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MicroReview

Sensing the light: photoreceptive systems and signal transduction in cyanobacteria

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Summary

Photosynthetic prokaryotes have highly developed abilities to detect and react to environmental signals. Light sensing is one of the most important capabilities of organisms that use light for photosynthesis and photomorphogenesis. This review addresses photoreception in cyanobacteria from the perception of light through the physiological responses observed in response to light-dependent signalling. Recent progress made in our understanding of the structure and function of photosensory receptors and their downstream effector molecules is discussed.

Introduction

All photosynthetic organisms possess finely tuned capacities to sense and respond to changes in their environment. The perception of light and the developmental changes that occur as a response to light signals are arguably the most important adaptive responses of any organism that uses light for carbon fixation and generation of reductant. Thus, photosynthetic organisms contain a range of proteins that allow them to detect and respond to light, including photosynthetic and photosensory proteins.

As the sequencing of prokaryotic genomes has progressed, it has become apparent that many bacterial systems possess photoreceptors that are related in sequence and function to eukaryotic photoreceptors. As these data began to emerge, it had already been noted that complementary chromatic adaptation, a specific form of photomorphogenesis in some cyanobacteria, was under the control of a biliprotein photoreceptor with significant similarity to plant phytochromes (Kehoe and Grossman, 1996). In higher plants, the biliprotein phytochromes covalently bind a linear tetrapyrrole chromophore (bilin) and control many aspects of growth and development, from seed germination through senescence. Although phytochromes and phytochrome-related proteins have been found in a wide range of organisms from eubacteria through higher plants, cyanobacteria contain a larger number of proteins harbouring chromophore-binding GAF domains than any other group of organisms examined to date (Ohmori *et al*., 2001; Okamoto and Ohmori, 2003; see description of GAF domains in Box 1). These organisms also contain three recognized classes of flavin-based blue light receptors: cryptochromes, phototropin-like photoreceptors and BLUF proteins (van der Horst and Hellingwerf, 2004). Cyanobacterial genomes also possess genes encoding sensory rhodopsins (reviewed by Spudich, 2006) and other novel photosensory proteins. In recent years, a great deal of progress has been made in the study of cyanobacterial photoreceptors and light sensing in these organisms. In this review, I will summarize the recent developments that have advanced our understanding of photosensory proteins and the photomorphogenetic responses they control in cyanobacteria.

Overview of photosensing and photomorphogenesis in cyanobacteria

Cyanobacteria are aquatic, Gram-negative prokaryotes that range from unicellular/colonial to filamentous to branched filamentous in form. Cyanobacteria are autotrophic and utilize oxygen-evolving photosynthesis to produce fixed carbon, as do higher plants. Cyanobacteria possess photosynthetic light-harvesting antennae called phycobilisomes. These phycobilisomes contain light-absorbing phycobiliproteins that have covalently attached, linear tetrapyrrole chromophores. The phycobiliproteins absorb light and transfer light energy to photosystem II for photosynthesis.

In addition to carrying out photosynthesis, cyanobacteria exhibit many acclimation or adaptation responses to light. For example, many cyanobacteria possess the ability to alter dramatically the composition of their

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Box 1. Conserved domains in cyanobacterial photosensory proteins.

Photosensory domains

BLUF domain
Sensors of blue light using FAD domains bind FAD non-covalently (Gomelsky and Klug, 2002). BLUF domains represent novel flavin-binding domains distinct from PAS and photolyase domains.

GAF domain (cGMP phosphodiesterase/adenyl cyclase/FhlA)
Similar to PAS domains in structure, GAF domains (Aravind and Ponting, 1997) are found in cGMP-specific phosphodiesterases and phytochromes and phytochrome-related proteins. In phytochrome-class receptors, GAF domains covalently bind a linear tetrapyrrole chromophore.

LOV domains
LOV domains, involved in sensing light, oxygen and voltage, are a subgroup of the larger PAS domain family (Crosson *et al*., 2003). These domains are conserved FMN-binding domains found in blue light-responsive phototropins.

PAS domains (Per/ARNT/Sim)
PAS domains are involved in sensing changes in light, oxygen, redox potential and the binding of small ligands (Taylor and Zhulin, 1999). The roles of PAS domains are undefined for most receptors in which they are found, although a PAS domain has been shown to act as the site of chromophore attachment for at least one bacterial photoreceptor, resulting in the production of a blue light photosensor (Baca *et al*., 1994). More recently, novel bacteriophytochromes have been shown to utilize their PAS domains to regulate gene expression through light-regulated protein–protein interactions (Giraud *et al*., 2002). Other recent results suggest that the PAS domain of RcaE is critical for light-dependent regulation of CCA in the cyanobacterium *Fremyella diplosiphon* (J.R. Bordowitz and B.L. Montgomery, unpubl. data).

Output domains

Histidine kinase domains
Two-component sensory kinases autophosphorylate on a histidine residue and transfer the phosphate to an aspartate residue of a cognate response regulator (reviewed in West and Stock, 2001). These phosphorylation events initiate a signalling cascade that ultimately results in metabolic and/or physiological cellular responses in an organism. The output domain for many photosensory proteins is a histidine kinase domain. These light sensors often serve as sensor kinases that are involved in two-component systems.

Serine/threonine kinase domains
Many proteins also have been shown to be phosphorylated on serine or threonine residues in cyanobacteria in response to growth under various light conditions (Zhang *et al*., 2005). Notably, two proteins encoding serine/threonine kinases, which also contain classical two-component sensor domains, were found to be induced by red light in the cyanobacterium *F. diplosiphon* (Stowe-Evans *et al*., 2004). These results suggest that the regulation of phosphorylation events at serine and threonine residues is likely important for photosensing in cyanobacteria.

GGDEF and EAL domains
These domains, which are named after conserved amino acid residues, are involved in the turnover of bis-(3′,5′)-cyclic dimeric guanosine monophosphate (c-di-GMP). GGDEF domains participate in the synthesis/production of c-di-GMP, whereas EAL domains are involved in c-di-GMP hydrolysis to linear diguanylate pGpG. A significant number of cytoplasmic PAS and GAF domain-containing proteins also contain GGDEF and/or EAL domains (Romling *et al*., 2005).

Methyl-accepting chemotaxis protein (MCP) signalling domain
MCP signalling domains are conserved domains containing methyl-accepting sites (these are glutamate residues) that undergo reversible methylation in response to environmental stimuli (Bourret and Stock, 2002).

phycobilisomes in response to changes in the prevalent wavelengths of light in their ambient environment (Palenik, 2001; Everroad *et al*., 2006; Kehoe and Gutu, 2006). This ability, termed chromatic adaptation, is exhibited in a variety of forms in different species of cyanobacteria. Cyanobacteria also move in response to light in a process called phototaxis.

Cyanobacterial sensory proteins initiate a signal transduction cascade in response to an environmental signal. These signalling cascades generally consist of three steps: signal perception, signal transduction and cellular responses (see Fig. 1). Many of these pathways are phosphotransfer cascades commonly known as two-component systems. Two-component systems are based on two signal transduction components, including a sensor histidine kinase and a response regulator (reviewed by Stock *et al*., 2000; Mascher *et al*., 2006). Cyanobacteria contain large numbers of two-component signalling proteins (Ashby and Houmard, 2006). These proteins are involved in the regulation of many different physiological processes. Apart from phosphotransfer reactions, alternative transduction pathways are utilized in cyanobacterial signal transduction cascades, including bis-(3′,5′)-cyclic dimeric guanosine monophosphate (c-di-GMP) metabolism (reviewed by Romling *et al*., 2005; Ryan *et al*., 2006), cyclic AMP (cAMP) signalling and reversible methylation events (see Fig. 1).

Photoperception and photosensory proteins

Sensors used by cyanobacteria to perceive and respond to light include phytochromes and phytochrome-related receptors, blue light photosensors, rhodopsins and UV receptors. Cyanobacterial photoreceptors contain a

number of conserved domains involved in the perception and transduction of the light signal (see Box 1).

Phytochrome-class photoreceptors

RcaE (Regulator of chromatic adaptation) was the first functional phytochrome-like protein identified in a prokaryotic system (Kehoe and Grossman, 1996). RcaE, which controls complementary chromatic adaptation (CCA), is required for both red and green light responsiveness in *Fremyella diplosiphon*. RcaE binds a bilin chromophore on a conserved cysteine residue *in vivo*, much like higher-plant phytochromes. Molecular genetic studies show that the bilin chromophore specifically participates in green light responsiveness; some other domain of RcaE or an associated protein is responsible for red light responsiveness (Terauchi *et al*., 2004). Following the identification of *rcaE*, *plpA* (phytochrome-like protein) from *Synechocystis* sp. PCC 6803 was isolated and characterized (Wilde *et al*., 1997). Results from these studies indicate that the phytochrome-related PlpA is required for growth of *Synechocystis* under blue light (Wilde *et al*., 1997).

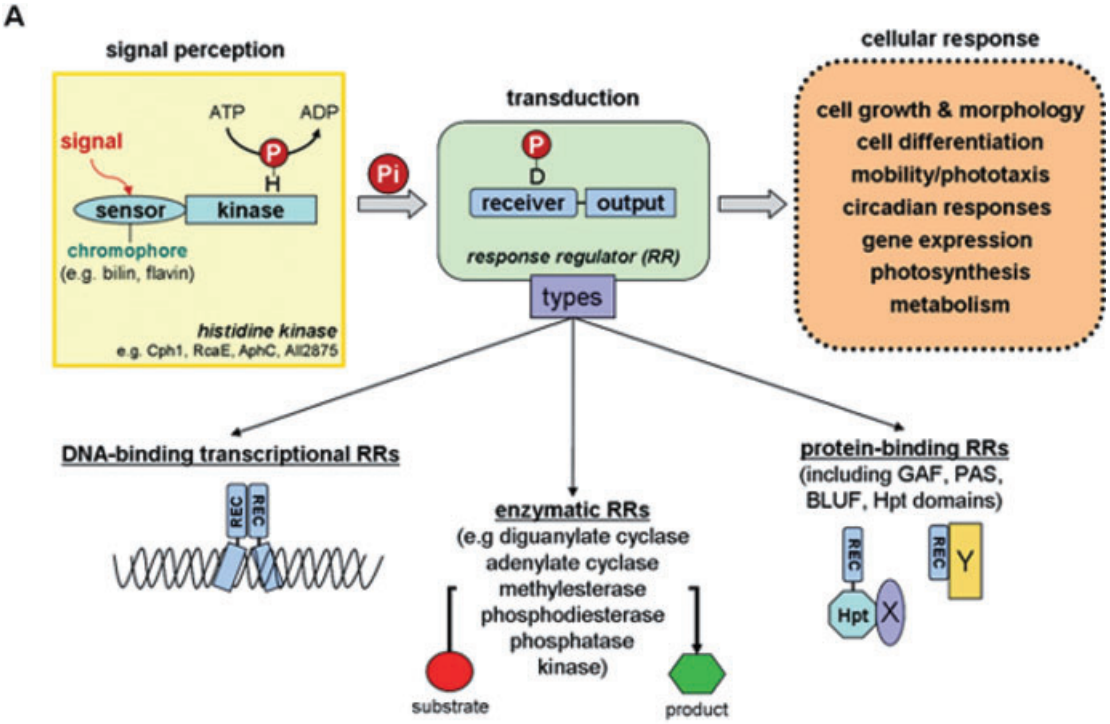
Following the genetic identification of physiological roles for the proteins encoded by *rcaE* and *plpA*, a gene encoding cyanobacterial phytochrome (*cph1*) was identified in the *Synechocystis* sp. PCC 6803 genome, based on sequence similarity to higher-plant phytochromes (Hughes *et al*., 1997; Yeh *et al*., 1997). Yeh *et al*. (1997) demonstrated that *cph1* occurs in an operon directly upstream of a gene that encodes response regulator Rcp1 (response regulator for cyanobacterial phytochrome). Cph1 is a light-regulated biliprotein kinase that is capable of phosphotransfer to Rcp1 *in vitro* (Yeh *et al*., 1997). *In vitro* spectral analysis of Cph1 demonstrated that it undergoes classic red/far-red reversibility, reminiscent of higher-plant phytochromes (Hughes *et al*., 1997; Yeh *et al*., 1997). The N-terminal domain of Cph1 has since been shown to dimerize in a light-dependent fashion. Pfr dimers, a molecular species containing two phytochrome molecules in the far-red absorbing state, are more stable than Pr dimers, which contain two phytochromes in the red-absorbing state (Strauss *et al*., 2005).

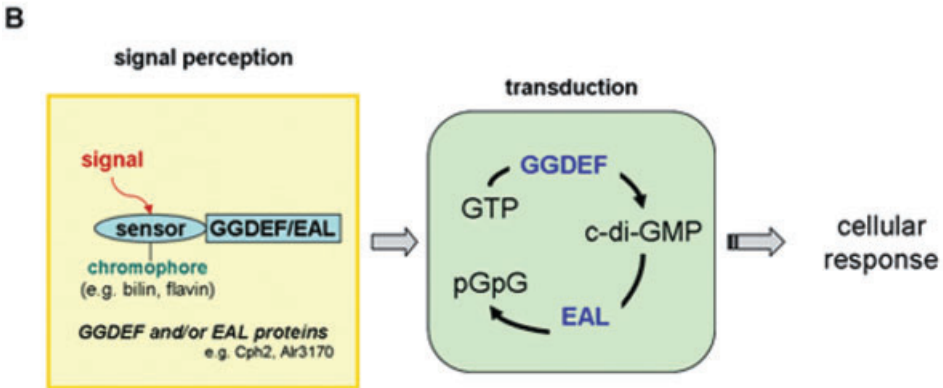
A second cyanobacterial phytochrome, Cph2, was also identified from *Synechocystis* and shown to covalently attach a bilin (Park *et al*., 2000). In contrast to Cph1, Cph2 lacks a histidine kinase domain and as an alternative contains GGDEF and EAL output domains (Montgomery and Lagarias, 2002; see description in Box 1). Recently, two prokaryotic phytochrome-like molecules containing a conserved chromophore-binding GAF domain and GGDEF and EAL output domains were identified in the anoxygenic photosynthetic bacterium *Rhodobacter sphaeroides* (Tarutina *et al*., 2006). These proteins were shown to possess red/far-red reversible photochromicity *in vitro* and a truncated derivative of one of these proteins, BphG1, shows light-dependent c-di-GMP phosphodiesterase activity (Tarutina *et al*., 2006). C-di-GMP is a novel second messenger in bacteria (reviewed by Romling *et al*., 2005; Ryan *et al*., 2006). This report of a light-regulated, non-kinase enzymatic activity for a phytochrome-related molecule and the occurrence of GGDEF and EAL domains suggests that Cph2 may also act as a light-responsive, non-kinase enzyme regulating a physiological response *in vivo*.

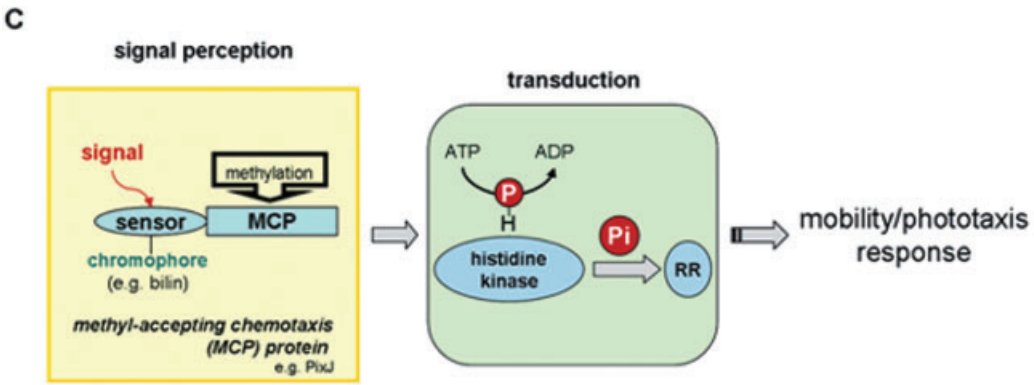
Based on sequence similarities to higher-plant phytochromes, two canonical phytochromes also have been reported for *F. diplosiphon* (also referred to as *Calothrix* sp. PCC 7601), which possesses the aforementioned phytochrome-like protein RcaE (Hübschmann *et al*., 2001). Both of these phytochromes: (i) are located in operons with cognate response regulators, (ii) autophosphorylate *in vitro* in an ATP-dependent fashion and (iii) transfer a phosphate to respective response regulators in a light-dependent manner (Hübschmann *et al*., 2001).

A cluster of genes that exhibit similarity to the *che* genes involved in chemotaxis in *Escherichia coli* were identified and found to be involved in positive phototaxis in *Synechocystis* sp. strain PCC 6803 (Yoshihara *et al*., 2000). One of the genes (*pisJ1*) encodes a protein containing domains with similarity to the chromophore-binding motif

Fig. 1. Summary of photosensory signalling in cyanobacteria. Photosensory signalling cascades consist of three major stages, including signal perception, transduction and cellular responses. Light signals are perceived by sensory molecules – chromophore-bound sensory proteins.
A. Many photosensory molecules are ATP-dependent sensory kinases that initiate phosphorelay cascades, which consist of transfer of a phosphate moiety in the transduction of the light signal. Many of the targets of the phosphorelay are response regulator (RR)-type molecules. A variety of response regulators exist in cyanobacteria, including DNA-binding, enzymatic and protein-binding RRs. The actions of the RRs ultimately result in observable cellular responses. GAF, domain present in phytochromes and cGMP-specific phosphodiesterases; PAS, Per/ARNT/Sim domain; BLUF, sensor of blue light using FAD; Hpt, histidine-containing phosphotransfer domain.
B. An alternative class of photosensory molecules utilizes GGDEF and EAL domains as output components. These domains participate in bis-(3′, 5′)-cyclic dimeric guanosine monophosphate (c-di-GMP) turnover. GGDEF activity results in the production of c-di-GMP from GTP, whereas EAL activity results in the production of diguanylate pGpG from c-di-GMP hydrolysis. The targets of c-di-GMP in cyanobacterial systems are still under investigation.
C. Cyanobacterial photosensory proteins also utilize methyl-accepting chemotaxis protein domains (MCP) as output components. Light absorption results in reversible methylation of the MCP domain. The signal is then transduced via the activity of a histidine kinase and response regulator to effect a cellular motility response.







of phytochromes and methyl-accepting chemotaxis protein receptors (see description in Box 1). In 2003, an independent report included evidence that TaxD1, which is the same as PisJ1, is involved in regulating positive phototaxis in response to red light that is photoreversed by exposure to far-red light (Ng *et al*., 2003). Subsequent biochemical characterization resulted in the novel finding that PixJ1, formerly identified as PisJ1, is a blue/green-photoreversible biliprotein that regulates positive phototaxis in *Synechocystis* sp. strain PCC 6803 (Yoshihara *et al*., 2004). No absorption of red or far-red light by PixJ1 was detected in this study. PixJ1 purified from *Synechocystis* contains a conserved cysteine in the second GAF domain that is necessary for covalent chromophore attachment (Yoshihara *et al*., 2004). Based on the wavelength maxima of the holoprotein of 425–435 nm in the blue region and 535 nm in the green region of the visible spectrum, the authors hypothesized that bilirubin may be the chromophore that attaches to PixJ1.

A subsequent report focused on reconstituting PixJ1 holoprotein in bilin-producing *E. coli* cells to determine definitively the structure of the PixJ1 chromophore (Yoshihara *et al*., 2006). For these studies, *pixJ1* was coexpressed in bacterial cells presumably accumulating one of four linear tetrapyrrole bilins – biliverdin IXα (BV), bilirubin (BR), phycocyanobilin (PCB) or phycocyanorubin (PCR) (Yoshihara *et al*., 2006). Based on their analyses, the authors suggest that PCB serves as the endogenous chromophore for PixJ1. As the expression of the cyanobacterial *bvdR* gene in the *E. coli* system described may not result in biliverdin reductase activity, the accumulation of rubins in this system is uncertain: *in vitro* BvdR exhibits a pH optimum of 5.8 and negligible activity above pH 7 (Schluchter and Glazer, 1997), whereas *E. coli* cells generally maintain an intracellular pH of 7.6–7.8 (Padan *et al*., 1981). Analyses of the homologous TePixJ from *Thermosynechococcus elongatus* strain BP-1 has been recently reported (Ishizuka *et al*., 2006). MALDI-TOF/MS analyses of heterologously expressed, affinity-purified TePixJ holoprotein indicates that a chromophore identical in size to PCB is covalently bound to a conserved cysteine of the GAF domain of TePixJ (Ishizuka *et al*., 2006). However, denaturation analysis of this protein shows that the chromophore structure differs significantly from PCB in its conjugated double-bond configuration, despite having an identical molecular mass (Ishizuka *et al*., 2006). Taken together, these PixJ studies leave the definitive identification of the PixJ chromophore unresolved. In summary, PixJ is largely responsive to blue and green light and, thus, is a membrane-localized, blue light-responsive receptor distinct from prototypical phytochrome-class family members.

In *Anabaena* sp. strain PCC 7120, a phytochrome-class protein, AphC, controls cAMP levels in response to far-red illumination (Okamoto *et al*., 2004). AphC contains three GAF domains, two with conserved cysteine residues and a histidine kinase domain. Based on mutant analyses, these authors show that AphC signals to an enzymatic response regulator, CyaC, a complex protein containing two response regulator domains, a histidine kinase domain, and an adenylate cyclase domain that likely functions to catalyse production of cAMP from ATP (Okamoto *et al*., 2004). Thus, AphC and CyaC likely participate in a light-responsive phosphorelay in the control of cAMP levels in *Anabaena*, though the *in vivo* chromophorylation state and kinase activity of AphC have not been reported.

To gain a greater understanding of the molecular mechanisms utilized by phytochromes and phytochrome-related proteins, studies on the photochemistry and structure of proteins from the phytochrome superfamily have been conducted. In a recent report, a conserved tyrosine residue was shown to have a direct role in the primary photochemistry of Cph1 and other phytochromes (Fischer and Lagarias, 2004). The residue is conserved among all members of the phytochrome superfamily. Replacement of this tyrosine residue, which is located in the bilin-binding GAF domain, results in an intensely fluorescent biliprotein (Fischer and Lagarias, 2004). This fluorescent molecule lacks the ability to photoisomerize in response to light exposure, releasing excess excitation energy as fluorescence. Furthermore, the recent structure analysis of the chromophore-binding domain of DrBphP implicates this conserved residue in an interaction with the D-ring of the attached chromophore (Wagner *et al*., 2005). Thus, the conserved tyrosine most likely has a direct role in the photoisomerization of the phytochrome chromophore.

Additional analyses of the conserved tyrosine residue (Tyr176) in plant phytochromes, cyanobacterial phytochrome Cph1 and bacteriophytochromes revealed that the role of this residue is likely to maintain the protonated, extended chromophore conformation (Fischer *et al*., 2005). The nearby Glu189 has been implicated as the proton donor in the Cph1 phytochrome (Fischer *et al*., 2005), while the structure of DrBphP implicates distinct residues, i.e. Asp207 or His260, as potential proton donors (Wagner *et al*., 2005; Rockwell and Lagarias, 2006). Although mutants lacking the Tyr residue in plant and cyanobacterial phytochromes exhibited enhanced fluorescence, replacement of this residue in a bacteriophytochrome from *Pseudomonas aeruginosa* did not result in increased fluorescence of the already fluorescent Pfr ground-state molecule (Fischer *et al*., 2005). This result indicates that the conserved residue may play distinct roles in bacteriophytochromes, as compared with the proposed gating mechanism it possesses in plant and cyanobacterial phytochromes. The proposed gating mechanism regulates the rate of the photochemical relaxation from Z- to E-conformation of the chromophore

in response to light. These results and additional structural features were presented in the aforementioned landmark report that described the first solved structure of a chromophore-binding domain of the *Deinococcus radiodurans* phytochrome (Wagner *et al*., 2005). Though not cyanobacterial in origin, the structures of the bilin-binding pockets of cyanobacterial and plant phytochromes are likely to be similar to those of bacteriophytochromes (Rockwell and Lagarias, 2006). Thus, the structural data reported by Wagner *et al*. (2005) are expected to inform generally about the phytochrome photocycle. In this regard, Wagner *et al*. (2005) report a high degree of residue conservation for the PAS and GAF domains, the bilin-binding domain and the domain that makes up the trefoil knot. Notably, the knot may be required for red/far-red photoreversibility, as it appears to be missing from phytochrome-like proteins that bind bilins but do not exhibit red/far-red-photoreversible properties. A recent review on the implications of the first solved phytochrome structure has been published (Rockwell and Lagarias, 2006).

In summary, although numerous phytochromes and phytochrome-like proteins have been isolated from cyanobacteria and other prokaryotes, and their photobiological activities characterized *in vitro*, many of these proteins remain unlinked to physiological responses *in vivo*. Only a limited number of phytochrome-related proteins, including RcaE, PlpA and PixJ1, have been linked to light-dependent phenotypes *in vivo* (Kehoe and Grossman, 1996; Wilde *et al*., 1997; Yoshihara *et al*., 2000), although none of these have been shown to have the red/far-red photochemistry similar to higher-plant and canonical cyanobacterial phytochromes Cph1 and Cph2. Thus, it has been proposed that many of the phytochromes and phytochrome-related proteins identified in cyanobacterial genomes, but lacking obvious regulatory roles in photomorphogenesis, may serve as regulators of bilin synthesis or as bilin-binding tetrapyrrole sensors in cyanobacteria rather than as light sensors themselves (Montgomery and Lagarias, 2002; Lamparter, 2004).

Light sensory phycobiliproteins

Mullineaux (2001) hypothesized that, separate from light-harvesting structural phycobiliproteins that function in photosynthesis, sensory phycobiliproteins likely operate in cyanobacteria. This hypothesis has been supported by the isolation of proteins such as AplA (Montgomery *et al*., 2004) and a green light-absorbing phycoerythrin, CpeB, in the cyanobacterium *Prochlorococcus* sp. MED4 (Steglich *et al*., 2005). AplA is a phycobiliprotein-related protein that is not found in the structural phycobilisomes and appears to have a photosensory rather than structural role in the cyanobacterium *F. diplosiphon* (Montgomery *et al*., 2004). AplA overexpression in wild-type cells results in a light-dependent phenotype under both red and green light, whereas expression in RcaE-null cells results in a phenotype only under GL conditions. Thus, AplA may be involved in green and/or red light sensing in *F. diplosiphon*. The high light-adapted marine cyanobacterium *Prochlorococcus* sp. MED4 contains a green light-absorbing phycoerythrin, although it lacks other phycobiliproteins and phycobilisomes (Steglich *et al*., 2005). Evidence that this protein may serve as a sensory phycobiliprotein in this organism includes the observations that the gene encoding the protein is expressed and that the protein binds a chromophore autocatalytically at identified residues (Steglich *et al*., 2005). As no PBSs are present in this organism, CpeB is expected to serve as a green light-absorbing member of the family of phycobiliprotein-related proteins with photosensory, rather than structural, functions.

Blue light photoreceptors: cryptochromes

Although a putative cryptochrome gene was identified in *Synechocystis* and reported to lack light-activated photolyase activity (Hitomi *et al*., 2000; Ng and Pakrasi, 2001), additional information about the physiological role(s) of this receptor *in vivo* has not been reported. More recently, structural and functional analyses revealed that this protein exhibits structural similarities to DNA-binding photolyases that are likely to allow for DNA interactions, but that the protein has amino acid replacements at key residues needed for light-activated DNA-repair activity (Brudler *et al*., 2003). Accordingly, the structural similarity is sufficient to impart the capacity of the cyanobacterial cryptochrome to bind DNA (Brudler *et al*., 2003). Structural differences were observed in residues involved in FAD binding, suggesting distinct FAD-binding characteristics for the cyanobacterial cryptochrome (Brudler *et al*., 2003). Comparative genomic analyses using wild-type cells and the cryptochrome knockout mutant showed that the cyanobacterial cryptochrome likely functions as a transcriptional repressor similar to animal cryptochromes (Brudler *et al*., 2003). Thus, the cyanobacterial cryptochrome likely functions as a light-responsive transcriptional regulator.

Blue light photoreceptors: phototropins

Until recently, the only reported phototropin-related gene product in a prokaryotic system was YtvA, a *Bacillus subtilis* LOV (light/oxygen/voltage) domain-containing protein that binds a FMN chromophore and exhibits spectroscopic and photochemical properties similar to higher-plant phototropin proteins (Losi *et al*., 2002). LOV domains, conserved FMN-binding domains in phototro-

pins, are a subgroup of the larger PAS (Per/ARNT/Sim) domain family (Taylor and Zhulin, 1999; Crosson *et al*., 2003; see description in Box 1). As a significant number of PAS domain-containing proteins exist in cyanobacteria (Narikawa *et al*., 2004), the occurrence of phototropin-like proteins in these organisms has been anticipated. A putative LOV domain-containing protein, encoded by gene *slr0359* in *Synechocystis*, has been reported (Fiedler *et al*., 2005). Slr0359 is predicted to have GAF, GGDEF and EAL domains, in addition to the putative LOV domain. Mutagenesis of the *slr0359* locus, however, did not result in the identification of a defect in blue light-dependent phototaxis. Additional analyses of these mutants for novel blue light-dependent phenotypic defects have not been reported.

Other LOV domain-containing proteins have been reported for *Anabaena* sp. PCC 7120, including those encoded by genes *alr1229*, *all2875* and *alr3170* (Ohmori *et al*., 2001). Notably, the proteins encoded by two of these genes, All2875 and Alr3170, recently have been shown to contain LOV domains that possess the conserved cysteine of photoactive LOV domains and to exhibit FMN-binding and blue light-dependent, phototropin-like photocycles (Narikawa *et al*., 2006). Both of these are GGDEF domain-containing proteins; Alr3170 also contains an EAF domain, and All2875 also contains a GAF domain and a canonical histidine kinase domain (Narikawa *et al*., 2006). The third protein, Alr1229, lacks the conserved cysteine in the LOV domain and, accordingly, does not exhibit flavin-binding or phototropin-like properties (Narikawa *et al*., 2006). Although additional experimentation is needed to confirm physiological roles for these phototropin-related proteins, these findings strongly suggest the occurrence of functional phototropins with diverse output domains in cyanobacteria.

Blue light photoreceptors: BLUF domain photosensors

Another class of blue light photosensors contains a conserved BLUF (sensors of blue light using FAD) domain. Whereas in a large number of these proteins the only recognized domain is a BLUF domain, in some proteins GGDEF and EAL domains are found linked to BLUF domains (see Box 1). In the former context, short BLUF domain proteins are likely to regulate downstream responses via protein–protein interactions, as these proteins lack DNA-binding or output domains. In the GGDEF- and EAL-containing proteins, c-di-GMP turnover may be regulated by blue light. BLUF domain proteins have been identified in cyanobacteria, including *Synechocystis* sp. PCC 6803 and *Synechococcus* sp. PCC 7002 (Fiedler *et al*., 2005; Okajima *et al*., 2005).

In *Synechocystis*, the identified BLUF protein (Slr1694 or PixD) appears to be involved in the regulation of positive phototaxis, as *slr1694* deletion mutants exhibit negative phototaxis in response to light (i.e. white illumination or illumination of 500–700 nm), in contrast to wild-type cells that exhibited positive phototaxis under these conditions (Masuda and Ono, 2004; Okajima *et al*., 2005). Spectroscopic analysis of heterologously expressed PixD yielded light-induced structural changes characteristic of a flavin-bound BLUF domain (Masuda *et al*., 2004). Additional biochemical analyses showed that PixD, which lacks an output domain, interacts with response regulator PixE in yeast two-hybrid assays (Okajima *et al*., 2005).

The first reported crystal structure for a BLUF domain was for the cyanobacterial BLUF protein Tll0078 from the cyanobacterium *T. elongatus* BP-1 (Kita *et al*., 2005). The structure, which included an intramolecular hydrogen-bonded FAD chromophore, and additional biochemical analyses implicated a conserved glutamine (Gln50) and tyrosine (Tyr8) as critical for the blue light-induced photochemical reaction (Kita *et al*., 2005; Okajima *et al*., 2006). The structure of *Synechocystis* PixD has also been reported (Yuan *et al*., 2006). The structure of the BLUF domain of this protein was very similar to that of Tll0078 and confirmed the proposed roles of the conserved Gln and Tyr in chromophore interactions. An additional tryptophan residue (Trp91) was also identified as interacting with the flavin and undergoing conformational reorientation in response to light absorption by PixD (Yuan *et al*., 2006).

UV receptors

A number of UV-B-dependent behavioural responses have been reported for cyanobacteria, including the photoavoidance migration response of *Microcoleus chthonoplastes* (Bebout and Garcia-Pichel, 1995) and the photosystem restructuring response of *Synechococcus* sp. PCC 7942 (Campbell *et al*., 1998). Evidence for the existence of a novel UV-B photoreceptor has been reported for the cyanobacterium *Chlorogloeopsis* PCC 6912 (Portwich and Garcia-Pichel, 2000). This photoreceptor is implicated in the induction of mycosporine-like amino acid (MAA) in this organism. Notably, MAA is a UV-absorbing compound and thus its induction is presumably a UV acclimation or sunscreen response. Generation of an action spectrum for the UV response led to the hypothesis that a reduced pterin may serve as the chromophore (Portwich and Garcia-Pichel, 2000). Additional support for this hypothesis came from studies that used an inhibitor of the pterin biosynthetic pathway and a pterin antagonist, which inhibit light-dependent induction of MAAs (Portwich and Garcia-Pichel, 2000). The detailed molecular structure of the photoreceptor is yet to be reported.

Rhodopsins

A sensory rhodopsin was identified as a green light-activated photoreceptor in *Anabaena* (*Nostoc*) sp. PCC 7120 (Jung *et al*., 2003). The gene encoding the membrane-bound rhodopsin is contained in an operon with a gene encoding a soluble 14 kDa protein. Heterologous expression of the cyanobacterial rhodopsin in *E. coli* resulted in a molecule that bound a retinal chromophore and exhibited characteristic photochemical responses of a functional sensory rhodopsin (Jung *et al*., 2003). Coexpression of the 14 kDa protein resulted in an accelerated photocycle for the rhodopsin, suggesting a novel mechanism, including interaction with a cytoplasmic partner, for this *Anabaena* rhodopsin (Jung *et al*., 2003).

The crystal structure of *Anabaena* rhodopsin was determined recently and shows a seven-helical, membrane-embedded structure similar to haloarchael rhodopsins (Vogeley *et al*., 2004). Detailed analyses of the photosensor showed that it can exist as two stable isoforms containing either an all-*trans* or a 13-*cis* chromophore configuration (Vogeley *et al*., 2004; Sineshchekov *et al*., 2005). These two forms are interconvertible by distinct wavelengths of light (i.e. 549 nm and 537 nm; Vogeley *et al*., 2004). This photointerconvertibility is similar to that observed for photochromic phytochromes and may indicate a role for *Anabaena* rhodopsin in the regulation of wavelength-responsive genes and/or proteins, e.g. phycobilisome components.

Recent studies of the photocycle of the photochromic rhodopsin from *Anabaena* indicate that deprotonation of the retinal chromophore is correlated with protonation of an aspartate residue on the cytoplasmic domain of the protein (Shi *et al*., 2006). Furthermore, chromophore deprotonation leads to a conformational change in the rhodopsin protein that may be associated with activation of the cognate, physically associated transducer (Shi *et al*., 2006). Additional insight into the comparison of the *Anabaena* rhodopsin to other sensory rhodopsins and more information about the recent progress made in understanding the roles of these microbial sensory rhopodsins are presented in a recent review (Spudich, 2006).

Output components and signal transduction

Response regulators

Many types of response regulators are contained in a large variety of prokaryotic species (Galperin, 2006). The types and combinations of domains found in bacterial response regulators are diverse. These domains include DNA-binding transcriptional, enzymatic and protein-binding regulators in cyanobacteria (see Fig. 1; Galperin, 2006). Controlled by the activity of an upstream environmental sensor, response regulators themselves regulate the activity of intramolecular effector domains or separate effector proteins via phosphorylation (reviewed by West and Stock, 2001). This phosphorylation event results in identifiable changes in the cellular responses of organisms. Response regulators operate in classical two-component cascades, as well as in chemotaxis cascades (see Fig. 1).

Second messengers

A large number of putative photosensory cyanobacterial proteins contain GGDEF and EAL domains that participate in c-di-GMP metabolism. Thus, c-di-GMP likely functions as a second messenger in a variety of signalling pathways. The specific cellular targets of c-di-GMP are being identified (Romling *et al*., 2005).

Cyclic AMP is another second messenger in cyanobacterial systems. Cellular cAMP content is under reversible control by red/far-red irradiation in the cyanobacterium *Anabaena cylindrica* (Ohmori *et al*., 2002). Under red light irradiation, cAMP content decreases, but far-red light irradiation leads to a rapid increase in cAMP content. This phenotype is likely to be controlled by an unidentified phytochrome-related photoreceptor. A similar cAMP response is under the control of a Cph2-related protein in *Anabaena* sp. strain PCC 7120 (Okamoto *et al*., 2004).

Calcium also is implicated as a second messenger in the regulation of photomovement in *Synechocystis* sp. PCC 6803 (Moon *et al*., 2004). By using calcium chelator EGTA, calcium ionophores or calcium channel inhibitors, Moon *et al*. (2004) demonstrated that the photoorientation response in *Synechocystis* is blocked when calcium is removed from the cell.

Cellular responses and growth and development changes

Complementary chromatic adaptation

Complementary chromatic adaptation is one of the best-studied photomorphogenetic processes that occur in cyanobacteria. CCA is controlled by the phytochrome-like protein RcaE in *F. diplosiphon* and has been the subject of a number of recent reviews (Stowe-Evans and Kehoe, 2004; Alvey *et al*., 2005; Kehoe and Gutu, 2006). Based on genetic data and mutant complementation analyses, it is commonly accepted that RcaE acts in a complex phosphorelay to transmit a light signal to downstream response regulators RcaF and RcaC (Kehoe and Grossman, 1997). Unlike other prokaryotic phytochromes that have most commonly been studied *in vitro*, biochemical analysis of RcaE, which led to its identification as a photosensory biliprotein, has been based largely on the ability

of mutated proteins to restore the CCA phenotype to a null mutant (Terauchi *et al*., 2004).

Recent studies indicate that a second regulatory pathway, named the Cgi (control of green light induction) pathway, is also involved in the regulation of CCA in response to green illumination. Identification of this pathway was based on the ability of *rcaE* null mutant cells to exhibit green light induction of the phycoerythrin operons, *cpeBA* and *cpeCDE* (reviewed by Alvey *et al*., 2005; Kehoe and Gutu, 2006). Thus, the regulation of CCA is more complex than initial studies on RcaE indicated, and additional photoreceptors may be involved in the regulation of CCA. The isolation of 17 novel open reading frames that are light responsive in *F. diplosiphon* was recently reported (Stowe-Evans *et al*., 2004). These genes were identified by microarray analyses and confirmed by Northern blot analyses. Included in this group are two novel two-component sensor kinases that may play a role in the light-dependent CCA response.

Other light-dependent cellular responses

Although holoprotein production, red/far-red-photoreversible spectral properties, and autophosphorylation and kinase activities have all been observed *in vitro* for a number of bacterial phytochromes (see section on phytochrome-class photoreceptors above), few of these proteins have been linked definitively to light-dependent responses *in vivo*. Notable exceptions, apart from the phytochrome-class protein RcaE (which regulates CCA), include the discovery that, in *Synechocystis*, Cph2 inhibits blue light-mediated phototaxis (Wilde *et al*., 2002; Fiedler *et al*., 2005). As Cph2 shows red/far-red photoreversibility in spectral assays, its involvement in a blue light response was unexpected for a phytochrome photoreceptor. Results from additional studies with *cph1* and *cph2* deletion mutants reported slightly reduced growth (~35% reduction) for *cph1* mutants under far-red light conditions, whereas *cph2* mutants exhibited defective growth under red light (~20% reduction) (Fiedler *et al*., 2004). The double mutants exhibited defects under both light conditions, although the observed growth defects were not significantly different from those of single mutants. Although the differences observed in growth for *cph1* and *cph2* mutants are not large, the mutated lines do show reduced fitness, as evident from competition experiments in which wild-type cells displaced *cph1* mutant cells after prolonged growth in far-red light and replaced *cph2* mutant cells after prolonged growth in red light (Fiedler *et al*., 2004).

A recent study used microarray analysis to determine the role of Cph1 and Cph2 in red and far-red light responsiveness in *Synechocystis* sp. PC 6803 (Hübschmann *et al*., 2005). In their studies of wild-type cells, this group showed that approximately 25% of the genome is responsive to red and far-red light. Red light induces genes related to light harvesting, photosynthesis and cellular metabolism, whereas far-red light exposure results in the induction of stress-related genes. Mutations in *cph1* and *cph2*, singly or in combination, resulted in relatively minor changes in gene expression in response to light – i.e. only 21 genes showed aberrations in differential expression, as compared with wild-type cells (Hübschmann *et al*., 2005). These 21 genes included genes involved in chlorophyll synthesis and translation for cells shifted from far red to red, and stress adaptation/high light-inducible proteins from cells shifted from red to far red (Hübschmann *et al*., 2005). Thus, other receptors or regulatory systems must be responsible for additional changes in the 25% of the genome that is light responsive in wild-type cells. These additional regulatory proteins may include phytochrome-like receptors found in this organism.

Up to 25% of the *Synechocystis* genome also is involved in sensing light-to-dark transitions as determined by DNA microarray analysis (Gill *et al*., 2002). Some of these genomic changes are likely to be directly regulated by light; others are hypothesized to be regulated by the stress associated with light-to-dark transitions. In addition to the genomic studies described above, Pandey *et al*. (2000) used 35S methionine and two-dimensional gel electrophoretic analyses to study the effects of blue, red and white light on the proteome of *Synechococcus* sp. PCC 7942. They identified a limited number of dark-adapted (8), blue light-specific (10) and red light-inducible (4) proteins (Pandey *et al*., 2000).

New approaches to address persisting questions

A number of proteins capable of detecting light have been identified in cyanobacterial genomes. *In vitro* characterization of these proteins has yielded valuable information about the potential light-sensing capabilities of these molecules. Definitive evidence of *in vivo* activity for most of these molecules, however, is lacking. Although some of these molecules may not be involved in photoreception or bilin sensing *in vivo*, it is more likely that the phenotypes associated with the activity of these proteins have yet to be uncovered. These proteins may regulate novel photoresponses that will not be uncovered using common protocols for assessment of photomorphogenetic mutants – i.e. screening under red and far-red light for potential phytochrome mutants and under standard blue light conditions for putative cryptochrome and phototropin mutants. Clearly, some phytochrome-related proteins regulate physiological effects in response to novel light conditions, including red and green light for RcaE (Kehoe and Grossman, 1996; Terauchi *et al*., 2004) and blue and green light for PixJ1 (Yoshihara *et al*., 2000; 2004).

Pioneering approaches, including systems biology methods, will be required to address these complex, unique regulatory systems. Although genomic studies, including microarray analyses, have become more common in the study of cyanobacterial genomes, proteomic and metabolic studies using cyanobacterial subjects are still rare (Burja *et al*., 2003). Such studies should prove powerful for gaining additional insight into photosensory proteins and the signal cascades that they control in the regulation of light-dependent responses.

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