Chromophore Association 726
Arrangement of Phycobiliproteins within the Light-Harvesting Complex 727
PBS Model 727
Characterization of PBS Polypeptides 728
X-Ray Crystal Structure 729
New Structure for Some PBS 729
ASSEMBLY OF PBS 730
CHROMOPHORE SYNTHESIS 730
GENES ENCODING PBS POLYPEPTIDES: ARRANGEMENT AND TRANSCRIPTION 730
Eukaryotic Red Algae 731
Cyanobacteria 731
CHROMATIC ADAPTATION 733
Historical Perspective 733
Gene Expression 734
Photobiology 736
Regulatory Mutants 736
Approaches for Isolating Regulatory Elements 737
Model Describing Regulation 738
DEGRADATION OF PBS AND RESPONSE OF CYANOBACTERIA TO NUTRIENT LIMITATION 740
Pigment Changes and Changes in Photosynthesis 740
PBS Degradation 740
CONCLUDING REMARKS 743
ACKNOWLEDGMENTS 743
REFERENCES 743

INTRODUCTORY REMARKS

Diverse pigments are used by photosynthetic organisms to efficiently harvest the spectrum of light energy that drives photosynthesis. Considerable progress has been made in the characterization of three of these light-harvesting complexes (also called antenna complexes). First, in terrestrial plants light harvesting is carried out by a macromolecular complex in which the major pigments are chlorophylls *a* and *b* and the polypeptide constituents of the complex are a group of related proteins called the chlorophyll *a*/*b*-binding (Cab) proteins (41, 102, 156). The Cab polypeptides are integral membrane proteins and are probably arranged in trimeric arrays (124). Genes encoding Cab polypeptides constitute a nuclear, multigene family (41, 102) that is regulated by light (208, 209). Second, in marine algae such as diatoms, dinoflagellates, brown algae, and chrysophytes, the major light-harvesting complex contains xanthophylls, such as fucoxanthin or peridinin, and chlorophylls *a* and *c* (64, 71, 125, 171). Like the Cab polypeptides, the constituents of the fucoxanthin-chlorophyll *a*/*c* complex are a family of low-molecular-weight hydrophobic proteins. They exhibit some homology to the Cab polypeptides (108), which may relate to the chlorophyll-binding function of both protein families. Third, in the prokaryotic cyanobacteria and eukaryotic red algae, light harvesting is carried out primarily by a group of pigmented proteins, called phycobiliproteins, that become constituents of a macromolecular complex called the phycobilisome (PBS) (30, 76, 77, 93–95, 100, 230). The constituents of this third type of light-harvesting complex are structurally very different from the polypeptides of the other antenna complexes. The major classes of phycobiliproteins present in the PBS are phycoerythrin (PE), phycocyanin (PC), and allophycocyanin (AP). These are brilliantly colored molecules (PE is red, PC is blue, and AP is bluish green) that are soluble in aqueous media. They are produced in massive amounts in a number of photosynthetic organisms and may constitute 50% of the soluble protein of the cell. In this review we discuss the processes involved in PBS biosynthesis and the way in which environmental factors modulate the levels of the different PBS components.

PBS STRUCTURE

In the eukaryotic red algae and the prokaryotic cyanobacteria, the outer surface of the thylakoid or photosynthetic membranes is covered by rows of closely spaced granular structures. These structures were first postulated to represent an ordered array of the pigmented phycobiliproteins

* Corresponding author.
† Carnegie Institution of Washington publication 1155.

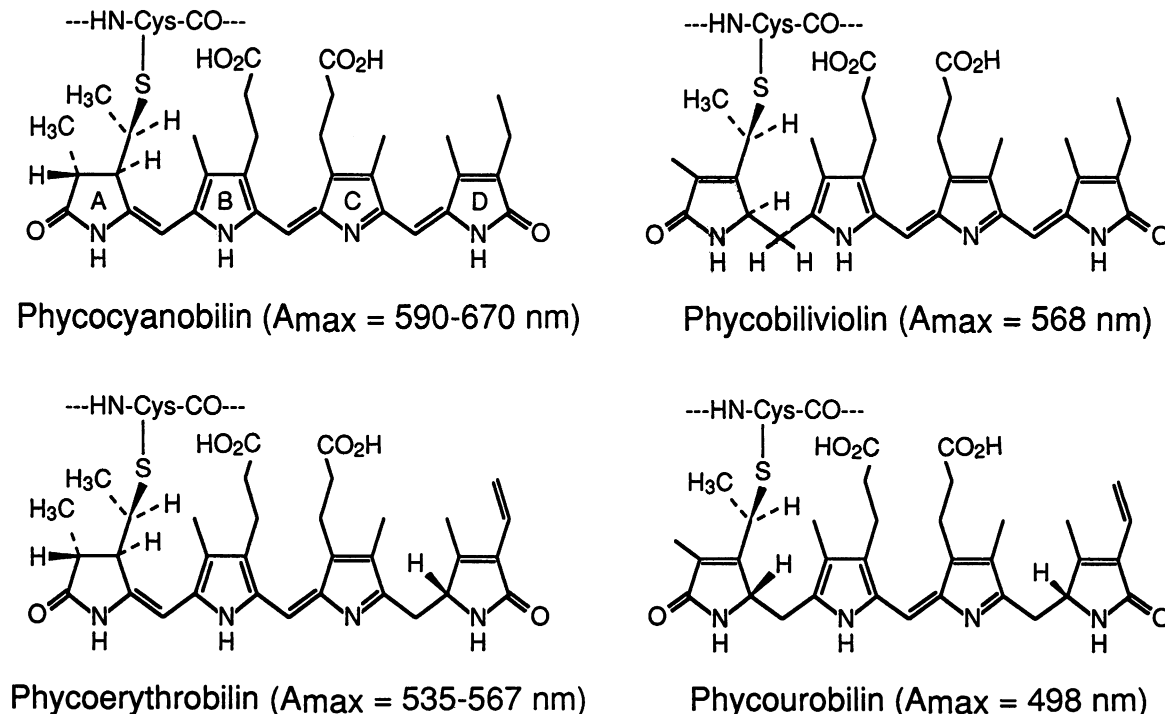

FIG. 1. Phycobilins and their peptide linkages in phycobiliproteins. The absorption maxima (A_{max}) for each of the bilins, presented under the structures, can vary considerably depending on its protein environment. The linkages presented here are between ring A and a cysteine residue of the phycobiliprotein, but phycoerythrobilin and phycourobilin chromophores can be linked to the apoprotein via both rings A and D (96).

that associate with and harvest light energy for the photosynthetic reaction centers embedded in the thylakoid membranes (79). In a series of studies Gantt and Conti (78–80) demonstrated that these granular structures were indeed composed of phycobiliproteins, and they named them phycobilisomes (PBS). PBS were first purified from the red alga *Porphyridium cruentum*. They were removed from the photosynthetic membranes with 0.5 M Triton X-100, fixed with glutaraldehyde, and separated from the rest of the solubilized cellular components by sucrose gradient centrifugation (79). A more general protocol for the isolation of PBS from both red algae and cyanobacteria was developed, which did not involve cross-linking the proteins of the complex (81). In this method, Triton X-100 was used for dissociating PBS from thylakoid membranes, high-molarity phosphate buffer was used to maintain the stability of the intact PBS, and sucrose gradient centrifugation was used to separate it from other cellular components. It was necessary to perform the isolations at room temperature since the PBS dissociated when exposed to the cold. Although additional isolation methods have been developed over the years, most are modifications of the procedure established by Gantt and coworkers (for a review of PBS isolation, see reference 97).

Chromophore Association

Numerous studies have been performed to determine the characteristics of the polypeptide components of the PBS and the nature of the chromophore molecules bonded to the pigmented phycobiliproteins. As a group, phycobiliproteins absorb visible light in the range of 450 to 660 nm. PE (A_{max} ≈ 560 nm), PC (A_{max} ≈ 620 nm), and AP (A_{max} ≈ 650 nm) are the prominent phycobiliproteins present in cyanobacteria and red algae. Phycoerythrocyanin (PEC; A_{max} ≈ 567) can replace PE in the PBS in some organisms, such as in the cyanobacterium *Mastigocladus laminosus* (229). Each of the phycobiliproteins is composed of two different subunits, termed α and β. Generally, the α subunit has a molecular mass of between 15 and 20 kDa and the β subunit has a molecular mass of between 17 and 22 kDa. The designations for the different subunits are given by the subunit type (α or β) and a superscript that indicates the class of phycobiliprotein (AP, PC, or PE). The chromophores attached to the phycobiliproteins are phycocyanobilin, phycoerythrobilin, phycouroblin, and phycobiliviolin (Fig. 1). The chromophores are linear tetrapyrroles that are bonded to the apoprotein via thioether linkages. Within the phycobiliproteins, the tetrapyrrole chromophores are maintained in an extended conformation. These chromophores have very similar characteristics to the chromophore associated with the photoreceptor phytochrome of higher plants. The numbers and types of chromophores that are associated with a particular type of phycobiliprotein subunit are usually invariant (but not always [see below]). In Fig. 1 the different tetrapyrrole chromophores are shown bonded to cysteine residues of the phycobiliprotein subunits via ring A. Some of the chromophores can be bonded to the apoprotein through both ring A and ring D (96).

The bilin chromophores associated with phycobiliproteins are isomers of each other, but their absorbance maxima differ. When linked to the phycobiliproteins the maximal absorbances of phycourobilin, phycobiliviolin, phycoerythrobilin, and phycocyanobilin are approximately 498, 568, 535 to 567, and 590 to 670 nm, respectively. The exact absorption maximum of each of the bilin chromophores is strongly influenced by its conformation and interactions with amino

acid residues of the protein moiety of the molecule, which in turn may be considerably affected by the aggregation state of the biliprotein subunits. The α and β subunits of both PC and AP are bonded to phycocyanobilin. Some exceptions to this generality have been noted in marine cyanobacteria, in which the α^{PC} may be associated with a phycourobilin or phycoerythrobilin chromophore (201). The α subunit of PC and the α and β subunits of AP are bonded to one chromophore, whereas the β subunit of PC is attached to two chromophores. In many cyanobacteria the chromophore phycoerythrobilin is bonded exclusively to α^{PE} and β^{PE}: two phycoerythrobilin molecules are attached to the α subunit, and three are attached to β. In a few marine cyanobacteria the PBS contains two different types of PE, and some attachment sites on the PE subunits may be occupied by phycourobilin chromophores rather than phycoerythrobilin. Furthermore, the α^{PE} in these organisms may be linked to three chromophores rather than two (167). In red algae, just as in marine cyanobacteria, PE subunits may contain both phycoerythrobilin and phycourobilin chromophores. Since the maximum transmission of light in seawater is near 500 nm and the phycourobilin chromophore has its peak absorbance at 498 nm, the replacement of phycoerythrobilin by phycourobilin in α^{PE}, which causes a blue shift in the absorbance maximum for the PBS, may increase the efficiency with which the light is harvested in a marine environment.

Arrangement of Phycobiliproteins within the Light-Harvesting Complex

Spectroscopic, biochemical, immunological, and X-ray crystallographic techniques have been used to establish the pathway for energy flow and the arrangement of the different phycobiliproteins within a PBS. Initial spectroscopic experiments on the function and organization of phycobiliproteins were performed in vivo. In intact red algal and cyanobacterial cells, light absorbed by PE is efficiently transferred to chlorophyll in the photosynthetic reaction centers (56). French and Young (70) established PC as an intermediate in the transmission of this excitation energy.

In vitro studies on the arrangement of the phycobiliproteins within the PBS were initiated following the successful isolation of the PBS. Using fluorescence emission spectroscopy, Gantt et al. demonstrated that the energy absorbed by PE in isolated PBS could be transferred to AP via PC and that most of the energy absorbed was emitted at 670 to 680 nm at room temperature and 678 to 685 nm at −196°C (82, 84). On dissociation of the PBS, fluorescence emission shifted to lower wavelengths; the 670 to 680-nm peak was lost, and increased fluorescence emission was observed from PE (575 nm), PC (640 to 650 nm), and AP (660 nm) (84). This hypochromatic shift is the consequence of uncoupling the energy transfer pathway established by the placement of the different phycobiliproteins within the PBS. These results, coupled with energetic considerations, strongly suggested the disposition of the different phycobiliproteins within the PBS and the pathway of energy flow given below.

PBS (PE, PC, AP)

PE → PC → AP → chlorophyll of the photosynthetic membranes

This arrangement also was supported by immunochemical studies. The intact, isolated PBS reacted with both PE and AP antibodies (82) but did not react with antibodies raised to PC. As the PBS was gradually dissociated by exposure to low salt concentrations, PC became accessible to antibodies (83, 85). Furthermore, when the PBS was attached to membrane vesicles, the AP antibodies did not react with the complex (85). Hence, AP was likely to be in direct contact with thylakoid membranes, PE was most peripheral to the complex, and PC served to link PE to AP. This order of the different phycobiliproteins within the PBS has been independently determined from studies of mutants that do not synthesize complete PBS (220, 226) and the characterization of PBS in organisms that display chromatic adaptation (36, 220) (and see below).

Work defining the building blocks of the PBS has helped us understand its architecture. Studies on phycobiliprotein aggregation states (17, 18, 142, 157, 189) suggested that the PBS were composed of trimeric $(\alpha\beta)_3$ (referred to as a disc in many texts) and hexameric $(\alpha\beta)_6$ (double disc) phycobiliprotein assemblages (Fig. 2). These assemblages can be reconstituted in vitro from isolated phycobiliprotein subunits. Purified PC subunits could form a mixture of monomers $(\alpha\beta)$, trimers, and hexamers in vitro. The hexamers and trimers were interconvertible, with the equilibrium between the forms being pH dependent (16, 99, 158).

In addition to the pigmented phycobiliproteins, the PBS contains nonpigmented polypeptides that have been named linker polypeptides (205). Linker polypeptides constitute approximately 15% of the total protein of the PBS and are strongly associated with phycobiliprotein hexamers. They may aid in assembly processes, stabilize interactions between phycobiliprotein hexamers, and modulate the absorption characteristics of the hexamers (95, 97). The absorbance modulation that is caused by the interaction of phycobiliproteins with linker polypeptides promotes the unidirectional, highly efficient transfer of energy both within the PBS and from the PBS to the chlorophylls of the thylakoid membranes; energy absorbed by the PBS may be transferred to the photosynthetic reaction centers with better than 95% efficiency (174, 190).

The structure of the PBS was further characterized by examination of images obtained from electron microscopy. As viewed laterally, the PBS of both red algae and cyanobacteria have a fanlike appearance, as depicted in Fig. 2. For the red alga *Rhodella violacea*, the PBS appeared to be composed of two structural domains. One domain contained three ring-shaped structures (which in three dimensions resemble cylinders) that are arranged in a triangle and make up the "PBS core." This domain is composed predominantly of AP. In some cyanobacteria, such as *Synechococcus* sp. strain PCC 6301, the core is composed of two cylinders (Fig. 2). The second domain is composed of six rodlike structures that radiate from that core (154). Both the core and the rods of the PBS are composed of stacked phycobiliprotein hexamers. The hexamers of the rods are either exclusively PC or both PC and PE (123), depending on the organism being studied.

PBS Model

A synthesis of the information generated from electron microscopy and biochemical and biophysical studies led to the development of a detailed model for PBS structure (36, 76, 77, 79, 80, 85, 93, 94, 97, 136–141). The polypeptide constituents of the PBS rods and core of *Synechococcus* sp. strain PCC 6301 (and the closely related organism *Synechococcus* sp. strain PCC 7942, a widely used experimental organism) are shown in Fig. 2. *Synechococcus* sp. strain

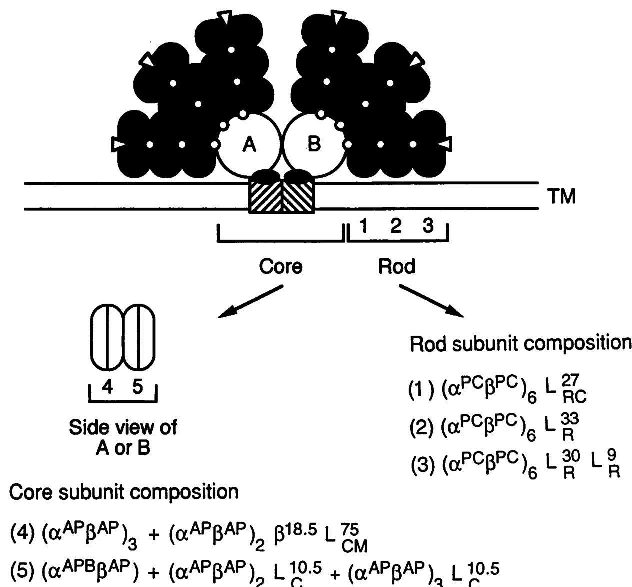

FIG. 2. Structure of the simple PBS from the cyanobacterium *Synechococcus* sp. strain PCC 6301 (and *Synechococcus* sp. strain PCC 7942). The two substructures of the PBS are the dicylindrical core and the six peripheral rods that radiate from the core. A side view of one of the two (A and B) cylinders of the core, each composed of two hexamers, is shown, along with details of the composition of the hexamers (hexamers 4 and 5). The high-molecular-weight polypeptide that may anchor the PBS to the thylakoid membranes (TM) is represented as two solid ovals in association with both the core and the thylakoid membranes (denoted L^{75}_{CM} in the core subunit composition). The cross-hatched rectangle within the thylakoid membranes represents the reaction center and associated chlorophylls. The composition of the three hexamers of the rods (hexamers 1, 2, and 3) is also presented, with hexamer 1 being most proximal to the core and hexamer 3 being most distal. Each of the three PC hexamers in a given rod is associated with a specific linker polypeptide (the linker polypeptides are designated L). The rod linker polypeptides, depicted as open circles, are mostly buried within the phycobiliprotein hexamers. Linker polypeptides are also associated with the core of the PBS. Subscripts to L, which denote the position of the linker in the PBS are C for core, CM for between the core and the thylakoid membranes, RC for between the rods and core, and R for in the rods. The superscript to L gives the molecular mass of the specific linker polypeptide. The 27-kDa polypeptide L_{RC} is involved in the attachment of the core-proximal hexamer of the rod to the PBS core. The second hexamer of the rods is held to the core-proximal PC hexamer by the 33-kDa L_R polypeptide. The terminal hexamer is linked to the rods via the 30-kDa L_R polypeptide. A small linker polypeptide of approximately 9.0 kDa may terminate the rod substructure (represented as an open triangle associated with the hexamers most distal to the core). In addition to containing AP subunits, the PBS core contains AP-like proteins that function as terminal energy acceptors for the PBS. These are α^{APB} (not shown), $\beta^{18.5}$ (not shown), and the 75-kDa polypeptide, L_{CM}, which has both phycobiliprotein and linker polypeptide characteristics. There is also a small (≈10.5-kDa) linker polypeptide, L_C, that is present in the core (not shown).

PCC 6301 has a relatively simple PBS composed of the phycobiliproteins PC and AP; the PBS of this organism does not contain PE. The fan-like complex contains a dicylindrical core and a set of six cylindrical rods radiating from that core. This PBS is hemidiscoidal, as in many cyanobacteria, although PBS may also be hemielliptical, as in many red algae (77), or resemble bundles of rod-shaped elements (110). The PBS of *Synechococcus* sp. strain PCC 6301 is relatively small, with a molecular mass of 7×10^6 Da (100). In general, PBS can have a mass of 7×10^6 to 15×10^6 Da (77, 94), a diameter of 32 to 70 nm, a height of 25 to 45 nm, and a thickness of 12 to 40 nm (depending on both the specific organism being examined and the conditions under which the organism is grown) (77). The phycobiliprotein hexamers are stacked to form the cylinders of the peripheral rod or core substructures. The linker polypeptides of the rods are mostly buried within the phycobiliprotein hexamers and reside in a channel at the center of the hexamer (the hexamer can be visualized as a doughnut). The core is in direct contact with thylakoid membranes and may serve to lock the PBS onto the membranes via large proteins (L_{CM} in Fig. 2 and Table 1) that have domains with homology to both phycobiliproteins and linker polypeptides. The most prominent component of the core is AP, which is sometimes assembled into two cylinders (as in the case of *Synechococcus* sp. strain PCC 6301 and *Synechococcus* sp. strain PCC 7942) but is most commonly assembled into three cylinders (see the structure of the *Fremyella diplosiphon* PBS in Fig. 4); each cylinder contains two hexameric assemblages.

Characterization of PBS Polypeptides

The phycobiliprotein subunits make up a group of related proteins. The complete or partial amino acid sequences of both PC and AP subunits from a number of different organisms have been reported (68, 69, 136, 194, 212–214, 221). These studies demonstrated that specific phycobiliproteins, such as PC, are very similar among different organisms. Furthermore, the subunits of all of the phycobiliproteins are related to each other and probably arose via duplications of a single ancestral gene (92, 194). Information concerning the similarities among the phycobiliprotein subunits has expanded considerably over the last 10 years with the characterization of numerous phycobiliprotein genes (see below).

Linker polypeptides also make up a group of related proteins. Those that are present in the rods usually have molecular masses of between 25 and 40 kDa. They are basic proteins that form ionic bonds with the acidic residues of the biliproteins that are exposed in the central channel of a hexamer. Specific linker proteins are associated with specific phycobiliprotein hexamers in the rod substructure. Characterizations of PBS dissociation and the reconstitution of phycobiliprotein-linker assemblages in vitro (140) have been valuable in establishing the locations of the linker polypeptides within the PBS. The positions of linker polypeptides in PBS also have been determined from the characterization of mutants that make smaller or aberrant PBS (88, 226) or by analyses of PBS from cells grown in medium that lacks a specific nutrient (225). In *Synechococcus* sp. strain PCC 6301 and *Synechococcus* sp. strain PCC 7942 there are four linker polypeptides associated with the rods (Fig. 2). A 27-kDa linker polypeptide serves in the attachment of PC hexamers to the PBS core (this linker polypeptide is designated L^{27}_{CM}). Extension of each rod occurs by the sequential addition of a 33-kDa linker–$(\alpha^{PC}\beta^{PC})_6$ and then a 30-kDa linker–$(\alpha^{PC}\beta^{PC})_6$. A small 9-kDa linker polypeptide is located at the most distal position of the rod substructure and may play some role in terminating the growth of the rods. It has been shown that the linker polypeptides share several conserved domains (65, 91).

A variety of protein types make up the PBS core. In addition to AP, the core contains polypeptides with linker characteristics (L_C [107, 116, 118]), phycobiliprotein characteristics (α^{APB}, $\beta^{18.5}$ [117]), or both linker and phycobilipro-

tein characteristics (L_{CM} [38, 115]). The α^{APB} and $\beta^{18.5}$ are specialized phycobiliproteins of the core that may serve as terminal energy acceptors in the PBS (30). L_C, a linker polypeptide of ≈10.5 kDa that is located in the core, may stabilize the core substructure. In a mutant of *Synechococcus* sp. strain PCC 7002 that lacked this polypeptide, the PBS was more susceptible to dissociation by heat and the mutant grew 25% more slowly than the wild type did (145). L_{CM}, also called the anchor protein (176), may play a role in the association of PBS with thylakoid membranes. Like other specialized biliproteins of the core, it is important in transferring energy from the PBS to the photosynthetic reaction centers. It is a large protein with a molecular mass between 75 and 120 kDa (38, 89), depending on the organism being studied. Conserved regions of the L_{CM} are homologous to both AP subunits and linker polypeptides (38, 115). A phycocyanobilin chromophore is bonded to the AP-like domain of L_{CM}. The environment around the chromophore in L_{CM} is somewhat different from that of the α and β subunits of AP, so that the peak absorbance of the chromophore is shifted to higher wavelengths in L_{CM} than in AP. The linker polypeptide-like domains of L_{CM} may stabilize the core of the PBS by binding to AP hexamers (38). Each PBS contains two L_{CM} polypeptides (97), and in many cases each L_{CM} has three repeats of the conserved linker-like domains. The PBS core contains six AP-containing hexamers, with each of the linker domains of L_{CM} binding a single hexamer. Most of the sequences separating the conserved linker domains are highly divergent, although the lengths of these spacer regions are similar among the different L_{CM} polypeptides that have been characterized. The nonconserved spacer regions may be positioned on the surface of the PBS and establish the proper orientation of the phycobiliprotein and linker-like sequences to allow for efficient PBS assembly, help stabilize the structure, and enable it to function efficiently in the transfer of energy to the chlorophyll complexes of the thylakoid membranes.

Depending on the organism, L_{CM} has between two and four linker-like domains. The number of linker-like domains is correlated with the number of cylinders in the core and the number of rods that associate with the core. When there are two linker-like domains in L_{CM} the PBS core is dicylindrical. When L_{CM} has four linker-like domains, the core is tricylindrical and is associated with as many as eight peripheral rods. Two of the linker-like domains probably interact with AP hexamers that become part of the peripheral rods (see below). In mutants lacking L_{CM}, the PBS was not able to assemble, although unassembled PC and AP still accumulated in the cells (29, 30).

X-Ray Crystal Structure

High-resolution X-ray crystallography coupled with knowledge of the amino acid sequence of the proteins (often via gene analysis) has provided a detailed picture of the arrangement and interactions of phycobiliproteins within the rods of the PBS. X-ray structures have been determined from trimers of PC and PEC isolated from *M. laminosus* (54, 181, 184), PC hexamers of *Synechococcus* sp. strain PCC 7002 (185), and PC hexamers from *F. diplosiphon* (55, 186), which is very similar to *Calothrix* sp. strain PCC 7601 (these two organisms are considered as a single organism in this review). These studies have revealed sites of potential protein-protein interactions between the phycobiliprotein assemblages. Phycobiliprotein trimers are composed of three identical αβ heterodimers (each heterodimer is referred to as a monomer, by convention) arranged around a threefold symmetry axis. The trimer is 110 Å (11 nm) wide and 30 Å thick with a central channel which is 35 Å in diameter. The hexamers are composed of two trimers associated in a face-to-face arrangement (the hexamer is 110 by 60 Å). Each of the phycobiliprotein subunits has eight α-helical domains separated by nonhelical loops. The phycobilin chromophores are covalently bound via thioether linkages to cysteinyl residues of the apoprotein and are maintained in an extended conformation. This conformation is stabilized by interaction of the propionic acid side chains on the tetrapyrroles with arginine residues in the protein moiety of the phycobiliprotein and perhaps also because of interactions of the pyrrole nitrogen atoms with aspartate residues (186). Since PE has considerable similarity to PC and forms analogous trimeric and hexameric structures, knowledge of the crystal structure of PC could be used to suggest the disposition of the PE subunits within hexamers and trimers.

The structural information obtained by solving the X-ray crystal patterns of phycobiliprotein hexamers, coupled with spectroscopic studies (186, 195), has suggested a pathway of energy migration within the peripheral rods of the PBS. For PC, the phycocyanobilin chromophores are attached to the cysteinyl residues at position 84 in the α subunit and positions 82 and 155 in the β subunit. The α-84 and β-155 chromophores are at the periphery of phycobiliprotein hexamers, whereas the phycocyanobilin attached to β-82 is positioned near the center of the hexamers. Excitation energy absorbed by the peripheral bilin chromophores is transferred to the centrally located chromophore at β-82. The excitation energy migrates down the rod via transmission from a PC β-82 chromophore of one hexamer to the analogous chromophore of the contiguous hexamer that is positioned more proximal to the core (98). The excitation energy captured by the core is then transferred to chlorophyll molecules associated with the reaction centers of the photosynthetic membranes.

New Structure for Some PBS

Although a PBS with a tricylindrical core and six peripheral rods radiating from the core has been observed in a number of organisms, there are variations on this structure. For example, as presented in Fig. 2, the PBS of some cyanobacteria have a dicylindrical core substructure. Recent results suggest that the PBS of some organisms have more than six peripheral rods radiating from a tricylindrical core. Evidence for a PBS with more than six rods comes from studies with *M. laminosus* and *Anabaena* sp. strain PCC 7120. In PBS from these organisms the phycobiliprotein hexamers at the core-proximal position of the rod substructure may be composed of AP 25% of the time (rather than PC) (119). The L_{CM}, which is 120 kDa, probably contains four linker domains; one of those domains may interact with the core-proximal AP hexamer present in the rods. Furthermore, unlike *Synechococcus* sp. strain PCC 7002, which has only a single L_{RC} protein that is encoded by the gene *cpcG*, both *Anabaena* sp. strain PCC 7120 and *M. laminosus* appear to have four *cpcG* genes. The L_{RC} polypeptide mediates the interaction of the AP of the core with the core-proximal PC hexamers of the rods. For *Anabaena* sp. strain PCC 7120, the four *cpcG* genes have been isolated and sequenced and at least three of the gene products have been found in the PBS. These four genes are part of a large operon, *cpcBACDEFG1G2G3G4*, which encodes several

PBS structural components as well as polypeptides involved in the chromophorylation of the α^{PC} subunit. For *M. laminosus*, three distinct *cpcG* genes have been sequenced and a fourth gene is probably present on the cyanobacterial genome. The PBS of both *M. laminosus* and *Anabaena* sp. strain PCC 7120 also appear different from those of other cyanobacteria such as *Synechocystis* sp. strain PCC 6701 in electron micrographs. Largely on the basis of these data, Glauser et al. (89) proposed a new model for the structure of the PBS of *M. laminosus* and *Anabaena* sp. strain PCC 7120 in which eight peripheral rods are postulated to radiate from the core. In this model the linker domains of the two L_{CM} proteins present in the PBS core bind both to the AP discs present in the core and to the two AP hexamers that are associated with the peripheral rods. One of the four L_{RC} proteins facilitates the interaction of these rod-associated AP hexamers with a PC hexamer. The other three L_{RC} polypeptides occupy specific pairs of positions on the PBS core and serve to bind the core-proximal PC hexamers of the six remaining peripheral rods. This newly characterized, more complicated PBS could provide a larger pigment bed and more effective capture of light energy.

ASSEMBLY OF PBS

Assembly of the peripheral rods appears to be the consequence of a series of specific interactions between phycobiliproteins and linker polypeptides. The α and β subunits of the phycobiliproteins combine to form $\alpha\beta$ monomers. These assemble into trimers, which in turn strongly interact with linker polypeptides. The resulting $(\alpha\beta)_3L_R$ complex serves as a template for the formation of hexamers. The positions of phycobiliprotein hexamers in peripheral rods is dictated by the linker polypeptide with which the hexamer associates.

The successful in vitro assembly of purified phycobiliproteins into trimers and hexamers has helped to spawn the idea that assembly of the specific components of the complex might occur spontaneously in vivo. The association of PC hexamers into rods was shown to require the linker polypeptides (93). Although little is known about the assembly of the core, a mutant in which the L_{CM} is no longer synthesized will not assemble a stable core (37), suggesting that L_{CM} plays a crucial role in core assembly and may even serve as a scaffold that facilitates the proper interactions among different core constituents.

The short doubling time of many cyanobacteria and the preponderance of PBS in the cell make it imperative that the cell be capable of rapid synthesis and maturation of PBS polypeptides, assembly of PBS substructures, and ultimately, biogenesis of a mature light-harvesting complex. Although some processes involved in the construction of a PBS may be spontaneous, it is unclear whether all aspects of PBS biosynthesis go unassisted. Some studies have demonstrated that linker polypeptides are required for building the peripheral rods from phycobiliprotein hexamers. It is still unknown whether proteins other than the linker polypeptides aid in the assembly process. The biogenesis of other macromolecular complexes is assisted by proteins that do not remain associated with the mature complex. These proteins, called molecular chaperones, help maintain newly synthesized polypeptides in an assembly-competent conformation and aid in oligomeric assembly reactions (111). For example, certain complexes of the chloroplast in higher plants, such as ribulose-1,5-bisphosphate carboxylase holoenzyme, require molecular chaperones for their biogenesis. In the case of ribulose-1,5-bisphosphate carboxylase, the large subunit associates with a macromolecular chaperone complex prior to assembly with the small subunit (113). Since the linker polypeptides are insoluble unless associated with phycobiliprotein trimers, it would not be surprising if chaperones suppressed the precipitation of linker polypeptides and held them in a conformation that would allow assembly with the appropriate phycobiliprotein trimers. However, it is also possible that a tight coordination of the synthesis of linker polypeptides and phycobiliproteins obviates the need for chaperone-assisted assembly.

CHROMOPHORE SYNTHESIS

The formation of tetrapyrroles such as heme, chlorophyll, and phycobilins is initiated with the synthesis of δ-aminolevulinic acid. Considerable progress has been made in our understanding of the biosynthesis of the phycocyanobilin chromophore from work with the unicellular red alga *Cyanidium caldarium*. In vivo studies demonstrated that both protoheme and biliverdin IXα could serve as precursors in the production of phycocyanobilin (10, 23–26, 188). In vitro studies with protein extracts from *C. caldarium* demonstrated the existence of activities that could convert heme to biliverdin IXα (10) and biliverdin IXα to phycocyanobilin (13). These in vitro conversions required NADPH, ferredoxin, ferredoxin-$NADP^+$ reductase, and either a heme oxygenase activity or a phycobilin formation enzyme (11, 48). Recently it was shown that these in vitro reactions can be driven by reduced ferredoxin and that the NADPH was not directly required to support the synthesis of phycocyanobilin from protoheme (178) but served to reduce ferredoxin. Furthermore, a number of steps involved in the conversion of biliverdin IXα to 3(*E*)-phycocyanobilin were inferred from the detection of the intermediate molecules 15,16-dihydrobiliverdin IXα, 3(*Z*)-phycoerythrobilin, and 3(*Z*)-phycocyanobilin (12). Since the conversion of heme to biliverdin IXα and that of biliverdin IXα to phycocyanobilin are sequential, ferredoxin-linked processes, Beale and Cornejo proposed that the heme oxygenase and biliverdin reductase activities were present in the same multiprotein complex (10).

Under most conditions, bilin chromophore and apophycobiliprotein synthesis are coordinated (211). Troxler et al. (215) provide direct evidence for the involvement of heme in the regulation of apophycobiliprotein synthesis. In *C. caldarium*, light is required for the accumulation of AP and PC, as well as for the accumulation of mRNA encoding these proteins. However, in the absence of light, exogenous heme can trigger the accumulation of both of these biliproteins and their mRNAs. This is a specific response since heme does not alter the expression of genes encoding other components of the photosynthetic apparatus. The exact role of heme in controlling the expression of the biliprotein genes is not known, although it may function as a cofactor in the binding of regulatory elements to *cis*-controlling DNA sequences. Considerable evidence in animal systems implicates free heme in the control of gene expression (1, 19, 57, 197, 227). Unraveling the mechanisms by which heme may control biliprotein gene expression is an exciting new area of investigation.

GENES ENCODING PBS POLYPEPTIDES: ARRANGEMENT AND TRANSCRIPTION

Over the last 10 years, essentially all of the genes encoding structural components of the PBS have been isolated and

characterized from a number of different organisms (5–7, 15, 30, 32–35, 37, 45–47, 50, 51, 53, 65, 89, 91, 104, 106, 107, 115–118, 121, 126–128, 131–134, 147, 149, 150, 172, 206, 207, 219). This work has added enormously to our knowledge of phycobiliprotein structure, our ideas about the evolution of the different genes encoding PBS components (both linker and phycobiliprotein genes), and our understanding of the ways in which the levels of the different phycobiliprotein components are modulated. Initially, PC and AP gene sets, *cpcBA* and *apcAB*, respectively, were cloned from the plastid genome of a eukaryotic alga, *Cyanophora paradoxa* (34, 131, 132). The *B* and *A* in the gene designation correspond to the β and α subunit genes, respectively. Genes encoding PC and AP subunits were also cloned from numerous cyanobacteria, such as *Synechococcus* sp. strain PCC 7002 (50, 172), *Synechococcus* sp. strain PCC 6301 (118, 133), *F. diplosiphon* (45, 46, 104, 116, 206, 207), and *Synechocystis* sp. strain PCC 6701 (6). The PE genes, *cpeBA*, were cloned and characterized from the cyanobacteria *Synechocystis* sp. strain PCC 6701 (7) and *F. diplosiphon* (107, 147) and the unicellular marine cyanobacteria *Synechococcus* sp. strains WH8020 (219) and WH8103 (51). In all cases examined, the α and β subunit genes of a given phycobiliprotein are contiguous and cotranscribed.

Eukaryotic Red Algae

In eukaryotic red algae, the phycobiliprotein subunits are translated within plastids (14, 58, 198) and genes encoding phycobiliprotein subunits are on the plastid genome (8, 101, 179, 193). In *Porphyra purpurea* there are two distinct PC gene sets (193), although it is not known whether they are differentially expressed under different environmental conditions. As on the cyanobacterial genomes, the genes for the α and β subunits of a given phycobiliprotein are linked and cotranscribed. However, the individual phycobiliprotein gene sets are not contiguous. The amino acid sequences of the phycobiliproteins from the different red algae, as deduced from the gene sequences, are highly homologous among themselves, as well as to those of cyanobacteria. The linker polypeptide genes *apcE* and *cpcG*, encoding L_{CM} and L_{RC}, respectively, are on the plastid genome of *Aglaothamnion neglectum*. The *apcE* gene is 5′ to and in the same operon as *apcAB*, an arrangement observed in a number of cyanobacteria (116, 118). An exception to this occurs in *Synechococcus* sp. strain PCC 7002, in which *apcE* is not adjacent to the *apcAB* operon (30). In *A. neglectum* the *cpcG* gene is 5′ to the operon encoding the PE subunits but is transcribed from the opposite DNA strand. Both proteins encoded by these genes are approximately 50% identical to the analogous polypeptides of cyanobacteria. Some cyanobacteria, such as *Anabaena* sp. strain PCC 7120, have multiple *cpcG* genes (89, 91) whereas others have only a single *cpcG* gene (30). It is likely that only one *cpcG* gene is present in the red alga *A. neglectum* (9), suggesting that this one gene product is sufficient for linking the peripheral rods to any of the attachment sites on the PBS core.

Some of the genes encoding the linker polypeptides in eukaryotic algae are present on the nuclear genome (58, 109). These linker polypeptides are synthesized on 80S cytoplasmic ribosomes and must be transported into the plastid, where they assemble with the phycobiliproteins. Recently, a nuclear gene encoding a γ subunit of PE was isolated from *A. neglectum* (9a). The γ subunit is thought to possess some functions that are analogous to cyanobacterial linker polypeptides (it is probably localized in the central channel of the PE discs and may serve in assembly processes) but is chromophorylated like the phycobiliproteins. Some marine cyanobacteria also contain γ subunits that are bonded to chromophores (168). The chromophore-binding sites on the γ subunits were inferred from analyses of PE chromopeptides of the red alga *Gastroclonium coulteri* (122). The *A. neglectum* gene sequence for one of the γ subunits predicts a protein with four of the five inferred binding sites (9a). One region of the γ subunit has limited homology with conserved region III (91) of linker polypeptides. Both the origin of the γ subunit and its exact role in the structure and energy transfer characteristics of PBS rods have yet to be established.

Cyanobacteria

In cyanobacteria the genes encoding the PBS polypeptides are often clustered on the genome. Clustering was first noted in *F. diplosiphon*, where genes for PC and AP were localized to a large genomic fragment (47, 134). Phycobiliprotein and linker polypeptide gene clusters from a number of different cyanobacteria and the different-sized mRNAs transcribed from these clusters are shown in Fig. 3. Note that the gene designations and the proteins that they encode are cross-referenced in Table 1. The genes *apcAB*, encoding the α and β subunits of AP, as well as genes for other components of the core of the PBS, are constitutively expressed. The linker polypeptide genes are frequently contiguous to and cotranscribed with the phycobiliprotein genes. Contiguous to the *apcAB* genes of *F. diplosiphon* are genes encoding both L_C (*apcC*), the small linker polypeptide of the core, and L_{CM} (*apcE*), the anchor protein (116); *apcC* is 3′ and *apcE* is 5′ to *apcAB*. A similar arrangement of genes encoding core components occurs on the genome of *Synechococcus* sp. strain PCC 6301 (118), although the transcript patterns may be different (compare references 118 and 149). Different-sized RNA species span the *apcEABC* gene cluster in both *F. diplosiphon* and *Synechococcus* sp. strain PCC 6301. In *F. diplosiphon* there is a 1.4-kb transcript encoding both α^{AP} and β^{AP}, a 1.8-kb transcript encoding the AP subunits plus L_C, a 0.4-kb transcript that encodes only L_C, a 3.2-kb transcript that encodes L_{CM} (anchor protein), and longer transcripts of approximately 6 kb that probably cover the entire *apcEABC* operon. The accumulation of each of these transcripts may be a consequence of regulation of transcription termination and/or RNA degradation and processing. A gene encoding the α^{APB} of the core, *apcD*, has been characterized in *F. diplosiphon* (117). This gene is not closely linked to *apcAB* and is transcribed as a monocistronic mRNA. The protein encoded by this gene may, like the L_{CM} protein, serve as a terminal energy acceptor in the PBS.

A number of organisms contain more than one *cpcBA* gene set; for example, *F. diplosiphon* has three gene sets that encode PC. The arrangement of these genes and their transcriptional properties are shown in Fig. 3A. Two of the gene sets encoding PC subunits in *F. diplosiphon*, *cpcB1A1* and *cpcB2A2*, are linked on the cyanobacterial genome (47). The former gene set is constitutively expressed, whereas the latter is expressed only upon red illumination and is cotranscribed with the three PC linker genes *cpcH2I2D2* (discussed further in the section on chromatic adaptation). The PC subunits encoded by these two gene sets are greater than 80% identical (46, 149). The differences are mostly in the surface exposed residues of β^{PC}, which are involved primarily in the interactions between hexamers (55). Differences in this region may ultimately explain differences in the stacking

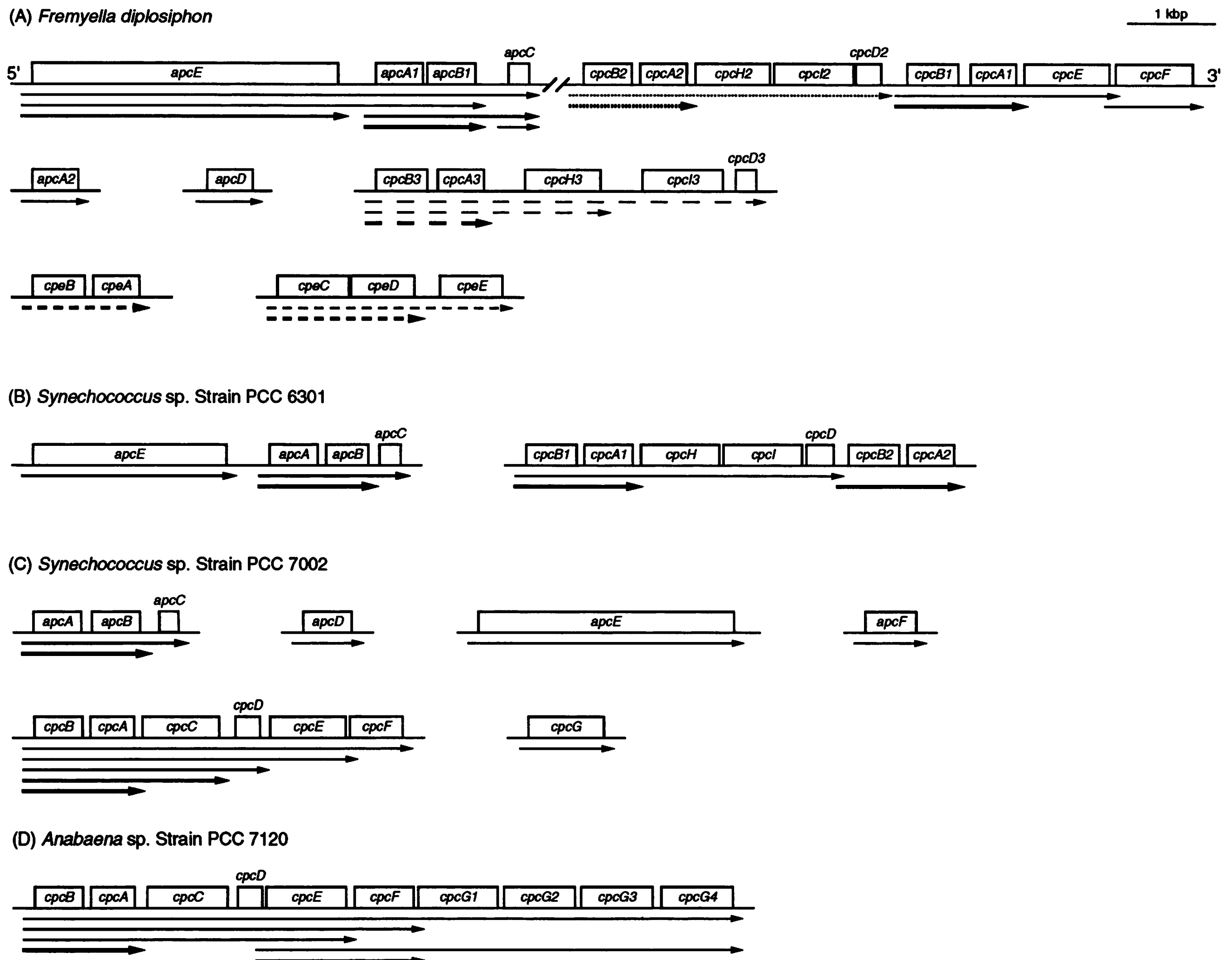

FIG. 3. Physical maps of the genes and gene clusters encoding protein components of the PBS from *F. diplosiphon* (A), *Synechococcus* sp. strain PCC 6301 (B), *Synechococcus* sp. strain PCC 7002 (C), and *Anabaena* sp. strain PCC 7120 (D). The gap between the *apcEA1B1C* and *cpcB2A2H2I2D2* gene clusters of *F. diplosiphon* is approximately 4 kbp. The specific protein encoded by each of the genes is noted in the text and in Table 1. The different-sized transcripts that emanate from each of the gene clusters are represented by the lengths of the bars. Some of these transcripts may have two start sites displaced from each other by 100 to 200 nucleotides. This is not indicated in the figure. The thickness of the arrow used for a given transcript is a qualitative indication of the abundance of that transcript. For *F. diplosiphon*, the regulation of transcription for each of the genes (constitutive, active in RL, active in GL) is noted by the type of line used to trace the transcript. The dotted transcripts accumulate in RL, and the small dashed transcripts accumulate in GL. Transcripts from the *cpcB3A3H3I3D3* operon (large dashes) of *F. diplosiphon* are controlled by the sulfur status of the environment.

characteristics of hexamers of the two PC types and the distribution of different PC hexamers within the *F. diplosiphon* PBS. A third gene set, *cpcB3A3*, encodes PC subunits that are devoid of sulfur amino acids except for the cysteine residues required for chromophore attachment. Contiguous with these genes are the linker genes *cpcH3I3D3*. Transcripts from the *cpcB3A3H3I3D3* operon appear only when *F. diplosiphon* is maintained in medium deficient in sulfur. This operon may offer *F. diplosiphon* an adaptive advantage during sulfur-limited growth (150).

Synechococcus sp. strain PCC 6301 and the closely related *Synechococcus* sp. strain PCC 7942 contain two PC gene sets (121, 128). These gene sets, *cpcB1A1* and *cpcB2A2*, are separated by 2.5 kbp. They are both transcriptionally active and produce prominent transcripts of approximately 1.4 kb (Fig. 3B). The two sets of PC subunits encoded by these genes are identical. A large mRNA of 3.7 kb is also transcribed from this region and covers *cpcB1A1* and the sequence sandwiched between the PC gene sets. This latter region encodes the linker polypeptides that associate with PC in the rod (121).

Linker polypeptide genes are also located downstream of the PC genes in *Synechococcus* sp. strain PCC 7002 (37) (Fig. 3C) and *Anabaena* sp. strain PCC 7120 (15, 89) (Fig. 3D). The *cpcBA* genes in *Synechococcus* sp. strain PCC 7002 are followed by the gene encoding the 33-kDa (*cpcC*) and 9-kDa (*cpcD*) linker polypeptides (37) present in the PBS rods. In *Anabaena* sp. strain PCC 7120 the *cpcBA* gene set is also followed by *cpcC* and *cpcD* (15). Other genes involved in the biosynthesis of the PBS can be located downstream of the linker genes. In some organisms, PC linker genes are followed by two genes, *cpcE* and *cpcF* (30, 37, 91), that are

involved in the attachment of the phycocyanobilin chromophore to α^{PC} (37, 206, 228). Recent evidence from Glazer's laboratory has demonstrated that the proteins encoded by *cpcE* and *cpcF* are directly involved in phycocyanobilin attachment at residue 84 of α^{PC}. Insertional inactivation of *cpcE* and *cpcF* in the marine unicellular cyanobacterium *Synechococcus* sp. strain PCC 7002 results in the production of α^{PC} that has no phycocyanobilin attached at residue 84 (202, 228). The CpcE and CpcF proteins synthesized in *E. coli* were capable of specifically catalyzing the attachment of phycocyanobilin to the cysteine at position 84 of α^{PC} in vitro (63). This reaction was reversible and required the protein products of both genes. Hence, CpcE and CpcF are components of a lyase involved in a specific chromophore attachment reaction. The specificity of this lyase suggests that there are a number of different genes encoding lyases that function in site-specific bilin attachment. In an organism such as *Synechococcus* sp. strain PCC 7002, which contains seven different polypeptides to which phycocyanobilin is attached and eight distinct bilin attachment sites, the cells may require as many as 16 polypeptides for the process of bilin attachment.

CHROMATIC ADAPTATION

Many environmental parameters alter the composition or abundance of PBS. Generally, low light intensities stimulate the synthesis of PBS and the rods may increase in length (135, 169, 170). Increased phycobiliprotein content is also observed in cyanobacteria that receive a preponderance of photosystem I (PS I) light. An increase in the size or number of the PBS produced under such conditions may help balance the electron flow between the two photosystems (143, 144, 151). Macronutrient limitation results in extensive PBS breakdown and a chlorosis or bleaching response (3, 44, 67, 222, 223, 225) is observed. The breakdown of the PBS under these conditions may allow for recycling of amino acids into proteins that aid in the acclimation process and/or may be important in preventing cellular damage via photooxidation. Finally, many cyanobacteria can alter the composition of the PBS in response to light quality (22, 104, 204). In this section we will focus on how certain cyanobacteria acclimate to different wavelengths of light by the process of chromatic adaptation.

Historical Perspective

It was reported over a century ago that certain photosynthetic organisms can acclimate to their light environment by changing their pigmentation (59–62, 72–75). Changes in pigmentation in photosynthetic organisms in response to light quality have been termed chromatic adaptation. The cyanobacteria can be classified into three groups, depending on their pigmentation when grown in different wavelengths of light. Group I cyanobacteria cannot alter their PBS composition when grown in different light qualities. Group II cyanobacteria can alter the levels of PE only, whereas group III organisms can modulate both the PE and PC levels. When the levels of the PE and PC pigments in the PBS are modulated in opposite directions, as in group III organisms, the process is termed complementary chromatic adaptation. Mechanisms involved in complementary chromatic adaptation have been most extensively examined in the cyanobacterium *F. diplosiphon*, although the phenomenon has been observed in a number of cyanobacteria (203) such as *Pseudanabaena* sp. strain PCC 7409 (28, 31). The process of

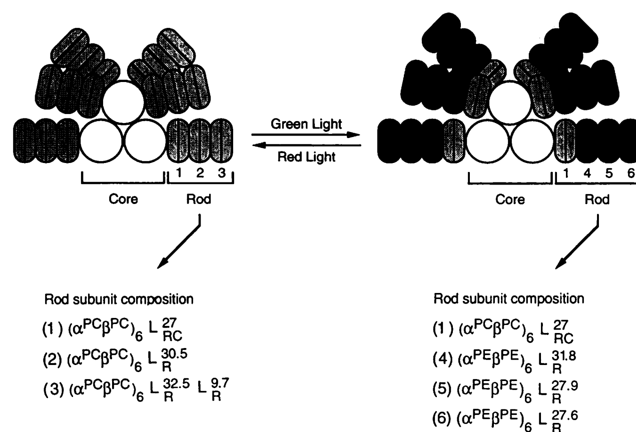

FIG. 4. Chromatic adaptation and the composition of the *F. diplosiphon* PBS from cells maintained in GL or RL. The compositions of the peripheral rods from RL-grown cells are given by 1, 2, and 3, and the compositions of the rods in GL-grown cells are given by 1, 4, 5, and 6. The linker polypeptides are not shown but are included in the "rod subunit composition" lists. Note that the PBS rods from RL-grown cells are composed exclusively (or almost exclusively) of PC hexamers, whereas the rods from GL-grown cells are composed primarily of PE hexamers. In GL only the most core-proximal double disc is PC. The composition of the core does not vary.

complementary chromatic adaptation is most clearly demonstrated when the adapting organisms are compared after growth in red light (RL) and green light (GL). In RL, *F. diplosiphon* accumulates high levels of the blue pigment PC with very little of the red pigment PE. Conversely, in GL the organism has low levels of PC and high levels of PE. The composition of the PBS from *F. diplosiphon* grown in RL and GL is shown in Fig. 4. The change in character of the light-harvesting complex in response to the different wavelengths of light affords the cells an adaptive advantage, since PC can efficiently absorb RL and PE can efficiently absorb GL, but not vice versa.

The initial events that trigger changes in phycobiliprotein gene expression during chromatic adaptation involve the absorption of light by a photoreceptor. The acclimation process can be blocked by inhibitors such as rifampin (87) and therefore requires de novo transcript synthesis. Different types of experiments have been performed to elucidate the nature of the photoreceptor. Early work concentrated on determining the action maxima for the light-triggered responses. The action maximum for the synthesis of PE for both *F. diplosiphon* (112, 216) and *Tolypothrix tenuis* (52) is between 540 and 550 nm, and the maximum for PC synthesis is between 650 and 660 nm. The accumulation of PC could also be promoted by short-wavelength light of 360 nm (166, 216). It is not known whether the short-wavelength response is governed by the same regulatory elements as the RL/GL photoreversible response. Bryant and Cohen-Bazire (31) first noted that more than one type of α^{PC} and β^{PC} was synthesized in chromatically adapting organisms grown in RL whereas only a single α^{PC} and β^{PC} was present in cells grown in GL. These results suggested the possibility that multiple PC genes were differentially controlled by light quality, an interpretation that has been substantiated by more recent work (see below).

The action spectrum for the accumulation of phycobiliproteins indicates that the photoreceptor involved in chro-

TABLE 1. Genes encoding *F. diplosiphon* phycobilisome polypeptides

Protein	Gene	Transcripts	Regulation[a]		References
			Red	Green	
AP and associated linker polypeptides					38, 107, 115–117
$\alpha\beta^{AP}$	*apcA1B1*	1.4-kb *apcAB*, 1.8-kb *apcABC*, ~6.0-kb *apcEABC*	++	++	
$L_C^{7.8}$ (core linker)	*apcC*	0.4-kb *apcC*, 1.8-kb *apcABC*, ~6.0-kb *apcEABC*	+	+	
L_{CM}^{120} (anchor protein)	*apcE*	3.2-kb *apcE*, ~6.0-kb *apcEABC*	++	++	
α^{APB}	*apcD*		++	++	
α^{AP2}	*apcA2*		++	++	
PC and associated linker polypeptides					45–47, 134, 149, 150
$\beta\alpha^{PC_c}$ (constitutive PC)	*cpcB1A1*	1.5-kb *cpcB1A1*	++	++	
$\beta\alpha^{PC_i}$ (RL-inducible PC)	*cpcB2A2*	1.6-kb *cpcB2A2*, 3.7-kb *cpcB2A2H2I2D2*	++	–	
$\beta\alpha^{PC_s}$[b]	*cpcB3A3*	1.6-kb *cpcB3A3*, 3.3-kb *cpcB3A3H3*, 5.0-kb *cpcB3A3H3I3D3*	SL	SL	
$L_R^{30.5}$	*cpcH2*	3.7-kb *cpcB2A2H2I2D2*	++	–	
$L_R^{30.5}$	*cpcI2*		++	–	
$L_R^{9.7}$	*cpcD2*		++	–	
PC_s linkers	*cpcH3*	3.3-kb *cpcB3A3H3*	SL	SL	
	cpcI3	5.0-kb *cpcB3A3H3I3D3*	SL	SL	
	cpcD3		SL	SL	
PE and associated linker polypeptides					65, 66, 107, 147
$\beta\alpha^{PE}$	*cpeBA*	1.4-kb *cpeBA*	–/+	++	
$L_R^{31.8}$	*cpeC*	2.1-kb *cpeCD*, 3.2-kb *cpeCDE*	–/+	++	
$L_R^{27.9}$	*cpeD*		–/+	++	
$L_R^{27.6}$	*cpeE*		–/+	++	
Proteins involved in posttranslational modification or regulation during chromatic adaptation					39, 46, 149
CpcE	*cpcE*	Cotranscribed with *cpcA1B1* (part of a system			
CpcF	*cpcF*	involved in placing chromophore on α^{PC}			
RcaC	*rcaC*	Regulatory protein involved in chromatic adaptation			

[a] Red and green indicate the light quality in which the cyanobacterium was grown. Symbols: ++, high levels of transcript; moderate levels of transcript; –, no detectable transcript; +, moderate levels of transcript; –/+ low levels of transcript; SL, expressed under sulfur-limiting conditions.
[b] $\beta\alpha^{PC_s}$ is used to denote the PC subunits that are expressed under sulfur-limiting conditions.

matic adaptation is most probably a phycobiliprotein, like phytochrome, the photoreceptor present in higher plants that controls many physiological and developmental processes (182). Attempts to isolate the photoreceptor that controls chromatic adaptation suggested that AP was directly involved in photocontrol (20, 162, 183). Cell extracts containing AP exhibited photoreversible absorption changes, as expected of a photoreceptor. However, photoreversibility of AP was observed only after partial denaturation or decomposition of AP and was not detectable within cells maintained under normal growth conditions (163–165). Hence, the initial biochemical search for a photoreceptor was confounded by artifact. It was also likely that the photoreceptor would not be required at high levels and would be extremely difficult to identify among the sea of phycobiliproteins that are structural components of the PBS. It is only now, with the isolation of genes encoding both phycobiliproteins and linker polypeptides, the use of mutants that exhibit aberrant light responsiveness, and the development of gene transfer technology, that the processes controlling chromatic adaptation are more amenable to detailed analysis.

Gene Expression

Since almost all of the work referred to in this section concerns *F. diplosiphon* (*Calothrix* sp. strain PCC 7601), we have included a table (Table 1) that shows the different phycobiliprotein and linker polypeptide genes that have been isolated from *F. diplosiphon*. We have also tabulated the transcriptional characteristics of these genes. Nearly all the genes listed in Table 1 encode structural components of the PBS. They have been characterized with respect to their sequences and the relative levels of their transcripts that accumulate in cells grown in RL and GL. Only recently have the characteristics of some proteins that may be involved in the regulation of chromatic adaptation been defined. One such gene, included in Table 1, is denoted *rcaC*.

Workers in our laboratory (104, 107) and others (206, 207) have isolated and characterized genes encoding PE, PC, and linker polypeptides from *F. diplosiphon*. *F. diplosiphon* contains three PC gene sets (Fig. 3), two of which are important to our discussion of chromatic adaptation. The mRNA from one, *cpcB1A1* (the protein is PC_c), accumulates constitutively, whereas the mRNA from a second, *cpcB2A2* (the protein is PC_i), accumulates only in cells maintained in RL (45). The subscripts c and i denote constitutive and inducible, respectively, and will be used to distinguish the PC proteins in this review.

Several observations suggest that PBS biosynthesis in the different light qualities is, to a great extent, a consequence of the differential transcription of *cpcB2A2* and *cpeBA*. First, transcripts from *cpeBA*, which encode the PE subunits, accumulate to high levels only in GL (147, 159, 160). Second, the abundance of the mRNAs from *cpcB1A1*, *cpcB2A2*, and *cpeBA* reflects the polypeptide composition of the PBS

under a given light condition. Third, the half-lives of the mRNAs encoding PC_i, PC_c, and PE are not altered significantly by exposure to different wavelengths of light (159).

Since specific linker polypeptides are associated with PC_i and PE, the expression of the genes encoding these linker polypeptides was also expected to be regulated by light quality. The genes encoding linker polypeptides associated with PC_i are downstream of *cpcB2A2* (134). This region of the genome has overlapping transcripts: one transcript of 1.6 kb encodes the α and β subunits of PC_i, and a second transcript of 3.7 kb encodes both the α and β subunits of PC_i and the three linker polypeptides that are required for assembly of PC_i into rods (Fig. 3A). These linker polypeptides, $L_R^{30.5}$ $L_R^{32.5}$, and $L_R^{9.7}$, are encoded by the genes *cpcH2*, *cpcI2*, and *cpcD2*, respectively. The molecular masses of each linker protein, indicated by the superscript to L, were calculated from the gene sequences. The order of genes in the operon is *cpcB2A2H2I2D2*. There is the potential to form relatively stable hairpin-loop structures at the 3′ ends of both the small (1.6-kb) and large (3.7-kb) transcripts. The ratio of the 1.6- to 3.7-kb mRNAs from this operon approximately reflects the ratio of the PC_i subunits to the linker polypeptides in the PBS; the level of the 3.7-kb species is about 10% of that of the 1.6-kb species. The larger mRNA may be generated by transcriptional readthrough 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 (46) may be a transcription termination signal, a recognition site for RNA processing, or a structure that prevents further 3′ exonuclease activity. This structure has a predicted ΔG of −31.7 kcal (−132.6 kJ) and is followed by a string of U residues that is characteristic of a prokaryotic termination signal (173). The larger transcript of 3.7 kb also terminates with an inverted repeat that has the potential to form a stable hairpin-loop structure with a predicted ΔG of −93 kcal (−389 kJ). This structure is likely to serve as a terminator since no longer transcripts have been detected from this operon. The role of the hairpin-loop structures in controlling the levels of the two transcripts and consequently the ratio of the PC subunits and linker polypeptides requires further investigation.

The genes encoding PE subunits, *cpeBA*, have been sequenced in *F. diplosiphon*. This gene set is transcribed as a 1.5-kb mRNA (107, 147) that is abundant in GL and present at low levels in RL. The *cpeBA* gene set from *Synechocystis* sp. strain PCC 6701 also has been characterized and encodes a 1.4-kb transcript (7). Like the *F. diplosiphon* genes, the transcript levels are elevated in GL. *Synechocystis* sp. strain PCC 6701 is a group II organism, so although the level of the mRNA encoding PE is elevated in GL, light quality does not change the level of PC mRNA (6).

Recently, Glauser et al. reported the presence of three linker polypeptides associated with PE hexamers of *F. diplosiphon* (90). The genes encoding these linker polypeptides have been characterized (65, 66). The protein sequences for the PE linker polypeptides, as deduced from the nucleotide sequences of the genes, are similar to each other and also show homology with linker polypeptides that associate with PC. These genes are not contiguous to *cpeBA* (in contrast to the PC_i linker polypeptide genes *cpcH2I2D2*, which are immediately downstream of *cpcB2A2*) but are clustered with each other. The PE linker genes are denoted *cpeC*, *cpeD*, and *cpeE*, and two overlapping transcripts from the operon containing these three genes are 2.1 and 3.2 kb long (Fig. 3A). The levels of these transcripts are elevated in coordination with the levels of *cpeBA* mRNA (65). The 2.1-kb transcript, which covers *cpeCD*, can accumulate to higher levels than the 3.2-kb transcript (65). The 3.2-kb transcript covers *cpeCDE* (66). Located 3′ of the translation stop codon of *cpeD* is a sequence that has a high potential to form a hairpin-loop structure (predicted $\Delta G = -101.4$ kcal [−424.3 kJ]). This structure is followed by a string of U residues characteristic of a prokaryotic terminator (173). The occurrence of overlapping *cpeCDE* transcripts that accumulate to different steady-state levels resembles the situation described above for the operon encoding PC_i and the PC_i linker polypeptides and may reflect a common mode of regulating the levels of different constituents of cyanobacterial macromolecular complexes.

The 5′ ends of transcripts from *cpeBA* and *cpeCDE* have been mapped. The 1.4-kb mRNA from *cpeBA* (Fig. 3) begins 62 to 64 bases upstream of the translation initiation codon. The *cpeCDE* transcript has an mRNA leader of 187 bp. This leader sequence contains several small overlapping open reading frames (ORFs) (14 to 21 amino acids) that are associated with potential ribosome-binding sites. Although the significance with respect to the *cpeCDE* transcript is not known, such ORFs can play a role in translational control in other bacteria. Similar ORFs are not present in the leader region of the *cpeBA* mRNA.

Sequences upstream of the transcription start sites of operons that are regulated in a similar manner may exhibit common regulatory motifs. In an effort to delineate sequence motifs that might be important for the light-regulated expression of the phycobiliprotein genes, the regions upstream of the transcription start sites for the genes encoding the various PBS polypeptides were examined. The constitutive PC gene set, *cpcB1A1*, may have both *Escherichia coli* −10 (TATaat) and −35 (TTGaca) sequences. At position −10 to −15 is the sequence TATAGT, whereas the sequence TTGACA is positioned −43 to −48 nucleotides relative to the transcription start site. Sequences similar to the *E. coli* general transcription signals are associated also with some of the light-regulated genes. For example, the *cpcB2A2H2I2D2* operon has a TTGCAC at −29 to −35, the *cpeBA* operon has a TATGTT sequence at −8 to −13, and the *cpeCDE* operon has a TTGATG sequence at −35 to −40. It has not been established that these sequences play a functional role in the transcription of *F. diplosiphon* genes. If they are important for the transcription of *F. diplosiphon* genes, as in *E. coli*, they would probably serve as general transcription signals. The light-regulated genes may contain additional *cis*-acting sequences upstream of these general motifs. Such sequences may be similar for genes that are regulated in a similar manner. However, too few light-regulated genes have been identified and characterized and few attempts have been made to identify upstream sequences that confer specific regulation to these genes. One candidate for an element that confers light regulation is TCCCCAGTCCCCAATCC. It is located 83 bp upstream of the transcription start site of *cpeBA* and, in the reverse complement, 195 bp upstream of the transcription start site of the *cpeCDE* operon. Although these operons respond similarly to light conditions, the function of this element in controlling GL-regulated transcription of *cpeBA* is questionable since it resembles repetitive sequences that flank other genes in the *F. diplosiphon* genome that are not light regulated (148). The 17-bp element may represent contiguous repetitive elements described by Mazel et al. (148). The sequence CCCCA(A/G)T, which is

repeated 10 times in the region that precedes *cpeBA*, has been classified as a short, tandemly repeated, repetitive sequence. These sequences are scattered throughout the *F. diplosiphon* genome and have also been identified in the genomes of other heterocyst-producing cyanobacteria (148). A sequence of unknown significance, GGATCAGG, is repeated five times upstream of the *cpeCDE* gene cluster (65). Ultimately, the proof of the significance of any sequence in the regulation of genes that are differentially controlled by specific wavelengths of light will rely on the demonstration of sequence function in vivo. Such an analysis will entail the evaluation of gene expression after regions of the promoter are deleted or modified.

Recently, proteins have been shown to bind to the promoter regions of both *cpcB2A2* and *cpeBA* and possibly alter the transcriptional activity of these genes (38a, 196a). Transcriptional control by such factors may be modulated by light-driven phosphorylation events (see below). Similar factors may control the expression of *cpeCDE*.

Photobiology

Action spectra for PE and PC synthesis in both *F. diplosiphon* and *Tolypothrix tenuis* have been known for many years (52, 112, 183, 216). Detailed photobiological characterizations of chromatic adaptation provide clues to the nature of the photoreceptor and its associated signal transduction pathway.

Photobiological characterizations of chromatic adaptation (159–161) show the following. (i) Transcription from both *cpcB2A2* and *cpeBA* can be triggered by a pulse of inductive light followed by darkness. For both gene sets, the transcript population increases immediately following transfer to inductive light and reaches a maximum within 2 h at 25°C. At elevated temperatures (32°C) the response is more rapid. (ii) The fluence required for altered transcription from the two gene sets is 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}, whereas the response for altering transcription from *cpeBA* is saturated at 6×10^3 μmol m^{-2}. The different fluences required to stimulate the *cpcB2A2* and *cpeBA* responses suggest that two distinct photoreceptors regulate expression from the two different phycobiliprotein gene sets or that there is a complex signal transduction chain between photoperception and the control of transcriptional activity that results in the different light responsiveness observed. Several observations support the concept of a single photoreceptor (see below). (iii) The kinetics of change in the rate of transcription from *cpcB2A2* and *cpeBA* is different when cells are transferred from inductive to noninductive light. Transcription from *cpcB2A2* declines rapidly on transfer to noninductive light and is barely detectable after 2 h. The rapid decline in transcriptional activity of *cpcB2A2* on shifting from inductive to noninductive light suggests that this gene set is controlled by 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. We favor the idea of control of *cpcB2A2* by a negative regulatory element. In contrast, transcription from *cpeBA* is still high even 10 h after the shift to noninductive light. On being transferred to noninductive light, the level of transcription from *cpeBA* decreases in concert with the generation time of the cells, suggesting that a factor involved in positive regulation of the gene set is becoming progressively more dilute during cell growth.

The alteration in the state of the photoreceptor by different wavelengths of light and the way in which the photoreceptor communicates these changes to the transcriptional machinery of the cell are not known. Many laboratories have attempted to isolate the photoreceptor by identifying a GL/RL photoreversible molecule, but they have been unrewarded in these attempts (as reviewed by Björn and Björn [20]). Although this photoreversible control system probably involves a bilin-associated photoreceptor that may resemble phytochrome, there are a number of differences between the two photoresponsive systems. (i) The action maxima for the cyanobacterial photoreceptor are 540 nm (GL) and 640 nm (RL), whereas those for phytochrome are 660 nm (red [R]) and 730 nm (far red [FR]). (ii) Both forms of the cyanobacterial photoreceptor are biologically active in the sense that specific gene sets are transcriptionally active at both action maxima. For the classic phytochrome response, the RL-activated species (FR absorbing form, P_{fr}) promotes the biological response. (iii) In the phytochrome system, a period of darkness following a terminal pulse of activating light results in a diminution of photoreversibility; there is an "escape" from photocontrol. There is no escape from photocontrol in the cyanobacterial system. Transcription from *cpcB2A2* and *cpeBA* remains fully on or off immediately following the terminal irradiation, and it is possible to fully reverse the effect with a pulse of complementary light, even after several hours of darkness following the terminal light pulse. (iv) Plants possess multiple genes encoding different forms of phytochrome, many of which exhibit tissue specificity and are developmentally regulated (210). It is unknown whether the photoreceptor involved in chromatic adaptation is a single molecule. It is also unclear whether other processes, such as the formation of hormogonia (49), are controlled by this species.

Despite extensive characterization of complementary chromatic adaptation at the level of gene expression, little is known about the photosensory and signal transfer components that govern this process. Photoperception and photoregulation of cellular processes are well documented for a number of higher-plant systems; however, the way in which the photoreceptor communicates with the transcriptional machinery of the cell remains largely unknown. The photobiological analysis of complementary chromatic adaptation in *F. diplosiphon* suggests that the state of the photoreceptor is linked to the activity of gene-specific transcriptional regulators. Since cyanobacteria are amenable to standard prokaryotic genetic manipulation, they can be used as a relatively simple model to investigate photoresponsiveness at the molecular level. Especially beneficial for the analysis of this control process is the acquisition of mutants that display aberrant chromatic adaptation.

Regulatory Mutants

Many *F. diplosiphon* mutants that exhibit aberrations in chromatic adaptation have been isolated (27, 43, 204). These mutants can be visually identified and may arise spontaneously. This spontaneous, low-frequency mutation rate may be a response of the cells to adverse environmental conditions such as desiccation (which may occur in the course of culture maintenance). The exposure of wild-type *F. diplosiphon* cells to mutagenic agents or electric shock significantly increases the frequency at which pigment mutants appear. A genetic characterization of such mutants is extremely important in defining the molecular details of chromatic adaptation. Cobley and Miranda (43) were the first to report the

characterization of pigment mutants after treatment of wild-type cells with UV irradiation. Three classes of mutants were defined on the basis of the pigmentation of the cells. In "green" mutants, the synthesis of PE did not occur in either GL or RL whereas PC synthesis was normal under both light conditions. In "blue" mutants, both photoinduction of PE and photorepression of PC synthesis were impaired. In a "black" mutant, PE was partially induced and PC partially repressed in RL. Hence, the black mutant responded to light quality in the opposite way to the response of wild-type cells. In 1983 a review article by Tandeau de Marsac described six classes of PBS regulatory mutants (204). These strains, isolated after exposure of wild-type cells to nitrosoguanidine, were postulated to represent six of eight possible mutant phenotypes in which PC_i and PE are abnormally regulated. At this point, a lack of detailed information about the different phenotypes does not allow for a critical evaluation of the model.

We have also isolated numerous mutants and have focused on the characterization of these mutants as a primary means of examining the regulatory mechanisms governing chromatic adaptation in *F. diplosiphon*. These mutants were generated during optimization of conditions for the transfer of DNA into *F. diplosiphon* by electroporation. Three mutant classes were generated (red, blue, and green) and thoroughly characterized (27). This characterization included spectral analyses of whole cells and cell extracts, determination of phycobiliprotein and linker polypeptide composition of intact PBS, and quantification of transcripts from *cpeBA*, *cpcB1A1*, *cpcB2A2*, and *apcAB*, allowing us to distinguish mutants with lesions in the structural genes of the PBS and assembly processes from regulatory mutants. Cells of the red mutant class (designated FdR, for *F. diplosiphon* red) have lost the ability to acclimate to RL. The FdR mutants have high levels of PE, normal levels of PC_c, and no detectable PC_i under conditions of both RL or GL; this pigment composition makes the cells appear red. The levels of the individual phycobiliproteins are reflected in the levels of the mRNAs encoding those proteins. Hence, FdR mutants are locked in the GL regulatory mode and are indifferent to the presence of RL. Blue mutants, designated FdB, exhibit normal regulation of *cpeBA* and elevated transcription from *cpcB2A2* in both RL and GL. Our FdB mutants do not exhibit the impaired PE synthesis observed for the blue mutant class reported by Cobley and Miranda (43). Cells of the green mutant class, designated FdG, have lost the ability to synthesize PE and normally regulate the levels of PC_i.

The characterization of these mutants has established two general features of chromatic adaptation. First, single lesions can alter the regulated transcription from both *cpcB2A2* and *cpeBA*. This is apparent in the phenotype of FdR strains, a phenotype frequently observed in mutant populations. This suggests the presence of common elements in the chain of events governing transcription of both *cpcB2A2* and *cpeBA*. Since expression of *cpeBA* and *cpcB2A2* in FdR is permanently locked in a mode in which the cells behave as if they are in GL, the putative activator that controls *cpeBA* and repressor that controls *cpcB2A2* remain active even in RL. Second, there are distinct events that specifically control the expression of *cpcB2A2* and *cpeBA*, as demonstrated in the phenotype of FdB. At this point we must still be tentative in stating that a repressor controls *cpcB2A2* and an activator controls *cpeBA*. Indeed, some results from both our laboratory (161) and the laboratory of others (196a) suggest that more than one regulatory element may be involved in controlling the expression of each of the biliprotein genes. In this respect the mutants described above are valuable, since, in conjunction with a gene transfer system developed for *F. diplosiphon*, they will aid in the isolation of genes integral to the phenomenon of chromatic adaptation and help elucidate the details of photoperception and signal transduction.

The generation and analysis of double mutants and second-site revertants, created by chemical mutagens or DNA transposition events, is also a powerful method for analyzing chromatic adaptation and the processes involved in its regulation. When an FdR strain is used for the generation of double mutants, only the green mutant phenotype appears. Mutagenesis of the blue mutant FdB yields colonies of a purple phenotype (both PE and PC_i are expressed constitutively). We have thus far been unable to obtain an altered phenotype in a population of mutagenized green mutants. These results, which begin to establish a hierarchy of mutant phenotypes, suggest that the green phenotype is dominant over all of the other pigment phenotypes.

Approaches for Isolating Regulatory Elements

Isolation and characterization of pigment mutants coupled with the introduction of wild-type DNA into the mutant strains has been successfully used for the complementation of regulatory mutants. Unlike many gram-negative bacteria and a number of unicellular cyanobacterial strains, *F. diplosiphon* is not naturally competent for transformation with exogenous DNA. We have improved a gene transfer system, initially developed by Cobley (42), for the introduction of DNA into *F. diplosiphon*. This system is based on the use of a shuttle vector that can replicate both in *E. coli* and in the cyanobacterium. The shuttle vector that we are using, designated pPL2.7, harbors a 2.7-kbp fragment from an endogenous *F. diplosiphon* plasmid that contains the necessary genetic elements for replication in the cyanobacterium. This plasmid also contains the ColE1 replication origin; the *bom* (basis of mobility) region, which facilitates the conjugal transfer of the plasmid from an *E. coli* host to a recipient strain; and the gene encoding aminoglycoside 3'-phosphotransferase, which confers resistance to the antibiotic kanamycin. For complementation experiments a library of random *Sau*3A fragments of wild-type genomic DNA was ligated into the pPL2.7 vector and the population of recombinant molecules was introduced into *F. diplosiphon* by either electroporation or conjugation. Although the plasmids are stable in *F. diplosiphon* cells as long as the selection pressure in maintained (40), homologous recombination between the plasmid containing the library of wild-type DNA and the cyanobacterial chromosome has rarely been observed.

Complementation of regulatory mutants also relies on an appropriate screen for choosing transformants that exhibit the wild-type phenotype. The dramatic pigmentation differences between wild-type and PBS regulatory mutants provides a clear visual screen for complementation. For example, when grown in RL an FdR mutant appears red whereas wild-type cells appear blue-green. A genomic library could be introduced into an FdR strain and the transformed cells selected on plates containing kanamycin. All transformants will be kanamycin resistant, but only FdR mutants that had been complemented would form colonies that appear blue-green in RL; the other transformants would remain red. Similarly, in GL the complemented FdG mutants would be red among noncomplemented green cells.

Several genes encoding putative components of the signal

transduction pathway have been isolated following complementation of members of the FdR and FdB mutant classes. The most thoroughly characterized gene, designated *rcaC*, was localized on a large plasmid, rescued from a single complemented colony of the red mutant FdR1 (39). Reintroduction of this large complementing plasmid into the mutant strain resulted in a high frequency of complemented colonies. The selected plasmid also conferred the wild-type phenotype to cells of another red mutant, FdR2. Sequence analysis of the complementing fragment revealed that the *rcaC* gene encodes a protein that has strong sequence identity with the *Bacillus subtilis* PhoP protein (191), a member of the superclass of regulatory proteins associated with two-component regulatory systems in bacteria (reviewed in references 2 and 200). Most of these regulators have a conserved N-terminal domain containing an aspartate residue that can be phosphorylated. The RcaC protein is unusual in that it contains conserved domains with potentially phosphorylated aspartate residues at both the N and C termini. Southern blot analysis of genomic DNA isolated from the 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. It has been reported that *F. diplosiphon* possesses multiple copies of two transposable elements, which have recently been characterized (146), as well as uncharacterized forms of these elements. Our results suggest that electroporation of wild-type cells of *F. diplosiphon* had activated the movement of genetic elements into the *rcaC* locus, thereby creating the mutant phenotype.

Three genes whose products may be involved in chromatic adaptation were isolated following complementation of the blue mutant FdB1. Sequence analysis of the genomic DNA on the complementing plasmid revealed three ORFs, which are large and tandemly arranged. Two of the ORFs encode nearly identical proteins with strong identity to the *E. coli* NR_{II} protein (153), a member of a superclass of sensory proteins associated with the aforementioned bacterial two-component regulatory system. The third ORF encodes a protein with sequence identity to rat myosin light-chain kinase (180), a member of a eukaryotic class of serine/threonine kinases. A similar serine/threonine kinase has been described in another prokaryote, *Myxococcus xanthus* (155). Although this DNA sequence was present on plasmids from independently isolated, complemented strains, recomplementation of the mutant with the isolated plasmid was not achieved. Hence, the role of these kinases in the process of chromatic adaptation remains uncertain. Although it is possible that complementation of FdB1 was the consequence of marker rescue (which is probably a very rare event in *F. diplosiphon*), the presence of the kinase genes on the plasmid isolated from complemented strains of the blue mutant may be fortuitous.

Further insight into the signal transduction mechanism governing chromatic adaptation can be gained 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. These interactions can be examined by extracting proteins from cells and incubating these proteins with specific DNA fragments. The protein-DNA complex has altered electrophoretic mobility (gel retardation assay) in a polyacrylamide gel matrix. The site specificity and binding activity of a transcriptional regulatory protein can be defined by gel retardation assays, and the putative regulatory gene encoding the binding protein may be isolated by screening a recombinant expression library with labeled target DNA (generally an oligomer of the specific region of the DNA that binds the protein). Gel retardation studies with DNA fragments from the vicinity of the transcription initiation site for *cpeBA* demonstrated the binding of specific proteins, which are present only in extracts from GL-grown cells. No binding to the same region was observed by proteins extracted from RL-grown cells. These data suggest that a *trans*-acting element is present or active only in cells cultured in GL and that it binds to and may facilitate transcription from *cpeBA*. Additional evidence for regulatory factors that interact with regions that precede the transcription initiation sites of *cpcB2A2* and *cpeBA* comes from recent studies by Houmard et al. (196a) and Casey and Grossman (38a). Houmard et al. 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. The binding proteins have been isolated, characterized, and named RcaA and RcaB. One of the proteins loses its binding activity when it is treated with alkaline phosphatase (196a). Hence, two *trans*-acting proteins 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* (38a, 196a). 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 protein from cells grown in both GL and RL. In vivo analysis of chimeric genes in which regulated promoters are fused to reporter genes (β-glucuronidase [GUS], luciferase) will help establish the significance of both *cis*-acting DNA sequences and *trans*-acting proteins in the regulated expression of phycobiliprotein genes. With the aid of a genetic system and the capability to evaluate promoter activity in vivo, coupled with an in vitro biochemical analysis of *cis*- and *trans*-acting elements associated with the light-regulated genes, the features of the *cpcB2A2* and *cpeBA* promoter regions important for their light-regulated transcription should be rapidly delineated.

Model Describing Regulation

We are developing a testable model for the RL- and GL-regulated expression of genes during chromatic adaptation. A reasonable assumption in generating a model is that the activity or abundance of transcriptional regulators is influenced by light quality. Before presenting the model, we will recapitulate some of the salient features of chromatic adaptation that have been used in its development. Following the transfer of wild-type cells from GL to RL, transcription from *cpcB2A2* (the operon includes *cpcB2A2H2I2D2*) rapidly increases whereas transcription from *cpeBA* and *cpeCDE* slowly decreases. When wild-type cells are transferred from RL to GL, transcription from *cpcB2A2* rapidly decreases whereas transcription from *cpeBA* rapidly increases. It is plausible that a negative regulatory element controls transcription from the inducible PC operon whereas a positive regulator may control transcription from the inducible PE operon. The initial model, probably oversimplified (Fig. 5), involves gene-specific regulation that is governed by *trans*-acting regulatory elements. In RL neither the repressor of *cpcB2A2* nor the activator of *cpeBA* is functioning (represented as A and R, which are not bound to *cpeBA* and *cpcB2A2* genes in Fig. 5; this is only a pictorial

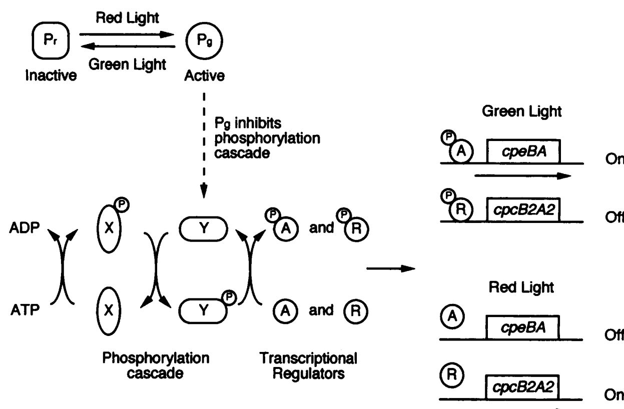

FIG. 5. Model for control of phycobiliprotein genes during chromatic adaptation. P_r and P_g are the RL- and GL-absorbing forms of a photoreceptor, respectively. X and Y are elements of the phosphorylation cascade. A and R are activator and repressor elements, respectively, that may exist in a phosphorylated or unphosphorylated form. Phosphorylation of these regulatory proteins makes them active; the activator triggers the expression of *cpeBA*, and the repressor prevents the expression of *cpcB2A2*. In this model, the active phosphorylated repressor and activator are shown to be in direct contact with the DNA (the genes are presented as lines with boxed portions that represent the coding regions), whereas the inactive nonphosphorylated forms are not bound to the DNA. This need not be the case, and both forms may bind to the DNA (the activity of the regulatory proteins may be a consequence of their conformations before and after phosphorylation).

representation, and the nonfunctional transcription regulators may still bind DNA). This situation in RL results in increased transcription from *cpcB2A2* and low levels of transcription from *cpeBA*. In GL the transcription regulators become active (represented as being attached to the DNA of the *cpeBA* and *cpcB2A2* genes in Fig. 5), resulting in elevated transcription from *cpeBA* and repression of *cpcB2A2*. The work of Houmard (114a) and Chiang et al. (39) and in vivo experiments in which proteins are phosphorylated in GL and RL (38a) suggest that a phosphorylation cascade is involved in controlling the activity of the regulatory elements. In Fig. 5 the phosphorylated elements function directly in the regulation of transcription. The phosphorylation cascade contains the two factors X and Y (the number of factors in the cascade is arbitrary), which mediate the phosphorylation and therefore the activity of A and R. Some of these factors might be histidine kinases, which are prominent in signal transduction pathways in many prokaryotes.

The next question concerns how we believe that photoperception modulates the phosphorylation cascade. On the basis of photobiology of chromatic adaptation and the studies of mutants that exhibit aberrant chromatic adaptation (27, 159–161), we propose that altered transcription from *cpcB2A2* and *cpeBA* is controlled by a single photoconvertible photoreceptor and that in GL this photoreceptor exists as P_r (inactive form). Both A and R are functioning under these conditions, and *F. diplosiphon* produces high PE and low PC levels. When the ratio of RL to GL becomes high, the photoreceptor is converted to P_g (active form), which causes inhibition of the phosphorylation cascade and prevents the phosphorylation of regulatory factors A and R. The transcription regulators would gradually become inactive, and the cells would synthesize high levels of PC_i and low levels of PE. Hence, the ratio of P_r to P_g, which is controlled by the ratio of RL to GL, would regulate the transcriptional activity of *cpeBA* and *cpcB2A2*.

Biochemical and photobiological studies, coupled with the characterization of PBS regulatory mutants, are consistent with the working model presented above. In the FdB mutants, *cpcB2A2* is expressed in both RL and GL whereas *cpeBA* is normally regulated. This phenotype could be the consequence of a lesion in a *cpcB2A2*-specific repressor, R, which prevents it from turning off *cpcB2A2* in GL. In the FdG mutants thus far characterized, there is normal regulation of *cpcB2A2* and no transcription from *cpeBA* in either RL or GL. This phenotype could be the consequence of inactivation of the positive regulator, A, which prevents it from turning on *cpeBA* in GL. Mutants of the FdR class exhibit constitutive expression of *cpeBA* and no expression of *cpcB2A2* in either RL or GL. Such a phenotype could be due to a lesion in the photoreceptor (making it impossible to suppress the phosphorylation cascade) or an intermediate that links the RL signal perceived by the photoreceptor to inhibition of the phosphorylation cascade.

The model is also supported by the determination of allowable phenotypes thus far observed in the creation of double mutants. For example, we observe that a FdR strain (constitutive functioning of the phosphorylation cascade) can be mutated to a FdG mutant (inactive phosphorylation cascade). This conversion would occur if the second lesion were in a component of the phosphorylation cascade, X and Y in the model. Second mutations in an FdB background could result in a new phenotype, in which the organism becomes purple when grown in both RL and GL (182a). If the second lesion were in the photoreceptor or the mechanism by which the photoreceptor regulates the phosphorylation cascade, *cpeBA* would be constitutively expressed. In conjunction with the lesion in FdB that caused constitutive expression of *cpcB2A2*, the second mutation would generate a new strain that would be purple when grown in both RL and GL.

As discussed above, we have isolated a gene, *rcaC*, that complements the FdR mutant. In our model the FdR phenotype reflects a constitutively active state for the regulators controlling *cpcB2A2* and *cpeBA*; A and R are both functional. Such a phenotype may be the consequence of a lesion in the photoreceptor or in components involved in the negative control mechanism immediately downstream of the photoreceptor. Hence, RcaC may be a transcriptional regulator necessary for expression of the photoreceptor gene or for genes encoding components of the pathway that cause inhibition of the phosphorylation cascade. It is also possible that RcaC is directly involved in modulating the activity of A and R. Continued genetic studies will elucidate the details of the signaling pathway.

In summary, we propose that altered transcription of *cpcB2A2* and *cpeBA* during chromatic adaptation is achieved by coordinated activation and deactivation of opposing gene-specific transcriptional regulatory elements in GL and RL, respectively. These regulators, A and R, which appear to control transcription from *cpeBA* and *cpcB2A2*, respectively, are controlled by a phosphorylation cascade. This cascade can be inactivated in RL as a consequence of the accumulation of the P_g form of the photoreceptor. Other environmental conditions such as light intensity and nutrient status of the cultures may also modulate the activity of this regulatory pathway.

DEGRADATION OF PBS AND RESPONSE OF CYANOBACTERIA TO NUTRIENT LIMITATION

Cyanobacteria exhibit a suite of responses during nutrient-limited growth that are either specific responses triggered by the depletion of a single nutrient or general responses that occur in medium that lacks any one of a number of different nutrients (3, 4, 177, 196). Examples of specific responses are increased synthesis of specific transport systems (e.g., sulfate transport during sulfur-limited growth [103, 130]) and enzymes that may help generate substrates that can be more readily transported into the cell (e.g., periplasmic alkaline phosphatase during phosphate-limited growth [21, 175]). General responses to nutrient limitation include changes in both cellular morphology and physiology.

Dramatic alterations in the ultrastructure of cyanobacterial cells grown under adverse nutrient conditions have been observed in the electron microscope. Sherman and Sherman (192) and Wanner et al. (217) demonstrated that iron-, nitrogen-, or sulfur-deficient cells had less than half of the normal complement of thylakoid membranes. The remaining membranes were disorganized and interspersed with large deposits of glycogen. There was also a decrease in the number of carboxysomes and increased formation of polyphosphate granules. Changes in the cell wall may also occur during nutrient-limited growth, although these changes have not been characterized at the biochemical level. Temporal studies of nitrogen-deficient *Synechococcus* sp. strain PCC 7002 demonstrated that removal of the nutrient caused a loss of PBS followed by a reduction in the level of ribosomes and thylakoid membranes (199). Both the chlorophyll and β-carotene levels in the cells have also been reported to decline, whereas the level of zeaxanthin relative to the other carotenoids increases (105). Maintenance of high zeaxanthin levels may be beneficial to nutrient-limited cells, since this xanthophyll can quench oxygen radicals generated by the nonproductive excitation of remaining chlorophyll molecules.

Pigment Changes and Changes in Photosynthesis

A dramatic response exhibited by cyanobacteria when they are limited for sulfur, nitrogen, phosphorus, carbon, or iron is a decrease in the abundance of pigment molecules in the cells. The first quantitation of pigment levels during nutrient-limited growth was performed by Allen and Smith (4). Cultures of *Anacystis nidulans* (*A. nidulans*, *Synechococcus* sp. strain PCC 7942, *Synechococcus* sp. strain PCC 6301 and *Synechococcus leopoliensis* are very similar strains that have been used in studies described below) maintained for 30 h in medium devoid of nitrogen had no detectable PC (4). The levels of carotenoids and chlorophyll did not change. Sulfur-deprived cultures also lose PC and cellular chlorophyll content declines during both nitrogen- and sulfur-limited growth, because cell division continues after pigment accumulation ceases (44, 217). The cells also accumulated reducing sugars when nutrient limited. Nutrient-deprived cells can remain viable for weeks after the cessation of growth.

During sulfur and nitrogen starvation, there is the rapid and near-complete degradation of the PBS. Degradation of the PBS could provide the cell with amino acids used for the synthesis of proteins important for the acclimation process. These amino acids may also be converted into carbon skeletons and used to produce other cellular constituents. The use of phycobiliproteins as amino acid storage molecules may be important for marine cyanobacteria, since nitrogen is frequently limiting in marine environments (224). 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. Recent studies demonstrate that the PBS are degraded in an ordered manner in both sulfur- and nitrogen-starved cells (44, 225). In contrast, cells deprived of phosphorus show only very limited degradation of the PBS (44); diminished PBS levels per cell are observed in phosphorus-limited cultures because the rate of PBS biosynthesis declines relative to the rate of cell division (at least five divisions occur following the elimination of phosphorus from the medium). Hence, general losses in pigmentation during macronutrient limitations are not all the same. Pigment changes and changes in the intracellular architecture of the cell may also be different during iron- or carbon-limited growth, although a detailed characterization of the morphological features and pigmentation alterations in such cells has not been performed.

In addition to the loss of the PBS antennae, photosynthetic electron transport is impaired when *Synechococcus* sp. strain PCC 7942 is starved for sulfur or nitrogen. Both sulfur and nitrogen starvation lead to a decrease in oxygen evolution to barely detectable levels as a consequence of a loss of PS II activity. Fluorescence emission studies show that in the early phases of nutrient stress, before the PBS is completely degraded, the PBS cannot efficiently transfer energy to the chlorophyll molecules of PS II. Photoacoustic measurements of stressed cells in which the PBS is completely degraded indicate that PS I is still active in the production of ATP (113a). The elimination of PS II function during nutrient-limited growth may be advantageous, since, in the absence of anabolic processes, the formation of excited PS II chlorophyll molecules would lead to the production of singlet oxygen, a highly reactive species that could inhibit the normal functioning of cellular processes. The continued production of ATP from cyclic electron flow around PS I would provide energy to drive metabolic processes that remain essential, even during nutrient-limited growth. This energy would also be required for transporting the limiting nutrient into the cell once it becomes available.

PBS Degradation

The degradation of the PBS during nitrogen- or sulfur-limited growth is an ordered process. The sequence of temporal events following the initial depletion of nitrogen or sulfur is depicted in Fig. 6. In initial studies, spectrophotometry was used to observe the loss of PC during nutrient-deprivation-triggered chlorosis (4, 120, 152, 187, 217). This loss is correlated with a loss of PC apoprotein, as determined immunologically (129). Furthermore, the synthesis of new PC is depressed during growth in nitrogen-deficient medium and is restored on readdition of the limiting nutrient to the medium. Work on nutrient-deprived cells has also demonstrated that elimination of nitrogen from the growth medium provokes a rapid degradation of the terminal hexamer of the PBS rods and its associated 30-kDa linker polypeptide (44, 225), as shown in Fig. 6, step 1. This is followed by degradation of the next PC hexamer and its associated 33-kDa linker polypeptide and elimination of entire rods (Fig. 6, step 2). The loss of these components results in a decrease in the PBS sedimentation coefficient and a reduction in the ratio of PC to AP. Continued stress conditions

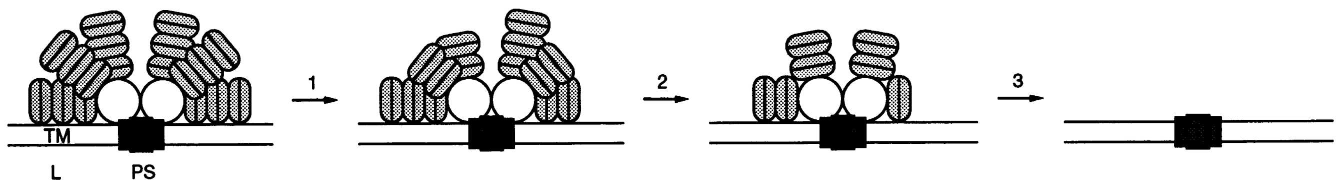

FIG. 6. Breakdown of the PBS during nutrient-limited growth. The time at which each of the events depicted in the figure occurs varies depending on the culture conditions and the specific nutrient that is removed from the growth medium. Generally, nitrogen deprivation is the most severe, and a loss of the terminal hexamer of the peripheral rods is observed prior to 12 h of deprivation (step 1). Continued loss of peripheral hexamers and the elimination of entire rods is observed by 24 h (step 2). Complete degradation of the PBS occurs within 36 h (step 3). L, PS, and TM are abbreviations for the lumen of the photosynthetic (thylakoid) membranes, the photosynthetic reaction centers, and the thylakoid membranes, respectively.

result in the complete destruction of the smaller PBS (Fig. 6, step 3). On addition of the limiting nutrient back to deprived cultures, new PBS are rapidly synthesized.

The levels of mRNAs encoding the phycobiliproteins also decline during nutrient-limited growth. de Lorimier et al. (50) reported that the level of PC mRNA decreased to nearly zero in nitrogen-starved *Synechococcus* sp. strain PCC 7002. This observation was used in a strategy to isolate the genes encoding the PC subunits from this organism. More detailed analyses have shown that by 3 to 5 h after the initiation of nitrogen deprivation, the transcripts from the *cpcBACDEF* operon are essentially undetectable (30). Data obtained by Gasparich et al. (86) with a *cpcB-lacZ* fusion suggest that nitrogen deprivation results in a marked decrease in transcription of the *cpcBA* gene set. Nitrogen-starved cultures of *Anabaena variabilis* also exhibit a loss of transcripts encoding the phycobiliprotein subunits (218). In *Synechococcus* sp. strain PCC 7942, the mRNA encoding both PC and AP declines rapidly during either nitrogen- or sulfur-limited growth and less rapidly during phosphorus-limited growth. However, levels of all of these mRNA species remain at 5 to 10% of that in nutrient-replete cells (44), even 48 h after the cells are 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. Although the PC and AP mRNA levels fall to 5% of the levels measured in nutrient-replete cells, the translation of phycobiliproteins cannot be detected at all (3a). This suggests that the production of phycobiliproteins during nutrient-limited growth is blocked by both transcriptional and posttranscriptional events.

The analysis of PBS biosynthesis and degradation under conditions in which the nutrient status of the medium is modulated provides us with the opportunity to examine events important for the production of this abundant macromolecular complex. In early studies it was suggested that a specific protease whose level increased four- to sixfold in nitrogen-limited *Anabaena* cells was responsible for PBS degradation (222, 223). Since the PBS is stable in nutrient-sufficient medium and extremely unstable in medium lacking either nitrogen or sulfur, it seemed unlikely that this increase in the level of a specific protease could fully account for the rapid PBS degradation initiated by exposure of *Synechococcus* sp. strain PCC 7942 to nutrient limitation. To examine this process from a different perspective, we (44a) mutated *Synechococcus* sp. strain PCC 7942 and screened for organisms unable to degrade their PBS. Such colonies appeared blue-green when allowed to grow on agar substantially free of sulfur, whereas wild-type colonies bleached. Some mutants did not degrade their PBS during either sulfur- or nitrogen-limited growth, although no new PBS were synthesized during the stress treatment. Surprisingly, these cells grew at a similar rate to wild-type cells and exhibited a similar susceptibility to high light levels during the acclimation process. Hence, even though the cells had a considerable number of PBS, they appeared to be no more photosensitive than normally acclimating cells were. Additional experiments suggested that the PBS present in the nutrient-deprived mutant organisms were not able to efficiently transfer harvested light energy to the reaction center of PS II; this uncoupling of the light-harvesting complex from the primary photochemical reactions of PS II may explain the lack of photosensitivity in these strains. To define the lesion, mutant strains were complemented back to the bleaching, wild-type phenotype and the DNA responsible for complementation was characterized. Initially, a 2.0-kbp fragment of genomic DNA was shown to be effective in complementation. Three genes, designated *orf2*, *nblA*, and *txlA*, were located on this fragment, as shown in Fig. 7. *orf2* is constitutively expressed and is not involved in the acclimation process. In contrast, the expression of both *txlA* and *nblA* is regulated by both the nitrogen and sulfur status of the medium. The sequence of the predicted gene product of *txlA* resembles both a thioredoxin and a protein disulfide isomerase. The *txlA* transcript is diminished in sulfur- or nitrogen-depleted cells. The role of *txlA* in the response of cells to nitrogen and sulfur limitations is still uncertain, although kinetic experiments suggest that it is involved in maintaining a stable linkage between the PBS and the PS II core complex or in the proper assembly of PS II.

The *nblA* gene contains an ORF of 59 amino acids, with an initiator methionine that is preceded by a perfect ribosome-binding site. A small transcript covering this gene accumulates at high levels only in cells starved for nitrogen or sulfur. Larger transcripts initiated at the beginning of *nblA* can extend through *txlA* (encoded on the opposite strand). Very low levels of the transcript can be detected in cells maintained in complete medium. Insertional inactivation of this gene results in a nonbleaching phenotype. Inactivation of the genes on either side of *nblA* does not prevent bleaching during nutrient-limited growth. The strain initially isolated as a nonbleaching mutant contained a single-base change in *nblA* that altered a serine to a phenylalanine. When the *nblA* gene is placed on a multicopy plasmid and put 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 do during nutrient-limited growth. The cells also degrade the PBS during phosphate stress if *nblA* is placed under the control of the derepressible alkaline phosphatase promoter. Hence, any condition that favors increased expression of *nblA* triggers the degradative process. No homology was observed between the protein

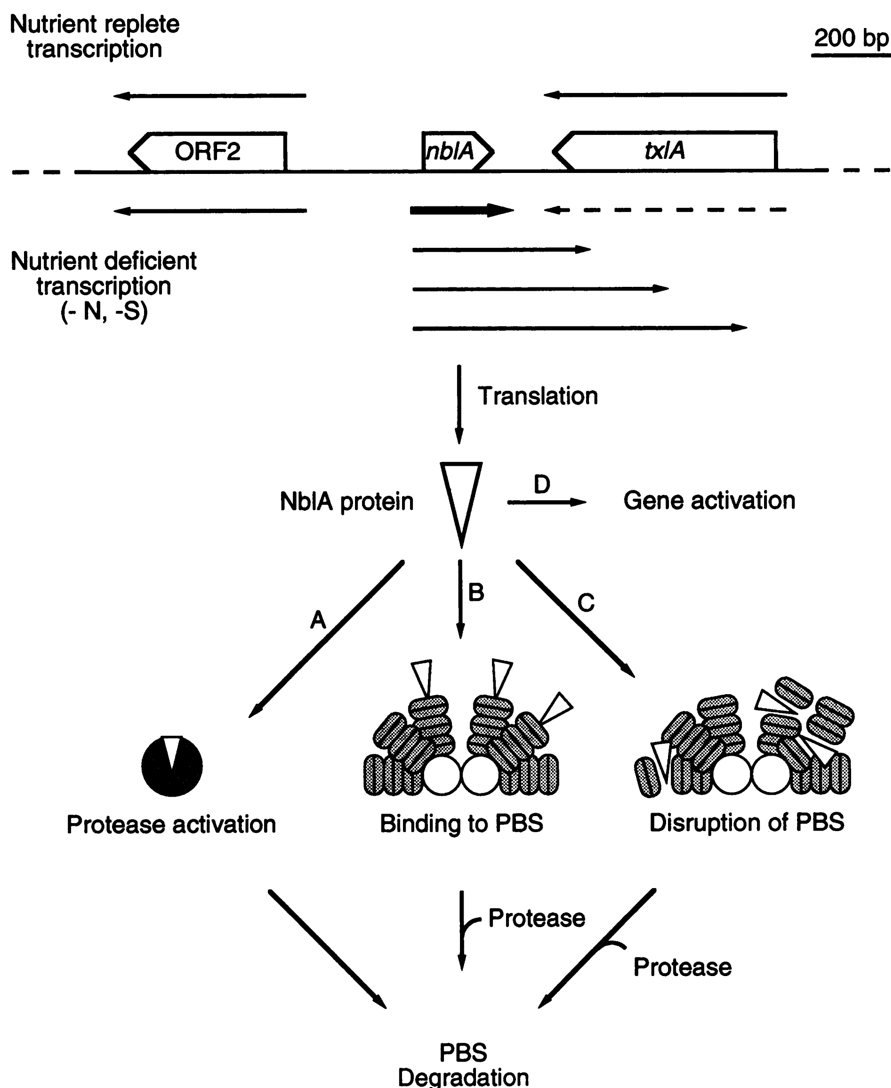

FIG. 7. Role of *nblA* in PBS degradation. Transcripts positioned above the 2-kbp gene cluster are readily detected in cells grown in nutrient-replete medium. Transcripts below the gene cluster are observed when the cells are grown in medium devoid of either nitrogen (−N) or sulfur (−S). The *nblA* transcript accumulates to high levels during both N- and S-depleted growth. It is transcribed at extremely low levels in cultures maintained on complete medium. Transcription past the end of the *nblA* coding region also occurs and may suppress transcription from *txlA*. Lower production of *txlA* might cause destabilization of the PBS/PS II interaction or eliminate the assembly of functional PS II units. The *nblA* gene product may cause the degradation of the PBS in one of four ways: activation of a specific protease(s) (A); tagging the structure for degradation in a manner analogous to that of ubiquitin (B); disruption of the PBS structure, making the components susceptible to proteolysis (C); or direct or indirect regulation of the transcription of other genes required for PBS degradation (D).

encoded by *nblA* and any sequence in the GenBank databases. A number of interesting ideas can be gleaned from these studies. First, since multiple copies of this gene can induce bleaching to some extent when cells are maintained in complete medium and since phosphate stress can cause PBS breakdown in a strain in which *nblA* is placed under the control of the alkaline phosphatase promoter, it is likely to be the only gene whose activity must be directly increased (NblA itself may be involved in the activation of other genes) during sulfur or nitrogen stress to provoke bleaching. Second, the small size of this protein and the lack of similarity to any known protease suggest that it is not a protease itself. There are at least four functions for this polypeptide that would explain why it is absolutely required for bleaching (Fig. 7). NblA may function to activate a protease, such as the one studied by Wood and Haselkorn (223), that subsequently degrades the PBS (Fig. 7A). NblA may trigger PBS degradation by interacting with the constituents of the complex and altering their susceptability to proteolysis. Such interactions may involve covalent attachment, similar to the binding of ubiquitin to proteins in eukaryotes, which marks them for degradation (114) (Fig. 7B). Alternatively, this small peptide may disrupt hydrophobic and/or ionic interactions among various constituents of the PBS, rendering them susceptible to degradation (Fig. 7C). Finally, NblA may be involved in activating other genes that are directly responsible for causing PBS degradation (Fig. 7D). In any case, these findings may be of general importance in understanding the degradation of macromolecular complexes in prokaryotic organisms.

CONCLUDING REMARKS

The size, structure, and number of PBS in cyanobacteria is exquisitely sensitive to the environmental milieu in which the organism is grown. The response of certain cyanobacteria to light quality may provide us with a broader understanding of photoperception in photosynthetic microorganisms 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 provide insights into processes involved in the targeting of macromolecular complexes for degradation and the machinery that implements this degradation. There is still little information available on factors that control the biosynthesis of a mature PBS. In our view, this 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 of the manuscript, and Glenn Ford was always available to aid us with computer-assisted analysis of the phycobilisome polypeptides and genes. We also thank the anonymous reviewer who helped to make this manuscript more accessible to a general readership.

We acknowledge The National Institutes of Health, The National Science Foundation, and The Carnegie Institution of Washington for providing funds that have made our participation in this work possible.

REFERENCES

1. **Alam, J., S. Shibahara, and A. Smith.** 1989. Transcriptional activation of the heme oxygenase gene by heme and cadmium in mouse hepatoma cells. J. Biol. Chem. **264:**6371–6375.
2. **Albright, L. M., E. Huala, and F. M. Ausubel.** 1989. Prokaryotic signal transduction mediated by sensor and regulator protein pairs. Annu. Rev. Genet. **23:**311–336.
3. **Allen, M. M.** 1984. Cyanobacterial cell inclusions. Annu. Rev. Microbiol. **38:**1–25.

3a. **Allen, T., J. L. Collier, and A. R. Grossman.** Unpublished data.

4. **Allen, M. M., and A. J. Smith.** 1969. Nitrogen chlorosis in blue-green algae. Arch. Microbiol. **690:**111–120.
5. **Anderson, L. K., and A. R. Grossman.** 1987. Phycocyanin genes in the cyanobacterium *Synechocystis* 6701 and a potential gene rearrangement in a pigment variant, p. 817–820. *In* J. Biggins, (ed.), Progress in photosynthesis research, vol. 4. Proceedings of the VIIth International Congress on Photosynthesis. Martinus Nijhoff Publishers, The Hague, The Netherlands.
6. **Anderson, L. K., and A. R. Grossman.** 1990. Genes for phycocyanin subunits in *Synechocystis* sp. strain 6701 and the assembly mutant UV16. J. Bacteriol. **172:**1289–1296.
7. **Anderson, L. K., and A. R. Grossman.** 1990. Structure and light-regulated expression of phycoerythrin genes in wild type and phycobilisome assembly mutants of *Synechocystis* sp. strain PCC 6701. J. Bacteriol. **172:**1297–1305.
8. **Apt, K. E., and A. R. Grossman.** 1993. Characterization and transcript analysis of the major phycobiliprotein subunit genes from *Aglaothamnion neglectum* (*Rhodophyta*). Plant Mol. Biol. **21:**27–38.
9. **Apt, K. E., and A. R. Grossman.** 1993. Gene encoding phycobilisome linker polypeptides on the plastid genome of *Aglaothamnion neglectum* (Rhodophyta). Photosynth. Res. **35:**235–245.

9a. **Apt, K., N. Hoffman, and A. R. Grossman.** The γ subunit of R-phycoerythrin and its possible mode of transport into the plastid. J. Biol. Chem., in press.

10. **Beale, S. I., and J. Cornejo.** 1984. Enzymic transformation of biliverdin to phycocyanobilin by extracts of the unicellular red alga *Cyanidium caldarium*. Plant Physiol. **76:**7–15.
11. **Beale, S. I., and J. Cornejo.** 1991. Biosynthesis of phycobilins. 15,16-Dihydrobiliverdin IXa is a partially reduced intermediate in the formation of phycobilins from biliverdin IXa. J. Biol. Chem. **266:**22341–22345.
12. **Beale, S. I., and J. Cornejo.** 1991. Biosynthesis of phycobilins. 3(*Z*)-Phycoerythrobilin and 3(*Z*)-phycocyanobilin are intermediates in the formation of 3(*E*)-phycocyanobilin from biliverdin IXa. J. Biol. Chem. **266:**22333–22340.
13. **Beale, S. I., and J. Cornejo.** 1991. Biosynthesis of phycobilins. Ferredoxin-mediated reduction of biliverdin catalyzed by extracts of *Cyanidium caldarium*. J. Biol. Chem. **266:**22328–22332.
14. **Belford, H. S., G. D. Offner, and R. F. Troxler.** 1983. Phycobiliprotein synthesis in the unicellular rhodophyte *Cyanidium caldarium*. Cell-free translation of the mRNAs for the α and β subunit polypeptides of phycocyanin. J. Biol. Chem. **258:**4503–4510.
15. **Belknap, W. R., and R. Haselkorn.** 1987. Cloning and light regulation of expression of the phycocyanin operon of the cyanobacterium *Anabaena*. EMBO J. **6:**871–884.
16. **Bennett, A., and L. Bogorad.** 1971. Properties of subunits and aggregates of blue-green algal biliproteins. Biochemistry **10:**3625–3634.
17. **Berns, D. S.** 1970. Protein aggregation in phycocyanin-osmotic pressure studies. Biochem. Biophys. Res. Commun. **38:**65–73.
18. **Berns, D. S., and E. Scott.** 1966. Protein aggregation in a thermophilic protein. Phycocyanin from *Synechococcus lividus*. Biochemistry **5:**1528–1533.
19. **Bhat, G. J., and G. Padmanaban.** 1988. Heme regulates cytochrome P-450 gene transcription elongation. Biochem. Biophys. Res. Commun. **151:**737–742.
20. **Björn, L. O., and G. S. Björn.** 1980. Photochromic pigments and photoregulation in blue-green algae. Photochem. Photobiol. **32:**849–852.
21. **Block, M. A., and A. R. Grossman.** 1988. Identification and purification of a derepressible alkaline phosphatase from *Anacystis nidulans* R2. Plant Physiol. **86:**1179–1184.
22. **Bogorad, L.** 1975. Phycobiliproteins and complementary chromatic adaptation. Annu. Rev. Plant Physiol. **26:**369–401.
23. **Brown, S. B., and J. A. Holroyd.** 1984. Biosynthesis of the chromophore of phycobiliproteins: a study of mesohaem and mesobiliverdin as possible intermediates and further evidence for an algal haem oxygenase. Biochem. J. **217:**265–272.
24. **Brown, S. B., J. A. Holroyd, R. F. Troxler, and G. D. Offner.** 1981. Bile pigment synthesis in plants: incorporation of haem into phycocyanobilin and phycobiliproteins in *Cyanidium caldarium*. Biochem. J. **194:**137–147.
25. **Brown, S. B., J. A. Holroyd, D. I. Vernon, and O. T. G. Jones.** 1984. Ferrochelatase activity in the photosynthetic alga *Cyanidium caldarium*: development of the enzyme during biosynthesis of photosynthetic pigments. Biochem. J. **220:**861–863.
26. **Brown, S. B., J. A. Holroyd, D. I. Vernon, R. F. Troxler, and K. M. Smith.** 1982. The effect of N-methylprotoporphyrin IX on the synthesis of photosynthetic pigments in *Cyanidium caldarium*: further evidence for the role of haem in the biosynthesis of plant bilins. Biochem. J. **208:**487–491.
27. **Bruns, B., W. R. Briggs, and A. R. Grossman.** 1989. Molecular characterization of phycobilisome regulatory mutants in *Fremyella diplosiphon*. J. Bacteriol. **171:**901–908.
28. **Bryant, D. A.** 1981. The photoregulated expression of multiple phycocyanin species: general mechanism for control of phycocyanin synthesis in chromatically adapting cyanobacteria. Eur. J. Biochem. **119:**425–429.
29. **Bryant, D. A.** 1988. Genetic analysis of phycobilisome biosynthesis, assembly, structure and function in the cyanobacterium *Synechococcus* sp. PCC 7002, p. 62–92. *In* S. E. Stevens, Jr., and D. A. Bryant (ed.), Light energy transduction in photosynthesis: higher plant and bacterial models. The American Society of Plant Physiologists, Rockville, Md.
30. **Bryant, D. A.** 1991. Cyanobacterial phycobilisomes: progress toward complete structural and functional analysis via molecular genetics, p. 257–300. *In* L. Bogorad and I. K. Vasil (ed.), Cell culture and somatic cell genetics of plants. Molecular

biology of plastids and mitochondria, vol. 7. Academic Press, Inc., San Diego, Calif.

31. **Bryant, D. A., and G. Cohen-Bazire.** 1981. Effects of chromatic illumination on cyanobacterial phycobilisomes: evidence for the specific induction of a second pair of phycocyanin subunits in *Pseudanabaena* 7409 grown in red light. Eur. J. Biochem. **119:**415–424.
32. **Bryant, D. A., R. de Lorimier, G. Guglielmi, V. L. Stirewalt, A. Cantrell, and S. E. Stevens.** 1987. The cyanobacterial photosynthetic apparatus, p. 749–755. *In* J. Biggins (ed.), Progress in photosynthesis research, vol. 4. Proceedings of the VIIth International Congress on Photosynthesis. Martinus Nijhoff Publishers, The Hague, The Netherlands.
33. **Bryant, D. A., R. de Lorimier, G. Guglielmi, V. L. Stirewalt, J. M. Dubbs, B. Illman, R. D. Porter, and S. E. Stevens.** 1985. Genes for phycobilisome components in *Synechococcus* 7002, *Pseudanabaena* 7409 and *Mastigocladus laminosus*. V International Symposium on Photosynthetic Prokaryotes, vol. (abstract) 103.
34. **Bryant, D. A., R. de Lorimier, D. H. Lambert, J. M. Dubbs, V. L. Stirewalt, S. E. Stevens, R. D. Porter, J. Tam, and E. Jay.** 1985. Molecular cloning and nucleotide sequence of the α and β subunits of allophycocyanin from the cyanelle genome of *Cyanophora paradoxa*. Proc. Natl. Acad. Sci. USA **82:**3242–3246.
35. **Bryant, D. A., R. de Lorimier, R. D. Porter, D. H. Lambert, J. M. Dubbs, V. L. Stirewalt, P. I. Field, S. E. Stevens, W. Liu, J. Tam, and E. W. K. Jay.** 1985. Phycobiliprotein genes in cyanobacteria and cyanelles, p. 249–258. *In* C. Arntzen, L. Bogorad, S. Bonitz, and K. Steinback (ed.), The molecular biology of the photosynthetic apparatus. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
36. **Bryant, D. A., G. Guglielmi, N. Tandeau de Marsac, A.-M. Castets, and G. Cohen-Bazire.** 1979. The structure of cyanobacterial phycobilisomes: a model. Arch. Microbiol. **123:**113–127.
37. **Bryant, D. A., J. Zhou, G. E. Gasparich, R. de Lorimier, G. Guglielmi, and V. L. Stirewalt.** 1990. Phycobilisomes of the cyanobacterium *Synechococcus* sp. PCC 7002. Structure, function, assembly and expression, p. 129–141. *In* G. Drews, (ed.), Molecular biology of membrane-bound complexes in photosynthetic bacteria. Plenum Publishing Corp., New York.
38. **Capuano, V., A. Braux, N. Tandeau de Marsac, and J. Houmard.** 1991. The "anchor polypeptide" of cyanobacterial phycobilisomes, molecular characterization of the *Synechococcus* sp PCC 6301 *apcE* gene. J. Biol. Chem. **266:**7239–7247.

38a. **Casey, E., and A. R. Grossman.** Unpublished data.

39. **Chiang, G. G., M. R. Schaefer, and A. R. Grossman.** 1992. Complementation of a red-light indifferent cyanobacterial mutant. Proc. Natl. Acad. Sci. USA **89:**9415–9419.
40. **Chiang, G. G., M. R. Schaefer, and A. R. Grossman.** 1992. Transformation of the filamentous cyanobacterium *Fremyella diplosiphon* by conjugation or electroporation. Plant Physiol. Biochem. **30:**315–325.
41. **Chitnis, P. R., and P. Thornber.** 1988. The major light-harvesting complex of photosystem II. Aspects of its molecular and cell biology. Photosynth. Res. **16:**41–63.
42. **Cobley, J. G.** 1985. Chromatic adaptation in *Fremyella diplosiphon*. I. Mutants hypersensitive to green light. II. Construction of mobilizable vectors lacking sites for FDI I and II, abstr. 105. Vth International Symposium on Photosynthetic Prokaryotes, 1985.
43. **Cobley, J. G., and R. D. Miranda.** 1983. Mutations affecting chromatic adaptation in the cyanobacterium *Fremyella diplosiphon*. J. Bacteriol. **153:**1486–1492.
44. **Collier, J. L., and A. R. Grossman.** 1992. Chlorosis induced by nutrient deprivation in *Synechococcus* sp. strain PCC 7942: not all bleaching is the same. J. Bacteriol. **174:**4718–4726.

44a. **Collier, J. L., and A. R. Grossman.** Unpublished data.

45. **Conley, P. B., P. G. Lemaux, and A. R. Grossman.** 1985. Cyanobacterial light-harvesting complex subunits encoded in two red light-induced transcripts. Science **230:**550–553.
46. **Conley, P. B., P. G. Lemaux, and A. R. Grossman.** 1988. Molecular characterization and evolution of sequences encoding light harvesting components in the chromatically adapting cyanobacterium *Fremyella diplosiphon*. J. Mol. Biol. **199:**447–465.
47. **Conley, P. B., P. G. Lemaux, T. L. Lomax, and A. R. Grossman.** 1986. Genes encoding major light-harvesting polypeptides are clustered on the genome of the cyanobacterium *Fremyella diplosiphon*. Proc. Natl. Acad. Sci. USA **83:**3924–3928.
48. **Cornejo, J., and S. I. Beale.** 1988. Algal heme oxygenase from *Cyanidium caldarium*. Partial purification and fractionation into three required protein components. J. Biol. Chem. **263:** 11915–11921.
49. **Damerval, T., G. Guglielmi, J. Houmard, and N. Tandeau de Marsac.** 1991. Hormogonium differentiation in cyanobacterium *Calothrix* sp. PCC 7601: a photoregulated developmental process. Plant Cell **3:**191–201.
50. **de Lorimier, R., D. A. Bryant, R. D. Porter, W. Liu, E. Jay, and S. E. Stevens.** 1984. Genes for α and β phycocyanin. Proc. Natl. Acad. Sci. USA **81:**7946–7950.
51. **de Lorimier, R., C. Chen, and A. N. Glazer.** 1992. Sequence comparison of two highly homologous phycoerythrins differing in bilin composition. Plant Mol. Biol. **20:**353–356.
52. **Diakoff, S., and S. Scheibe.** 1973. Action spectra for chromatic adaptation in *Tolypothrix tenuis*. Plant Physiol. **51:**382–385.
53. **Dubbs, J., and D. A. Bryant.** 1987. Organization of the genes encoding phycoerythrin and the two differentially expressed phycocyanins in the cyanobacterium *Pseudanabaena* PCC 7409, p. 761–764. *In* J. Biggins (ed.). Progress in photosynthesis research, vol. 4. Proceedings of the VIIth International Congress on Photosynthesis. Martinus Nijhoff Publishers, The Hague, The Netherlands.
54. **Duerring, M., R. Huber, W. Bode, R. Ruembeli, and H. Zuber.** 1990. Refined three-dimensional structure of phycoerythrocyanin from the cyanobacterium *Mastigocladus laminosus* at 2.7 Å. J. Mol. Biol. **211:**633–644.
55. **Duerring, M., G. B. Schmidt, and R. Huber.** 1991. Isolation, crystallization, crystal structure analysis and refinement of constitutive C-phycocyanin from the chromatically adapting cyanobacterium *Fremyella diplosiphon* at 1·66 Å resolution. J. Mol. Biol. **217:**577–592.
56. **Duysens, L. N. M.** 1951. Transfer of light energy within the pigment systems present in photosynthesizing cells. Nature (London) **168:**548–550.
57. **Dwarki, V. J., V. N. K. Francis, G. J. Bhat, and G. Padmanaban.** 1987. Regulation of cytochrome P-450 messenger RNA and apoprotein levels by heme. J. Biol. Chem. **262:**16958–16962.
58. **Egelhoff, T., and A. R. Grossman.** 1983. Cytoplasmic and chloroplast synthesis of phycobilisome polypeptides. Proc. Natl. Acad. Sci. USA **80:**3339–3343.
59. **Engelmann, T. W.** 1883. Farbe und Assimilation. Assimilation findet nur in den farbstoffhaltigen Plasmathielchen statt. II. Näherer Zusamennhang Zwischen Lichtabsorption und Assimilation. Bot. Z. **41:**1–13.
60. **Engelmann, T. W.** 1883. Farbe und Assimilation. III. Weitere Folgerungen. Bot. Z. **41:**17–29.
61. **Engelmann, T. W.** 1884. Untersuchungen über die qualitativen Beziehungen Zwischen Absorption des Lichtes und Assimilation in Planzenzellen. I. Das Mikrospectrophotometer ein Apparat zur quantitativen Mikrospectralanalyse. II. Experimentelle Grundlagen zur Ermittelung der quantitativen Beziehungen zwischen Assimilationsenergie und Absorptionsgrösse. Bot. Z. **42:**97–105.
62. **Engelmann, T. W.** 1902. Ueber experimentelle Erzeugung Zwechmässiger Aenderungen der Färbung pflanzenlicher Chromophylle durch farbiges Licht. Arch. Anat. Physiol. (physiological abstract) 1902:333–335.
63. **Fairchild, C. D., J. Zhao, J. Zhou, S. E. Colson, D. A. Bryant, and A. N. Glazer.** 1992. Phycocyanin α-subunit phycocyanobilin lyase. Proc. Natl. Acad. Sci. USA **89:**7017–7021.
64. **Fawley, M. W., and A. R. Grossman.** 1986. Polypeptides of light-harvesting complexes of the diatom *Phaeodactylum tri-*

cornutum are synthesized in the cytoplasm of the cell as precursors. Plant Physiol. 81:149–155.

65. **Federspiel, N. A., and A. R. Grossman.** 1990. Characterization of the light-regulated operon encoding the phycoerythrin-associated linker proteins from the cyanobacterium *Fremyella diplosiphon*. J. Bacteriol. 172:4072–4081.

65a. **Federspiel, N. A., and A. R. Grossman.** Unpublished data.

66. **Federspiel, N. A., and L. Scott.** 1992. Characterization of a light-regulated gene encoding a new phycoerythrin-associated linker protein from the cyanobacterium *Fremyella diplosiphon*. J. Bacteriol. 179:5994–5998.

67. **Foulds, I. J., and N. G. Carr.** 1977. A proteolytic enzyme degrading phycocyanin in the cyanobacterium *Anabaena cylindrica*. FEMS Microbiol. Lett. 2:117–121.

68. **Frank, G., W. Sidler, H. Widmer, and H. Zuber.** 1978. The complete amino acid sequence of both subunits of C-phycocyanin from the cyanobacterium *Mastigocladus laminosus*. Hoppe-Seyler's Z. Physiol. Chem. 359:1491–1507.

69. **Freidenreich, P., G. S. Apell, and A. N. Glazer.** 1978. Structural studies on phycobiliproteins. II. C-phycocyanin amino acid sequence of the β subunit. Specific cleavage of the α subunit. J. Biol. Chem. 253:212–219.

70. **French, C. S., and V. K. Young.** 1952. The fluorescence spectra of red algae and the transfer of energy from phycoerythrin to phycocyanin and chlorophyll. J. Gen. Physiol. 35:873–890.

71. **Friedman, A. L., and R. S. Alberte.** 1986. Biogenesis and light regulation of the major light harvesting chlorophyll-protein of diatoms. Plant Physiol. 80:43–51.

72. **Gaidukov, N.** 1903. Die Farberänderung bei den Prozessen der Komplementären chromatischen Adaptation. Ber. Dtsch. Bot. Ges. 21:517–522.

73. **Gaidukov, N.** 1903. Weitere Untersuchungen über den Einfluss farbigen Lichtes auf die Färbung der Oscillatarien. Ber. Dtsch. Bot. Ges. 21:484–492.

74. **Gaidukov, N.** 1904. Zur Farbenanalyse der Algen. Ber. Dtsch. Bot. Ges. 22:23–29.

75. **Gaidukov, N.** 1906. Die komplementäre chromatische Adaptation bei *Porphyra* und *Phormidium*. Ber. Dtsch. Bot. Ges. 24:1–5.

76. **Gantt, E.** 1975. Phycobilisomes: light harvesting pigment complexes. BioScience 25:781–787.

77. **Gantt, E.** 1981. Phycobilisomes. Annu. Rev. Plant Physiol. 32:327–347.

78. **Gantt, E., and S. F. Conti.** 1965. The ultrastructure of *Porphyridium cruentum*. J. Cell Biol. 26:365–381.

79. **Gantt, E., and S. F. Conti.** 1966. Granules associated with the chloroplast lamellae of *Porphyridium cruentum*. J. Cell Biol. 29:423–430.

80. **Gantt, E., and S. F. Conti.** 1966. Phycobiliprotein localization in algae. Brookhaven Symp. Biol. 19:393–405.

81. **Gantt, E., and C. A. Lipschultz.** 1972. Phycobilisomes of *Porphyridium cruentum*. I. Isolation. J. Cell Biol. 54:313–324.

82. **Gantt, E., and C. A. Lipschultz.** 1973. Energy transfer in phycobilisomes from phycoerythrin to allophycocyanin. Biochim. Biophys. Acta 292:858–861.

83. **Gantt, E., and C. A. Lipschultz.** 1977. Probing phycobilisome structure by immunoelectron microscopy. J. Phycol. 13:185–192.

84. **Gantt, E., C. A. Lipschultz, J. Grabowski, and B. K. Zimmerman.** 1979. Phycobilisomes from blue-green and red algae. Plant Physiol. 63:615–620.

85. **Gantt, E., C. A. Lipschultz, and B. Zilinskas.** 1976. Further evidence for a phycobilisome model from selective dissociation, fluorescence emission, immunoprecipitation and electron microscopy. Biochim. Biophys. Acta 430:375–388.

86. **Gasparich, G. E., J. S. Buzby, D. A. Bryant, R. D. Porter, and S. E. Stevens, Jr.** 1987. The effects of light intensity and nitrogen starvation on the phycocyanin promoter in the cyanobacterium *Synechococcus* sp. PCC 7002, p. 761–764. *In* J. Biggins (ed.), Progress in photosynthesis research, vol. IV. Martinus-Nijhoff, Dordrecht, The Netherlands.

87. **Gendel, S., I. Ohad, and L. Bogorad.** 1979. Control of phycoerythrin synthesis during chromatic adaptation. Plant Physiol. 64:786–790.

88. **Gingrich, J. C., L. K. Blaha, and A. N. Glazer.** 1982. Rod substructure in cyanobacterial phycobilisomes: analysis of *Synechocystis* 6701 mutant low in phycoerythrin. J. Cell Biol. 92:261–268.

89. **Glauser, M., D. A. Bryant, G. Frank, E. Wehrli, S. S. Rusconi, W. Sindler, and H. Zuber.** 1992. Phycobilisome structure in the cyanobacteria *Mastigocladus laminosus* and *Anabaena* sp. PCC 7120. Eur. J. Biochem. 205:907–915.

90. **Glauser, M., W. A. Sidler, K. W. Graham, D. A. Bryant, G. Frank, E. Wehrli, and H. Zuber.** 1992. Three C-phycoerythrin-associated linker polypeptides in the phycobilisome of green-light-grown *Calothrix* sp. PCC 7601 (cyanobacteria). FEBS Lett. 297:19–23.

91. **Glauser, M., V. L. Stirewalt, D. A. Bryant, W. Sidler, and H. Zuber.** 1992. Structure of the genes encoding the rod-core linker polypeptides of *Mastigocladus laminosus* phycobilisomes and functional aspects of the phycobiliprotein/linker-polypeptide interactions. Eur. J. Biochem. 205:927–937.

92. **Glazer, A. N.** 1980. Structure and evolution of photosynthetic accessory pigment systems with special reference to phycobiliproteins, p. 221–244. *In* D. S. Sigman and M. A. B. Brazier (ed.), The evolution of protein structure and function. Academic Press, Inc., New York.

93. **Glazer, A. N.** 1982. Phycobilisomes: structure and dynamics. Annu. Rev. Microbiol. 36:173–198.

94. **Glazer, A. N.** 1984. A macromolecular complex optimized for light energy transfer. Biochim. Biophys. Acta 768:29–51.

95. **Glazer, A. N.** 1985. Light harvesting by phycobilisomes. Annu. Rev. Biophys. Biophys. Chem. 14:47–77.

96. **Glazer, A. N.** 1987. Phycobilisomes: assembly and attachment, p. 47–77. *In* P. Fay and C. van Baalen (ed.), The cyanobacteria. Elsevier Biomedical Press, Amsterdam.

97. **Glazer, A. N.** 1988. Phycobilisomes. Methods Enzymol. 167:304–312.

98. **Glazer, A. N.** 1989. Light guides. Directional energy transfer in a photosynthetic antenna. J. Biol. Chem. 264:1–4.

99. **Glazer, A. N., and G. Cohen-Bazire.** 1971. Subunit structure of the phycobiliproteins of blue-green algae. Proc. Natl. Acad. Sci. USA 68:1398–1401.

100. **Glazer, A. N., D. J. Lundell, G. Yamanaka, and R. C. Williams.** 1983. The structure of a "simple" phycobilisome. Ann. Inst. Pasteur/Microbiol. 134B:159–180.

101. **Goff, L. J., and A. W. Coleman.** 1988. The use of plastid DNA restriction endonuclease patterns in delineating red algal species and populations. J. Phycol. 24:357–368.

102. **Green, B. R., E. Pichersky, and K. Kloppstech.** 1991. Chlorophyll-a/b-binding proteins—an extended family. Trends Biochem. Sci. 16:181–186.

103. **Green, L. S., and A. R. Grossman.** 1988. Changes in sulfate transport characteristics and protein composition of *Anacystis nidulans* R2 during sulfur deprivation. J. Bacteriol. 170:583–587.

104. **Grossman, A. R.** 1990. Chromatic adaptation and the events involved in phycobilisome biosynthesis. Plant Cell Environ. 13:651–666.

105. **Grossman, A. R., J. L. Collier, and D. E. Laudenbach.** The response of *Synechococcus* sp. strain PCC 7942 to sulfur-limited growth, p. 3–10. *In* N. Murata (ed.), Research in photosynthesis, vol. IV. Kluwer Academic Publishers.

106. **Grossman, A. R., P. G. Lemaux, and P. B. Conley.** 1986. Regulated synthesis of phycobilisome components. Photochem. Photobiol. 44:827–837.

107. **Grossman, A. R., P. G. Lemaux, P. B. Conley, B. U. Bruns, and L. K. Anderson.** 1988. Characterization of phycobiliprotein and linker polypeptide genes in *Fremyella diplosiphon* and their regulated expression during complementary chromatic adaptation. Photosynth. Res. 17:32–56.

108. **Grossman, A. R., A. Manodori, and D. Snyder.** 1990. Light-harvesting proteins of diatoms: their relationship to the chlorophyll *a/b* binding proteins of higher plants and their mode of transport into plastids. Mol. Gen. Genet. 224:91–100.

109. **Grossman, A. R., L. Talbot, and T. Egelhoff.** 1983. Biosynthesis of phycobilisome polypeptides of *Porphyridium aerugineum* and *Cyanophora paradoxa*. Carnegie Inst. Wash. Year Book. **82:**112–116.
110. **Guglielmi, G., G. Cohen-Bazire, and D. A. Bryant.** 1981. The structure of *Gloeobacter violaceus* and its phycobilisomes. Arch. Microbiol. **129:**181–189.
111. **Hartl, F. U., and J. Martin.** 1992. Protein folding in the cell: the role of molecular chaperones Hsp70 and Hsp60. Annu. Rev. Biophys. Biomol. Struct. **21:**293–322.
112. **Haury, J. F., and L. Bogorad.** 1977. Action spectra for phycobiliprotein synthesis in a chromatically adapting cyanophyte. Plant Physiol. **60:**835–839.
113. **Hemmingsen, S. M., C. Woolford, S. M. van der Vies, K. Tilly, D. T. Dennis, C. P. Georgopoulos, R. Hendrix, and R. J. Ellis.** 1988. Homologous plant and bacterial proteins chaperone oligomeric protein assembly. Nature (London) **333:**330–334.
113a. **Herbert, S., J. L. Collier, D. Fork, and A. R. Grossman.** Unpublished data.
114. **Hershko, A.** 1988. Ubiquitin-mediated protein degradation. J. Biol. Chem. **263:**15237–15240.
115. **Houmard, J., V. Capuano, M. V. Columbano, T. Coursin, and N. Tandeau de Marsac.** 1990. Molecular characterization of terminal energy acceptor of cyanobacterial phycobilisomes. Proc. Natl. Acad. Sci. USA **87:**2152–2156.
116. **Houmard, J., V. Capuano, T. Cousin, and N. Tandeau de Marsac.** 1988. Genes encoding core components of the phycobilisome in the cyanobacterium *Calothrix* sp. strain 7601. Occurrence of a multigene family. J. Bacteriol. **170:**5512–5521.
117. **Houmard, J., V. Capuano, T. Cousin, and N. Tandeau de Marsac.** 1988. Isolation and molecular characterization of the gene encoding allophycocyanin B, a terminal energy acceptor in cyanobacterial phycobilisomes. Mol. Microbiol. **2:**101–107.
118. **Houmard, J., D. Mazel, C. Moguet, D. A. Bryant, and N. Tandeau de Marsac.** 1986. Organization and nucleotide sequence of genes encoding core components of the phycobilisomes from *Synechococcus* 6301. Mol. Gen. Genet. **205:**404–410.
119. **Isono, T., and T. Katoh.** 1987. Subparticles of *Anabaena* phycobilisomes. II. Molecular assembly of allophycocyanin cores in reference to 'anchor' protein. Arch. Biochem. Biophys. **256:**317–324.
120. **Jensen, T. E., and J. W. Rachlin.** 1984. Effect of varying sulphur deficiency on structural components of a cyanobacterium *Synechococcus leopoliensis*: a morphometric study. Cytobios **41:**35–46.
121. **Kalla, R., L. K. Lind, and P. Gustafsson.** 1989. Genetic analysis of phycobilisome mutants in the cyanobacterium *Synechococcus* sp. 6301. Mol. Microbiol. **3:**339–347.
122. **Klotz, A. V., and A. N. Glazer.** 1985. Characterization of the bilin attachment sites in R-phycoerythrin. J. Biol. Chem. **260:**4856–4863.
123. **Koller, K. P., W. Wehrmeyer, and E. Mörschel.** 1978. Biliprotein assembly in the disc-shaped phycobilisomes of *Rhodella violacea*. On the molecular composition of energy-transferring complexes (tripartite units) forming the periphery of the phycobilisome. Eur. J. Biochem. **91:**57–63.
124. **Kuhlbrandt, W., and D. N. Wang.** 1991. 3-Dimensional structure of plant light-harvesting complex determined by electron crystallography. Nature (London) **350:**130–134.
125. **Larkum, A., and J. Barrett.** 1983. Light harvesting processes in algae. Adv. Bot. Res. **10:**1–219.
126. **Lau, P. C. K., J. A. Condie, G. Alvarado-Urbina, and R. H. Lau.** 1987. Nucleotide sequence of phycocyanin β subunit of cyanobacterium *Anacystis nidulans* strain R2. Nucleic Acids Res. **15:**2394.
127. **Lau, R. H., G. Alvarado-Urbina, and P. C. K. Lau.** 1987. Phycocyanin α-subunit gene of *Anacystis nidulans* R2: cloning, nucleotide sequencing and expression in *Escherichia coli*. Gene **52:**21–29.
128. **Lau, R. H., S. D. Blakeley, G. Alvarado-Urbina, J. E. Bailly, J. A. Condie, and P. C. K. Lau.** 1989. Duplication of the phycocyanin operon in the unicellular cyanobacterium *Anacystis nidulans* R2. Gene **80:**375–380.
129. **Lau, R. H., M. M. Mackenzie, and W. F. Doolittle.** 1977. Phycocyanin synthesis and degradation in the blue-green bacterium *Anacystis nidulans*. J. Bacteriol. **132:**771–778.
130. **Laudenbach, D. E., and A. R. Grossman.** 1991. Characterization and mutagenesis of sulfur-regulated genes in a cyanobacterium: evidence for function in sulfate transport. J. Bacteriol. **173:**2739–2750.
131. **Lemaux, P. G., and A. R. Grossman.** 1984. Isolation and characterization of a gene for a major light-harvesting polypeptide from *Cyanophora paradoxa*. Proc. Natl. Acad. Sci. USA **81:**4100–4104.
132. **Lemaux, P. G., and A. R. Grossman.** 1985. Major light-harvesting polypeptides encoded in polycistronic transcript in eukaryotic algae. EMBO J. **4:**1911–1919.
133. **Lind, L. K., S. R. Kalla, A. Lönneborg, G. Öquist, and P. Gustafsson.** 1985. Cloning of the β phycocyanin gene from *Anacystis nidulans*. FEBS Lett. **188:**27–32.
134. **Lomax, T. L., P. B. Conley, J. Schilling, and A. R. Grossman.** 1987. Isolation and characterization of light-regulated phycobilisome linker polypeptide genes and their transcription as a polycistronic mRNA. J. Bacteriol. **169:**2675–2684.
135. **Lönneborg, A., L. K. Lind, S. R. Kalla, P. Gustafsson, and G. Öquist.** 1985. Acclimation processes in the light-harvesting system of the cyanobacterium *Anacystis nidulans* following a light shift from white to red. Plant Physiol. **78:**110–114.
136. **Lundell, D. J., and A. N. Glazer.** 1981. Allophycocyanin B. A common β subunit in *Synechococcus* allophycocyanin B (λmax 670 nm) and allophycocyanin (λmax 650 nm). J. Biol. Chem. **256:**12600–12606.
137. **Lundell, D. J., and A. N. Glazer.** 1983. Molecular architecture of a light-harvesting antenna. Quaternary interactions in the *Synechococcus* 6301 phycobilisome core as revealed by partial tryptic digestion and circular dichroism studies. J. Biol. Chem. **258:**8708–8713.
138. **Lundell, D. J., and A. N. Glazer.** 1983. Molecular architecture of a light-harvesting antenna. Structure of the 18S core-rod subassembly of *Synechococcus* 6301 phycobilisomes. J. Biol. Chem. **258:**894–901.
139. **Lundell, D. J., and A. N. Glazer.** 1983. Molecular architecture of light-harvesting antenna. Core substructure in *Synechococcus* 6301 phycobilisomes: two new allophycocyanin and allophycocyanin B complexes. J. Biol. Chem. **258:**902–908.
140. **Lundell, D. J., R. C. Williams, and A. N. Glazer.** 1981. Molecular architecture of light harvesting antenna: *in vitro* assembly of the rod substructures of *Synechococcus* 6301 phycobilisomes. J. Biol. Chem. **256:**3580–3592.
141. **Lundell, D. J., G. Yamanaka, and A. N. Glazer.** 1981. A terminal energy acceptor of the phycobilisome: the 75,000 dalton polypeptide of *Synechococcus* 6301 phycobilisomes, a new biliprotein. J. Cell Biol. **91:**315–319.
142. **MacColl, R., J. J. Lee, and D. S. Berns.** 1971. Protein aggregation in C-phycocyanin. Studies at very low concentrations with the photoelectric scanner of the ultracentrifuge. Biochem. J. **122:**421–426.
143. **Manodori, A., and A. Melis.** 1986. Cyanobacterial acclimation to photosystem I or photosystem II light. Plant Physiol. **82:**185–189.
144. **Manodori, A., and A. Melis.** 1986. Light quality regulates photosystem stoichiometry in cyanobacteria, p. 653–662. *In* G. Akoyunoglou and H. Senger (ed.), Regulation of chloroplast differentiation. Alan R. Liss, Inc., New York.
145. **Maxson, P., K. Sauer, J. Zhou, D. A. Bryant, and A. N. Glazer.** 1989. Spectroscopic studies of cyanobacterial phycobilisomes lacking core polypeptides. Biochim. Biophys. Acta **977:**40–51.
146. **Mazel, D., C. Bernard, R. Schwarz, A. M. Castets, J. Houmard, and N. Tandeau de Marsac.** 1991. Characterization of two insertion sequences, IS701 and IS702, from the cyanobacterium *Calothrix* sp. PCC 7601. Mol. Microbiol. **5:**2165–2170.
147. **Mazel, D., G. Guglielmi, J. Houmard, W. Sidler, D. A. Bryant, and N. Tandeau de Marsac.** 1986. Green light induces transcription of phycoerythrin operon in the cyanobacterium *Calothrix*

7601. Nucleic Acids Res. 14:8279–8290.

148. **Mazel, D., J. Houmard, A. M. Castets, and N. Tandeau de Marsac.** 1990. Highly repetitive DNA sequences in cyanobacterial genome. J. Bacteriol. 172:2755–2761.
149. **Mazel, D., J. Houmard, and N. Tandeau de Marsac.** 1988. A multigene family in *Calothrix* sp. PCC 7601 encodes phycocyanin, the major component of the cyanobacterial light-harvesting antenna. Mol. Gen. Genet. 211:296–304.
150. **Mazel, D., and P. Marliere.** 1989. Adaptive eradication of methionine and cysteine from cyanobacterial light-harvesting proteins. Nature (London) 341:245–248.
151. **Melis, A., A. Manodori, R. E. Glick, M. L. Ghirardi, S. W. McCauley, and P. J. Neale.** 1985. The mechanism of photosynthetic membrane adaptation to environmental stress conditions: a hypothesis on the role of electron transport capacity and of ATP/NADPH pool in the regulation of thylakoid membrane organization and function. Physiol. Veg. 23:757–765.
152. **Miller, L. S., and S. C. Holt.** 1977. Effect of carbon dioxide on pigment and membrane content in *Synechococcus lividus*. Arch. Microbiol. 115:185–198.
153. **Miranda-Rios, J., R. Sanchez Pescador, M. Urdea, and A. Cavarrubias.** 1987. The complete nucleotide sequence of *glnALG* operon of *E. coli* K12. Nucleic Acids Res. 15:2757–2770.
154. **Mörschel, E., K. P. Koller, W. Wehrmeyer, and H. Schneider.** 1977. Biliprotein assembly in the disc-shaped phycobilisomes of *Rhodella violacea*. I. Electron microscopy of phycobilisomes in situ and analysis of their architecture after isolation and negative staining. Cytobiologie 16:118–129.
155. **Muñoz-Dorado, J., S. Inouye, and M. Inouye.** 1991. A gene encoding a protein-serine/threonine kinase is required for normal development of *M. xanthus*, a gram negative bacterium. Cell 67:995–1006.
156. **Murphy, D. J.** 1986. The molecular organization of the photosynthetic membranes of higher plants. Biochim. Biophys. Acta 864:33–94.
157. **Neufeld, G. S., and A. F. Riggs.** 1969. Aggregation properties of C-phycocyanin from *Anacystis nidulans*. Biochim. Biophys. Acta 181:234–243.
158. **O'Carra, P.** 1970. Algal biliproteins. Biochem. J. 119:2P.
159. **Oelmüller, R., P. B. Conley, N. Federspiel, W. R. Briggs, and A. R. Grossman.** 1988. Changes in accumulation and synthesis of transcripts encoding phycobilisome components during acclimation of *Fremyella diplosiphon* to different light qualities. Plant Physiol. 88:1077–1083.
160. **Oelmüller, R., A. R. Grossman, and W. R. Briggs.** 1988. Photoreversibility of the effect of red and green light pulses on the accumulation in darkness of mRNAs coding for phycocyanin and phycoerythrin in *Fremyella diplosiphon*. Plant Physiol. 88:1084–1091.
161. **Oelmüller, R., A. R. Grossman, and W. R. Briggs.** 1989. Role of protein synthesis in regulation of phycobiliprotein mRNA abundance by light quality in *Fremyella diplosiphon*. Plant Physiol. 90:1486–1499.
162. **Ohad, I., H. A. W. Schneider, S. Gendel, and L. Bogorad.** 1980. Light-induced changes in allophycocyanin. Plant Physiol. 65: 6–12.
163. **Ohki, K., and Y. Fujita.** 1979. *In vivo* transformation of phycobiliproteins during bleaching of *Tolypothrix tenuis* to forms active in photoreversible absorption changes. Plant Cell Physiol. 20:1341–1347.
164. **Ohki, K., and Y. Fujita.** 1979. Photoreversible absorption changes of guanidine-HCl-treated phycocyanin and allophycocyanin isolated from the blue-green alga *Tolypothrix tenuis*. Plant Cell Physiol. 20:483–490.
165. **Ohki, K., and Y. Fujita.** 1981. On the relationship between photocontrol of phycoerythrin formation and the photoreversible pigment of Scheibe. Plant Cell Physiol. 22:347–357.
166. **Ohki, K., M. Watanabe, and Y. Fujita.** 1982. Action of near UV and blue light on the photocontrol of phycobiliprotein formation: A complementary chromatic adaptation. Plant Cell Physiol. 23:651–656.
167. **Ong, L. J., and A. N. Glazer.** 1991. Phycoerythrins of marine unicellular cyanobacteria. I. Bilin types and locations and energy transfer pathways in *Synechococcus* sp. phycoerythrins. J. Biol. Chem. 266:9515–9527.
168. **Ong, L. J., A. N. Glazer, and J. B. Waterbury.** 1984. An unusual phycoerythrin from a marine cyanobacterium. Science 224:80–83.
169. **Öquist, G.** 1974. Distribution of chlorophyll between the two photoreactions in photosynthesis of the blue-green alga *Anacystis nidulans* grown at two different light intensities. Physiol. Plant. 30:38–40.
170. **Öquist, G.** 1974. Light-induced changes in pigment composition of photosynthetic lamellae and cell-free extracts obtained from the blue-green alga *Anacystis nidulans*. Physiol. Plant. 30:45–48.
171. **Owens, T. G., and E. R. Wold.** 1986. Light harvesting function in the diatom *Phaeodactylum tricornutum*. Plant Physiol. 80: 732–738.
172. **Pilot, T. J., and J. L. Fox.** 1984. Cloning and sequencing of the genes encoding α and β subunits from the cyanobacterium *Agmenellum quadruplicatum*. Proc. Natl. Acad. Sci. USA 81:6983–6987.
173. **Platt, T.** 1986. Transcription termination and the regulation of gene expression. Annu. Rev. Biochem. 55:339–372.
174. **Porter, G., G. J. Tredwell, G. F. W. Searle, and J. Barber.** 1978. Picosecond time-resolved energy transfer in *Porphyridium cruentum*. I. In the intact chloroplast. Biochim. Biophys. Acta 501:532–545.
175. **Ray, J. M., D. Bhaya, M. A. Block, and A. R. Grossman.** 1991. Isolation, transcription, and inactivation of the gene for an atypical alkaline phosphatase of *Synechococcus* sp. strain PCC 7942. J. Bacteriol. 173:4297–4309.
176. **Redlinger, T., and E. Gantt.** 1982. An M_r 95,000 polypeptide in *Porphyridium cruentum* phycobilisomes and thylakoids: possible function in linkage of phycobilisomes to thylakoids and in energy transfer. Proc. Natl. Acad. Sci. USA 79:5542–5546.
177. **Reithman, H., G. Bullerjahn, K. J. Reddy, and L. A. Sherman.** 1988. Regulation of cyanobacterial pigment-protein composition and organization by environmental factors. Photosynth. Res. 18:133–161.
178. **Rhie, G., and S. I. Beale.** 1992. Biosynthesis of phycobilins. Ferredoxin-supported NADPH-independent heme oxygenase and phycobilin-forming activities from *Cyanidium caldarium*. J. Biol. Chem. 267:16088–16093.
179. **Roell, M. K., and D. Morse.** 1991. Fractionation of nuclear, chloroplast and mitochondria DNA from *Polysiphonia boldii* (Rhodophyta) using a rapid and simple method for the simultaneous isolation of RNA and DNA. J. Phycol. 27:299–305.
180. **Roush, C. L., P. J. Kennelly, M. B. Glaccum, D. H. Hellman, J. D. Scott, and E. G. Krebs.** 1988. Isolation of cDNA encoding rat skeletal muscle light chain kinase. J. Biol. Chem. 263: 10510–10516.
181. **Rümbeli, R., T. Schirmer, W. Bode, W. Sidler, and H. Zuber.** 1985. Crystallization of phycoerythrocyanin from the cyanobacterium *Mastigocladus laminosus* and a preliminary characterization of two crystal forms. J. Mol. Biol. 186:197–200.
182. **Schäfer, E., and W. R. Briggs.** 1986. Photomorphogenesis from signal perception to gene expression. Photobiochem. Photobiophys. 12:305–320.

182a. **Schaefer, M. R., and A. R. Grossman.** Unpublished data.

183. **Scheibe, J.** 1972. Photoreversible pigment: occurrence in a blue-green alga. Science 176:1037–1039.
184. **Schirmer, T., W. Bode, R. Huber, W. Sidler, and H. Zuber.** 1985. X-ray crystallographic structure of the light-harvesting biliprotein C-phycocyanin from thermophilic cyanobacterium *Mastigocladus laminosus* and its resemblance to globin structure. J. Mol. Biol. 184:257–277.
185. **Schirmer, T., R. Huber, M. Schneider, W. Bode, M. Miller, and M. Hackert.** 1986. Crystal structure analysis and refinement at 2.5 Å of hexameric C-phycocyanin from the cyanobacterium *Agmenellum quadruplicatum*: the molecular model and its implication for light-harvesting. J. Mol. Biol. 188:651–676.
186. **Schirmer, T., and M. G. Vincent.** 1987. Polarized absorption and fluorscence spectra of single crystals of C-phycocyanin. Biochim. Biophys. Acta 893:379–385.

187. **Schmidt, A., I. Erdle, and H.-P. Kost.** 1982. Changes of c-phycocyanin in *Synechococcus* 6301 in relation to growth on various sulfur compounds. Z. Naturforsch. **37c**:870–876.
188. **Schuster, A., H. Köst, W. Rudiger, and J. Eder.** 1983. Investigations on the apoprotein of phycocyanin from *Cyanidium caldarium*. Arch. Microbiol. **135**:30–35.
189. **Scott, E., and D. S. Berns.** 1965. Protein-protein interaction. The phycocyanin system. Biochemistry **4**:2597–2607.
190. **Searle, G. F. W., J. Barber, G. Porter, and C. J. Tredwell.** 1978. Picosecond time-resolved energy transfer in *Porphyridium cruentum*. II. In the isolated light-harvesting complex (phycobilisomes). Biochim. Biophys. Acta **501**:246–256.
191. **Seki, T., H. Yoshikawa, H. Takahashi, and H. Saito.** 1987. Cloning and nucleotide sequence of *phoP*, the regulatory gene for alkaline phosphatase and phosphodiesterase in *Bacillus subtilis*. J. Bacteriol. **169**:2913–2916.
192. **Sherman, D. M., and L. A. Sherman.** 1983. Effect of iron deficiency and iron restoration on ultrastructure of *Anacystis nidulans*. J. Bacteriol. **156**:393–401.
193. **Shivji, M. S.** 1991. Organization of the chloroplast genome in the red alga *Porphyra vezoensis*. Curr. Genet. **19**:49–55.
194. **Sidler, W., J. Gysi, E. Isker, and H. Zuber.** 1981. The complete amino acid sequence of both subunits of allophycocyanin, a light harvesting protein-pigment complex from the cyanobacterium *Mastigocladus laminosus*. Hoppe-Seyler's Z. Physiol. Chem. **362**:611–618.
195. **Siebzehnrübl, S., R. Fischer, and H. Scheer.** 1987. Chromophore assignment in C-phycocyanin from *Mastigocladus laminosus*. Z. Naturforsch. Sect. C **42**:258–262.
196. **Simon, R. D.** 1987. Inclusion bodies in the cyanobacteria: cyanophycin, polyphosphate, polyhedral bodies, p. 199–225. *In* P. Fay and C. Van Baalen (ed.), The cyanobacteria. Elsevier Science Publishers B.V., Amsterdam.
196a. **Sobczyk, A., G. Schyns, N. Tandeau de Marsac, and J. Houmard.** 1993. Transduction of the light signal during complementary chromatic adaptation in the cyanobacterium *Calothrix* sp. PCC 7601: DNA binding proteins and modulation by phosphorylation. EMBO J. **12**:997–1004.
197. **Srivastava, G., I. A. Borthwick, D. J. Macquire, C. J. Elferink, M. J. B. Bawden, and B. K. May.** 1988. Regulation of 5-aminolevulinate synthase mRNA in different rat tissues. J. Biol. Chem. **263**:5202–5209.
198. **Steinmüller, K., M. Kaling, and K. Zetsche.** 1983. *In vitro* synthesis of phycobiliproteins and ribulose-1,5-bisphosphate carboxylase by non-polyadeylated RNA of *Cyanidium caldarium* and *Porphyridium aerugineum*. Planta **159**:308–313.
199. **Stevens, S. E., and D. A. M. Poane.** 1981. Accumulation of cyanophycin granules as a result of phosphate limitation in *Agmenellum quadruplicatum*. Plant Physiol. **67**:716–719.
200. **Stock, J. B., A. J. Ninfa, and A. M. Stock.** 1989. Protein phosphorylation and regulation of adaptive responses in bacteria. Microbiol. Rev. **53**:450–490.
201. **Swanson, R. V., L. J. Ong, S. M. Wilbanks, and A. N. Glazer.** 1991. Phycoerythrins of marine unicellular cyanobacteria. II. Characterization of phycobiliproteins with unusually high phycourobilin content. J. Biol. Chem. **266**:9528–9534.
202. **Swanson, R. V., J. Zhou, J. A. Leary, T. Williams, R. de Lorimier, D. A. Bryant, and A. N. Glazer.** 1992. Characterization of phycocyanin produced by *cpcE* and *cpcF* mutants and identification of an intergenic suppressor of the defect in bilin attachment. J. Biol. Chem. **267**:16146–16154.
203. **Tandeau de Marsac, N.** 1977. Occurrence and nature of chromatic adaptation in cyanobacteria. J. Bacteriol. **130**:82–91.
204. **Tandeau de Marsac, N.** 1983. Phycobilisomes and complementary adaptation in cyanobacteria. Bull. Inst. Pasteur **81**:201–254.
205. **Tandeau de Marsac, N., and G. Cohen-Bazire.** 1977. Molecular composition of cyanobacterial phycobilisomes. Proc. Natl. Acad. Sci. USA **74**:1635–1639.
206. **Tandeau de Marsac, N., D. Mazel, V. Capuano, T. Damerval, and J. Houmard.** 1990. Genetic analysis of the cyanobacterial light-harvesting antenna complex, p. 143–153. *In* G. Drews (ed.), The molecular biology of membrane-bound complexes in phototrophic bacteria. Plenum Publishing Corp., New York.
207. **Tandeau de Marsac, N., D. Mazel, T. Damerval, G. Guglielmi, V. Capuano, and J. Houmard.** 1988. Photoregulation of gene expression in the filamentous cyanobacterium *Calothrix* sp. 7601: light-harvesting complexes and cell differentiation. Photosynth. Res. **18**:99–132.
208. **Thompson, W. F., and W. J. White.** 1991. Physiological and molecular studies of light-regulated nuclear genes in higher plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. **42**:423–466.
209. **Tobin, E., and J. Silverthorne.** 1985. Light regulation of gene expression in higher plants. Annu. Rev. Plant Physiol. **36**:569–593.
210. **Tomizawa, K. I., A. Nagatani, and M. Furuya.** 1990. Phytochrome genes: studies using the tools of molecular biology and photomorphogenetic mutants. Photochem. Photobiol. **51**:265–276.
211. **Troxler, R. F.** 1972. Synthesis of bile pigments in plants. Formation of carbon monoxide and phycocyanobilin in wild type and mutant strains of the alga, *Cyanidium caldarium*. Biochemistry **11**:4235–4242.
212. **Troxler, R. F., A. S. Brown, and S. B. Brown.** 1979. Bile pigment synthesis in plants. Mechanism of ^{18}O incorporation into phycocyanobilin in the unicellular rhodophyte, *Cyanidium caldarium*. J. Biol. Chem. **254**:3411–3418.
213. **Troxler, R. F., M. M. Ehrhardt, A. S. Brown-Mason, and G. D. Offner.** 1981. Primary structure of phycocyanin from the unicellular rhodophyte *Cyanidium caldarium*. II. Complete amino acid sequence of the β subunit. J. Biol. Chem. **256**:12176–12184.
214. **Troxler, R. F., L. S. Greenwald, and B. A. Zilinskas.** 1980. Allophycocyanin from *Nostoc* sp. phycobilisomes. Properties and amino acid sequence at the NH_2 terminus of the α and β subunits of allophycocyanins I, II and III. J. Biol. Chem. **255**:9380–9387.
215. **Troxler, R. F., S. Lin, and G. D. Offner.** 1989. Heme regulates expression of phycobiliprotein photogenes in the unicellular rhodophyte, *Cyanidium caldarium*. J. Biol. Chem. **264**:20596–20601.
216. **Vogelman, T. C., and J. Scheibe.** 1978. Action spectrum for chromatic adaptation in the blue-green alga *Fremyella diplosiphon*. Planta **143**:233–239.
217. **Wanner, G., G. Henkelmann, A. Schmidt, and H.-P. Kost.** 1986. Nitrogen and sulfur starvation of the cyanobacterium *Synechococcus* 6301. An ultrastructural, morphometrical, and biochemical comparison. Z. Naturforsch. **41c**:741–750.
218. **Wealand, J. L., J. A. Myers, and R. Hirschberg.** 1989. Changes in gene expression during nitrogen starvation in *Anabaena variabilis* ATCC 29413. J. Bacteriol. **171**:1309–1313.
219. **Wilbanks, S. M., R. de Lorimier, and A. N. Glazer.** 1991. Phycoerythrins of marine unicellular cyanobacteria. III. Sequence of a class II phycoerythrin. J. Biol. Chem. **266**:9535–9539.
220. **Williams, R. C., J. C. Gingrich, and A. N. Glazer.** 1980. Cyanobacterial phycobilisomes. Particles from *Synechocystis* 6701 and two pigment mutants. J. Cell Biol. **85**:558–566.
221. **Williams, V. P., and A. N. Glazer.** 1978. Structural studies on phycobiliproteins. I. Bilin-containing peptides of C-phycocyanin. J. Biol. Chem. **253**:202–211.
222. **Wood, N. B., and R. Haselkorn.** 1979. Proteinase activity during heterocyst differentiation in nitrogen-fixing cyanobacteria, p. 159–166. *In* G. H. Cohen and H. Holzer (ed.), Limited proteolysis in microorganisms. U.S. Department of Health, Education, and Welfare Publication no. (NIH) 79-1591. National Institutes of Health, Bethesda, Md.
223. **Wood, N. B., and R. Haselkorn.** 1980. Control of phycobiliprotein proteolysis and heterocyst differentiation in *Anabaena*. J. Bacteriol. **141**:1375–1385.
224. **Wyman, M., R. P. F. Gregory, and N. G. Carr.** 1985. Novel role for phycoerythrin in a marine cyanobacterium, *Synechococcus* strain DC2. Science **230**:818–820.
225. **Yamanaka, G., and A. N. Glazer.** 1980. Dynamic aspects of phycobilisome structure. Phycobilisome turnover during nitro-

gen starvation in *Synechococcus* sp. Arch. Microbiol. **124**:39–47.

226. **Yamanaka, G., A. N. Glazer, and R. C. Williams.** 1980. Molecular architecture of a light-harvesting antenna. Comparison of wild type and mutant *Synechococcus* 6301 phycobilisomes. J. Biol. Chem. **255**:11004–11010.
227. **Yoshida, T., P. Biro, T. Cohen, R. M. Muller, and S. Shibihara.** 1988. Human heme oxygenase cDNA and induction of its mRNA by hemin. Eur. J. Biochem. **171**:457–461.
228. **Zhou, J., G. E. Gasparich, V. L. Stirewalt, R. de Lorimier, and D. A. Bryant.** 1992. The *cpcE* and *cpcF* genes of *Synechococcus* sp. PCC 7002. Construction and phenotypic characterization of interposon mutants. J. Biol. Chem. **267**:16138–16145.
229. **Zuber, H.** 1983. Structure and function of the light-harvesting phycobiliproteins from the cyanobacterium *Mastogocladus laminosus*, p. 23–41. *In* G. Papageorgiou and L. Packer (ed.), Photosynthetic prokaryotes: cell differentiation and function. Elsevier Science Publishing Co., Amsterdam.
230. **Zuber, H.** 1986. Structure of light harvesting antenna complexes of photosynthetic bacteria, cyanobacteria and red algae. Trends Biochem. Sci. **11**:414–419.