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REVIEW ARTICLE

Bacterial phytochromes: More than meets the light

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Abstract

Phytochromes are environmental sensors, historically thought of as red/far-red photoreceptors in plants. Their photoperception occurs through a covalently linked tetrapyrrole chromophore, which undergoes a light-dependent conformational change propagated through the protein to a variable output domain. The phytochrome composition is modular, typically consisting of a PAS-GAF-PHY architecture for the N-terminal photosensory core. A collection of three-dimensional structures has uncovered key features, including an unusual figure-of-eight knot, an extension reaching from the PHY domain to the chromophore-binding GAF domain, and a centrally located, long α-helix hypothesized to be crucial for intramolecular signaling. Continuing identification of phytochromes in microbial systems has expanded the assigned sensory abilities of this family out of the red and into the yellow, green, blue, and violet portions of the spectrum. Furthermore, phytochromes acting not as photoreceptors but as redox sensors have been recognized. In addition, architectures other than PAS-GAF-PHY are known, thus revealing phytochromes to be a varied group of sensory receptors evolved to utilize their modular design to perceive a signal and respond accordingly. This review focuses on the structures of bacterial phytochromes and implications for signal transmission. We also discuss the small but growing set of bacterial phytochromes for which a physiological function has been ascertained.

Keywords: *Photoreceptor; signal transduction; three-dimensional structure; tetrapyrrole; bilin lyase; GAF; PHY*

Introduction to phytochrome

Overview and distribution

Organisms perceive and respond to many variables in their environments including temperature, oxygen tension, iron availability, nutrient concentrations, and light quality and quantity. For photosynthetic organisms, light is a required source of radiation energy that is converted into chemical energy for cellular processes. For nonphotosynthetic organisms, light can act as a cue for optimal spatial positioning and orientation. Cells sense their light environment through a collection of photoreceptors, each tuned to a specific wavelength through a small molecule chromophore. Phytochrome is a photoreceptor that binds a linear tetrapyrrole (or bilin) as its chromophore through a thioether bond to a conserved cysteine residue. However, not all phytochrome family members retain the cysteine and function as light receptors, as discussed later in this review. In some unique examples, phytochromes act as redox sensors, collecting information about the metabolic status of the cell.

The prototypical phytochrome can be divided into an N-terminal photosensory core and a C-terminal regulatory domain (Quail, 1997; Montgomery & Lagarias, 2002). The chromophore resides in a pocket formed by the photosensory core, which in a classic phytochrome model consists of three conserved structural domains: PAS (Per-ARNT-Sim), GAF (cGMP phosphodiesterase/adenylate cyclase/FhlA), and PHY (phytochrome-specific). Bacteria utilize biliverdin IXα (BV) (Bhoo et al., 2001; Lamparter et al., 2004; Tasler et al., 2005) as their chromophore while cyanobacteria and plants use phycocyanobilin

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(PCB) (Hughes et al., 1997) or phytochromobilin (PΦB) (Lagarias & Rapoport, 1980), respectively. These chromophores combine with unique protein scaffolds to give phytochromes their light absorbing properties (Figure 1). Unlike protein-bound BV (max Pr Abs ~700 nm), the absorption profiles of protein-bound PΦB and PCB (max Pr Abs ~630 nm) overlap with that of chlorophyll, an important distinction with particular consequence for plants because it provides a means to sense shade from neighboring plants and thus avoid competition (Franklin & Whitelam, 2005). The evolutionary shift from use of BV in bacteria to PCB and PΦB in cyanobacteria and plants has been postulated to be the result of a switch from an anoxygenic to an oxygenic lifestyle, as BV may be sensitive to photodamage (Borucki et al., 2009).

Phytochrome chromophores are derived from heme and contain a system of conjugated double bonds, which provides their light absorbing properties (Figure 1). A comprehensive review on bilin biosynthesis can be found in Dammeyer and Frankenberg-Dinkel, 2008. In short, bilin biosynthesis is initiated by heme oxygenase with the regiospecific cleavage of heme to produce BV. In at least one case, phytochrome is required for product release from the heme oxygenase (Wegele et al., 2004). Subsequent reduction of BV by ferredoxin-dependent bilin reductases (FDBRs) produces PΦB, PCB, and phycoerythrobilin (PEB). PCB and PΦB are phytochrome chromophores, while PEB is found in macromolecular complexes involved in photosynthesis in cyanobacteria. Heme and chlorophyll share a biosynthetic precursor evidenced by their similar structures (Dammeyer & Frankenberg-Dinkel, 2008) (Figure 1). The overlapping biosynthetic pathways of the phytochrome chromophores, phycobilisome (PBS) pigments and chlorophyll implicates a link between light perception, photosynthesis, and redox homeostasis.

Phytochromes have historically been thought of as red/far-red reversible photosensors. The ground or dark state is the red light absorbing (Pr) form, which is converted into the far-red light absorbing (Pfr) form by irradiation with red light. The Pfr form is converted back to the Pr form by far-red light (Sage, 1992). Alternatively, phytochromes that maintain a dark adapted Pfr state and undergo Pfr/Pr photochemistry have also been isolated and due to the longer wavelength of their ground state are called bathyphytochromes (Karniol & Vierstra, 2003). Recent data suggest that these phytochromes are primarily found in nitrogen-fixing plant symbionts of the *Rhizobiales* order. Their dark adapted Pfr state fits well with their soil-dwelling symbiotic lifestyle as far-red light penetrates soil more effectively than red light and the roots of the plant host may even serve as fiberoptic cables for far-red light (Rottwinkel et al., 2010). Phytochromes absorbing at wavelengths other than red and far-red exist and will be discussed further in this review.

Phytochromes were first discovered in plants based on the red/far-red photoreversibilty of developmental processes such as germination (Butler et al., 1959). Likewise, a protocol for protein purification was initially developed using oat seedlings (Siegelman & Firer, 1964; Sage, 1992). The discovery of phytochromes outside the plant kingdom came much later with the identification of two phytochromes from cyanobacteria, photosynthetic bacteria known as the progenitors of plant chloroplasts. Regulator of Chromatic Adaptation (RcaE) from *Fremyella diplosiphon* (Kehoe & Grossman, 1996) and Cph1 (Cyanobacterial phytochrome) from *Synechocystis* sp. PCC6803 (Hughes et al., 1997) are both cyanobacterial phytochromes. In many cases, the overall architecture of cyanobacterial phytochromes varies from the typical PAS-GAF-PHY design (Table 1). Some lack the upstream PAS domain while maintaining the PHY domain. *Synechocystis* Cph2 defines this group. A number of phytochromes have been isolated from cyanobacteria that require only a GAF domain for proper photochemistry. These phytochromes are referred to as cyanobacteriochromes (CBCRs) (Ikeuchi & Ishizuka, 2008). RcaE is a CBCR as are PixJ proteins, which photoconvert between Pr or Pb (blue light absorbing) and Pg (green light absorbing) forms (Yoshihara et al., 2004; Ishizuka et al., 2006; Rockwell et al., 2008).

The discovery of PYP-phytochrome related (Ppr) from the purple photosynthetic bacterium *Rhodospirillum centenum* led to the addition of anoxygenic phototrophic bacteria to the list of phytochrome-containing organisms (Jiang et al., 1999) and marks the beginning of the expansion of phytochromes outside the plant and cyanobacterial realm. Ppr contains both a photoactive yellow protein (PYP) domain that binds p-hydroxycinnamic acid and a PAS-GAF-PHY phytochrome core that conserves a Cys residue between its PYP and PAS domains (Kyndt et al., 2004). Ppr displays a BV-dependent red absorption maximum but is bleached in red light rather than converting to a far-red absorbing form. The blue-light absorbing PYP domain accelerates recovery of red light absorption (Kyndt et al., 2007). Interestingly, Ppr was shown to regulate the expression of chalcone synthase, a flavonoid biosynthetic enzyme whose regulation in plants is controlled by phytochrome and the blue light photoreceptor cryptochrome (Jiang et al., 1999). Classic bilin-binding, red/far-red absorbing bacterial phytochromes were initially discovered in the nonphotosynthetic *Deinococcus radiodurans* and *Pseudomonas aeruginosa*. These so-called bacteriophytochromes (BphPs) (Davis et al., 1999) maintain a BV attachment site N-terminal to the PAS domain (Lamparter et al., 2004). This is in contrast to plant and Cph1-like phytochromes, which conserve a Cys residue in their phytochrome GAF domains as the chromophore attachment site (Lagarias & Rapoport,

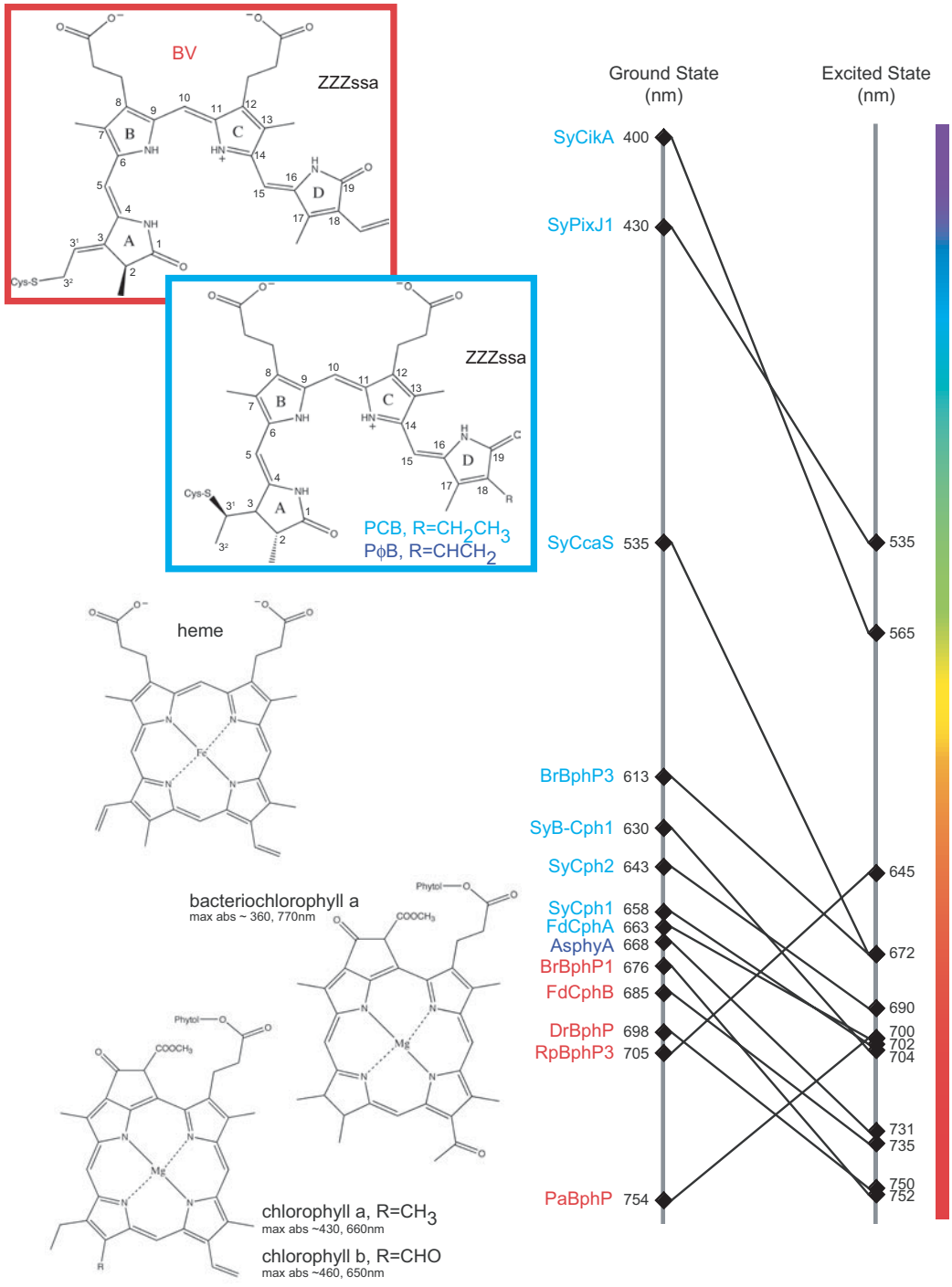


Figure 1. Because of its aromaticity, the porphyrin design is tailor-made to the absorption of light. Heme, chlorophyll, BV, PCB, and PΦB share a common precursor. Heme is crucial to redox chemistry, while chlorophyll and the phytochrome chromophores use their system of conjugated double bonds to capture light. PCB and BV are shown in their cysteine-ligated forms (Left). 'Spectral line' illustrating the varied ground (dark) and excited state wavelengths of selected phytochromes found in nature, color-coded to match corresponding physiological chromophore (Right). DrBphP (Bhoo et al., 2001)—*Deinococcus radiodurans*, PaBphP (Tasler et al., 2005)—*Pseudomonas aeruginosa*, RpBphP3 (Giraud et al., 2005)–*Rhodopseudomonas palustris*, FdCphA and FdCphB (Jorissen et al., 2002)—*Fremyella diplosiphon*, *Br*BphP1 (Giraud et al., 2002) and *Br*BphP3 (Jaubert et al., 2007)—*Bradyrhizobium* ORS278, AsphyA (Murphy & Lagarias, 1997)—*Avena sativa*, *Sy*Cph1 (Hughes et al., 1997), *Sy*Cph2 (Park et al., 2000b; Wu & Lagarias, 2000), *Sy*CcaS (Hirose et al., 2008), *Sy*PixJ1—(Yoshihara et al., 2004) and *Sy*CikA (Narikawa et al., 2008b)—*Synechocystis* sp. PCC6803, SyB-Cph1—*Synechococcus* OSB' (Ulijasz et al., 2008).

Table 1. Characteristics of biologically or biochemically validated phytochromes found in microbial systems.

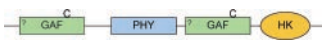
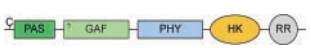
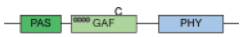
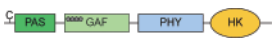
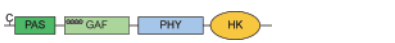
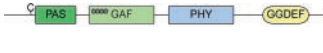
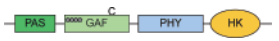
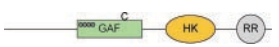
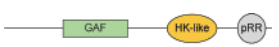
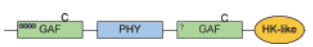
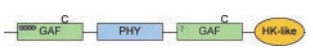
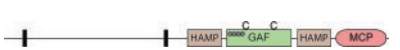
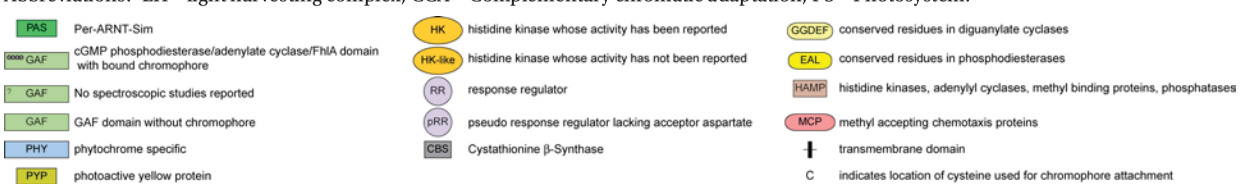
Species	Phytochrome	Chromophore chromicity	Domain architecture of full-length protein*	PDB code	Function	Ref.
Agrobacterium tumefaciens	Agp1	BV Pr/Pfr		—	Unknown	(Lamparter et al., 2002, Karniol & Vierstra, 2003)
Agrobacterium tumefaciens	Agp2	BV Pfr/Pr		—	Unknown	(Lamparter et al., 2002, Karniol & Vierstra, 2003)
Anabaena (Nostoc) sp. PCC 7120	AphA	PCB Pr/Pfr		—	Unknown	(Zhao et al., 2004)
Anabaena (Nostoc) sp. PCC 7120	AphC	?		—	cAMP induction	(Okamoto et al., 2004)
Anabaena (Nostoc) sp. PCC 7120	PixJ	PCB Pr/Pg		—	Unknown	(Narikawa et al., 2008a)
Bradyrhizobium ORS278	BrBphP1	BV Pfr/Pr		—	PS synthesis	(Giraud et al., 2002)
Bradyrhizobium ORS278	BrBphP2	?		—	Unknown	(Jaubert et al., 2007)
Bradyrhizobium ORS278	BrBphP3	PCB Po/Pr		—	Unknown	(Jaubert et al., 2007)
Deinococcus radiodurans	BphP	BV Pr/Pfr		1ZTU(Wagner et al., 2005), 2O9B, 2O9C (Wagner et al., 2007)	Carotenoid regulation	(Bhoo et al., 2001)
Fremyella diplosiphon	CphA	PCB Pr/Pfr		—	Unknown	(Hübschmann et al., 2001, Jorissen et al., 2002)
Fremyella diplosiphon	CphB	BV Pr/Pfr		—	Unknown	(Hübschmann et al., 2001, Jorissen et al., 2002, Quest et al., 2007, Quest & Gärtner, 2004)
Fremyella diplosiphon	RcaE	?		—	CCA	(Terauchi et al., 2004, Kehoe & Grossman, 1996)
Pseudomonas aeruginosa	BphP	BV Pfr/Pr		3C2W (Yang et al., 2008), 3IBR, 3G6O (Yang et al., 2009)	Unknown	(Wegele et al., 2004, Tasler et al., 2005, Bhoo et al., 2001)
Rhodobacter sphaeroides	BphG1	BV Pr/Pfr		—	Unknown	(Tarutina et al., 2006)
Rhodobacter sphaeroides	BphG2	?		—	Unknown	(Tarutina et al., 2006)
Rhodospirillum centenum	Ppr	BV? Pr/bleached		—	Chalcone synthase regulation	(Jiang et al., 1999, Kyndt et al., 2004, Kyndt et al., 2010)
Rhodopseudomonas palustris CEA001	RpBphP1	BV Pfr/Pr		—	Regulation of PS synthesis and respiration	(Giraud et al., 2002, Kojadinovic et al., 2008, Giraud, 2004)
Rhodopseudomonas palustris CGA009	RpBphP2	BV Pr/Pfr		—	Regulation of LH2 & LH4	(Giraud et al., 2005; Giraud, 2004)
Rhodopseudomonas palustris CGA009	RpBphP3	BV Pr/Pnr		2OOL (Yang et al., 2007)	Regulation of LH4	(Giraud et al., 2005; Giraud, 2004)
Rhodopseudomonas palustris CGA009/ CEA001	RpBphP4	none		—	Redox-dependent regulation of LH2	(Giraud, 2004; Vuillet et al., 2007)

Table 1. continued on next page

Table 1. Continued.

Species	Phytochrome	Chromophore chromicity	Domain architecture of full-length protein*	PDB code	Function	Ref.
Rhodopseudomonas palustris CGA009	RpBphP5	BV Pfr/Pr		___	Unknown	(Giraud, 2004)
Rhodopseudomonas palustris CGA009	RpBphP6	BV Pfr/Pr		___	Unknown	(Giraud, 2004)
Synechocystis sp. PCC 6803	Cph1	PCB Pr/Pfr		2VEA (Essen et al., 2008)	Unknown	(Hughes et al., 1997, Lamparter et al., 2001)
Synechocystis sp. PCC 6803	Cph2	PCB Pr/Pfr		___	Phototaxis	(Wu & Lagarias, 2000, Fiedler et al., 2005, Wilde et al., 2002)
Synechocystis sp. PCC 6803	TaxD1/PixJ1	PCB** Pb/Pg		___	Suppression of + phototaxis in blue light	(Bhaya, 2004, Yoshihara et al., 2004, Ikeuchi & Ishizuka, 2008)
Synechocystis sp. PCC 6803	CcaS	PCB Pg/Pr		___	CCA	(Hirose et al., 2008)
Synechocystis sp. PCC 6803	PlpA	?		___	Blue light dependent regulation of growth	(Wilde et al., 1997)
Synechocystis sp. PCC 6803	CikA	PCB Pv/Py		___	Unknown	(Narikawa et al., 2008b)
Synechocystis sp. PCC 6803	RGS	PCB Pr/Pg		___	Unknown	(Zhang et al., 2010)
Synechococcus elongatus	CikA	None– binds quinone in pRR		___	Redox sensor, circadian rhythm	(Schmitz et al., 2000; Narikawa et al., 2008a)
Synechococcus OSA	SyA-Cph1	PCB Pr/Pfr		___	Unknown	(Ulijasz et al., 2008)
Synechococcus OSB'	SyB-Cph1	PCB Pr/Pfr		2K2N (Cornilescu et al., 2008), 2KOI, 2KLI (Ulijasz et al., 2010)	Unknown	(Ulijasz et al., 2008)
Thermochromatium tepidium	Ppd	?		___	Unknown	(Kyndt et al., 2004; Kyndt et al., 2005)
Thermosynechococcus elongatus BP-1	Tlr0924	PCB** Pb/Pg		___	Unknown	(Rockwell et al., 2008)
Thermosynechococcus elongatus BP-1	PixJ	PCB** Pb/Pg		___	Unknown	(Ishizuka et al., 2006; Ishizuka et al., 2007; Ulijasz et al., 2009)

*Domain architecture denoted as determined by pfam (Finn et al., 2010) and primary literature.
Abbreviations: LH—light harvesting complex; CCA—Complementary chromatic adaptation; PS—Photosystem.



1980; Lamparter et al., 2001). In many cases, the gene encoding heme oxygenase is located in the same operon as the BphP (Bhoo et al., 2001; Karniol & Vierstra, 2003; Wegele et al., 2004; Karniol et al., 2005).

Phytochromes have likewise been found in fungi (Blumenstein et al., 2005). *Aspergillus nidulans* FphA (fungal phytochrome) most closely resembles BphPs, with a PAS-GAF-PHY photosensory core at its N-terminus and a histidine kinase domain at its C-terminus. Moreover, FphA attaches its BV chromophore to a Cys residue corresponding to the attachment site in BphPs (Blumenstein et al., 2005). Phytochrome in fungi is thought to play a role in the switch between sexual and asexual development (Rodriguez-Romero et al., 2010).

Thus, phytochromes can be divided into three categories: those containing PAS, GAF, and PHY domains (e.g. plant phytochromes, BphPs and Cph1), PAS-less phytochromes (e.g., *Sy*Cph2), and phytochromes that require only a GAF domain for proper photochemistry (CBCRs) (Table 1) (Rockwell & Lagarias, 2010). A number of excellent reviews have been published recently with focus on plant (Rockwell & Lagarias, 2006; Möglich et al., 2010b) and fungal (Rodriguez-Romero et al., 2010) phytochromes. This review focuses on phytochromes from bacterial species with special emphasis on observations gained through three-dimensional structural analysis and on phytochromes to which a physiological function has been assigned. For further reference, the reader is also directed to several recent reviews on cyanobacterial phytochromes and BphPs (Montgomery, 2007; van der Horst et al., 2007; Rockwell et al., 2008; Ikeuchi & Ishizuka, 2008; Giraud & Vermeglio, 2008; Losi & Gärtner, 2008; Hughes, 2010; Rockwell & Lagarias, 2010; Scheerer et al., 2010).

Structural studies

Phytochrome architecture

The PAS, GAF, and PHY domains that make up the photosensory core share a five-stranded antiparallel sheet (order 2-1-5-4-3) with a conserved helix (Figure 2, αC) joining the outside strands. The PAS is the smallest of the domains, with no additional elements (Wagner et al., 2005; Essen et al., 2008). PAS domains are sensor modules implicated in sensing redox potential, oxygen, light, and small molecules as well as providing a scaffold for protein interactions. Many PAS domains are contained within proteins that serve as receptors or transcription factors (Taylor & Zhulin, 1999). A single polypeptide may contain multiple PAS domains. A survey of the Pfam database yields many proteins with up to eight PAS domains, and several longer ones including a predicted signaling kinase with 26 PAS domains (Finn et al., 2010).

GAF domains, while structurally similar to PAS domains (Ho et al., 2000), contain three additional helices that in phytochrome form the dimer interface (Figure 2, helices αA, αB and αE). The bilin lyase GAF domain is the heart of every phytochrome as it houses the bilin chromophore (Aravind & Ponting, 1997; Wu & Lagarias, 2000). The chromophore is substantially positioned within the GAF domain with covalent attachment to a Cys residue either N-terminal to the PAS domain (as seen in BphPs and fungi) or within the GAF domain (as seen in cyanobacteria and plants), with linkage occurring through an intrinsic lyase activity to the bilin A-ring vinyl side chain (Lagarias & Lagarias, 1989) (Figure 1).

The first atomic resolution three-dimensional structure of a phytochrome was the chromophore binding domain (CBD is PAS and GAF domains only) of BphP from *D. radiodurans* (Wagner et al., 2005) (Figure 3A). The DrBphP-CBD structure surprised researchers by containing a figure-of-eight knot, a feature that has since been confirmed in all PAS-containing phytochromes studied structurally (Yang et al., 2007; Essen et al., 2008; Yang et al., 2008) (Figure 2). The PAS and GAF domains interact at the knot where the ~35 N-terminal residues pass through a 'lasso' loop contributed by the GAF domain (Wagner et al., 2005) (Figure 2). The function of the knot remains unknown, although it does not imbue phytochrome with unusually high mechanical stability (Bornschlögl et al., 2009). Because the Cph2 and CBCR classes do not contain a PAS domain, they cannot adopt this complex topology, thus the knot is not a universal feature of phytochromes. Interestingly, Cph2 PAS-less phytochromes such as *Synechococcus* sp. OS-A *Sy*A-Cph1 and *Synechococcus* sp. OS-B' *Sy*B-Cph1 retain the GAF sequence that would form the lasso portion of the knot (Ulijasz et al., 2008).

Bacterial and cyanobacterial phytochrome structures containing the entire photosensory core domain (PCD), including PAS, GAF, and PHY offered the first views of the PHY domain, its interactions with the GAF domain, and confirmation of the structural similarity of the GAF and PHY domains (Essen et al., 2008; Yang et al., 2008) (Figure 2). As expected, the PHY domain is closely related to the GAF domain (Montgomery & Lagarias, 2002), including the additional helices αA and αB before β1 and αE after β5. As in the GAF domain, these helices pack against each other. Because the αE helix of the GAF domain is continuous with the αA helix of the PHY domain, a single long helix runs the length of the photosensory core, and the PAS, GAF, and PHY domains are arrayed along it (Figure 2). This striking 66 Å α-helix connecting the PHY domain to the rest of the photosensory core is a feature that had previously been observed

in adenylyl cyclase (Martinez et al., 2005) and proteins containing tandem GAF domains [i.e., phosphodiesterase (Martinez et al., 2002)].

The PHY domain has a unique 49-residue extension between its β5 and αE that reaches back to the CBD, connecting what would otherwise be two spatially separate domains of the protein (Figure 2). An intriguing interaction not observable in CBD structures due to their truncated nature is a salt bridge between the Asp207 carboxylate and Arg472. Asp207 is within the GAF domain and part of the highly conserved DIP motif while Arg472 is in the PHY domain extension (Figure 3B). Interestingly, dimer formation was altered and minor changes in absorption were seen in an Arg472Ala variant, highlighting the importance of this PHY domain position (Essen et al., 2008).

The PHY extension also serves to cover the solvent accessible chromophore (Essen et al., 2008) (Figure 2). The exclusion from solvent is thought to confer stability to the Pfr conformation of bound bilin as evidenced by the bleached far-red spectrum of truncated phytochromes lacking PHY domains (Karniol et al., 2005). However, a GAF-only truncation of SyB-Cph1 does retain some photoconvertibility (Ulijasz et al., 2008). Despite high sequence homology, the secondary structure of the PHY extension differs in PaBphP (Yang et al., 2008) and Cph1 (Hughes, 2010) but structurally serves the same purposes (Figure 2).

Chromophore configuration

In BphPs, the thioether bond is between a Cys thiol and the $C3^2$ carbon of BV, whereas in cyanobacterial phytochromes the bond is instead to the $C3^1$ carbon of PCB (Wagner et al., 2007; Essen et al., 2008) (Figure 1). It is worth noting that the chemical structure of each free chromophore is altered upon ligation, with

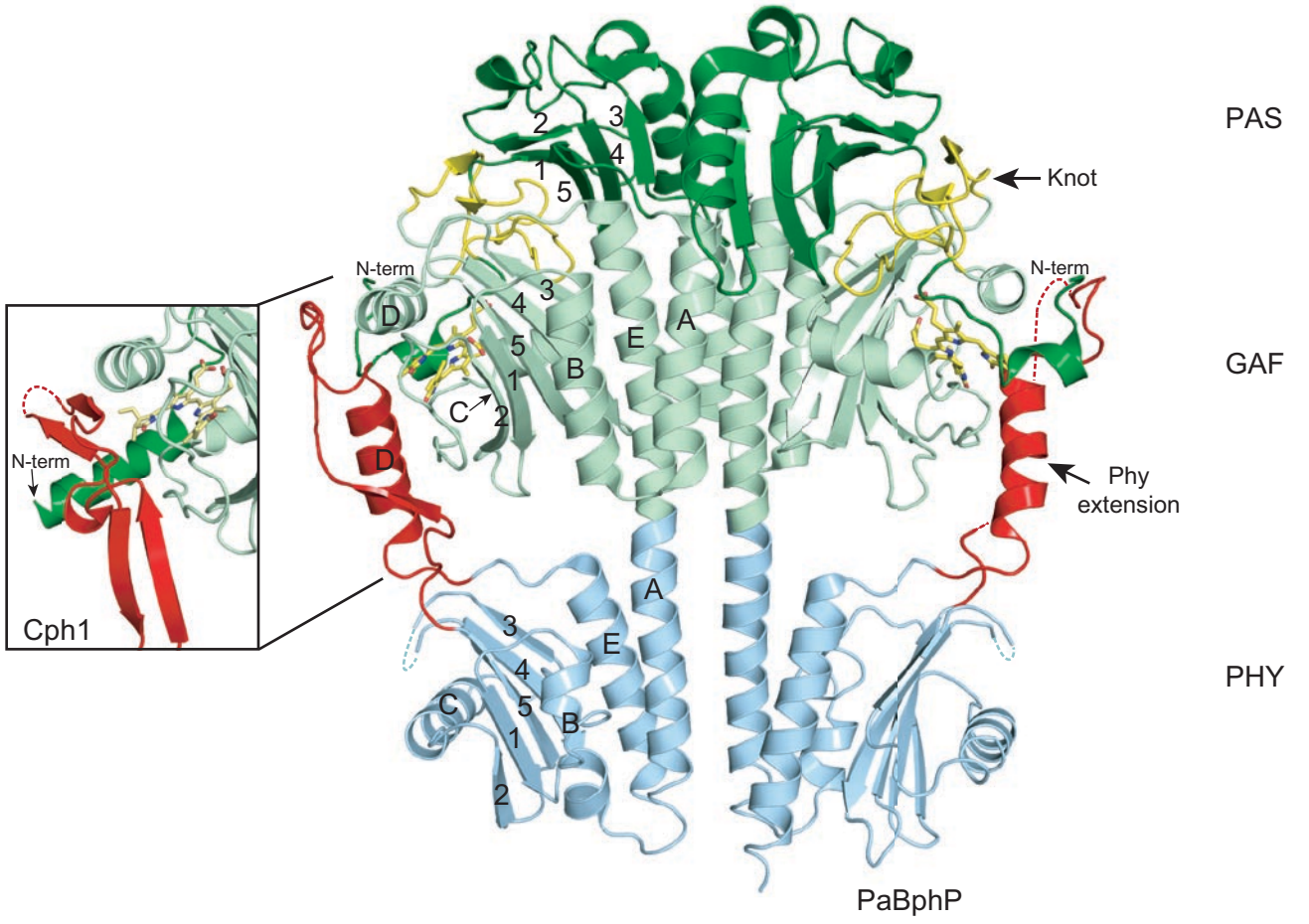


Figure 2. Structural architecture of PAS (dark green), GAF (light green), and PHY (blue) domains as seen in PaBphP dimer (pdb code 3C2W). Each domain consists of a five-stranded β-sheet in the order 2-1-5-4-3 with intervening helices. GAF domain helix αE forms a long continuous helix with PHY helix αA. PHY extension is shown in red and the residues making up the knot are in yellow. (Inset) The PHY extension adopts a different secondary structure in Cph1 (pdb code 2VEA). The Cph1 N-terminus adopts a well-ordered α-helical fold as opposed to the random coil present in DrBphP-CBD. It is not known whether the helical conformation exists in DrBphP when the PHY domain is present and/or whether the structural differences correlate with Pr vs. Pfr ground state.

corresponding affects on the overall conjugated system. For example, after nucleophilic attack by the Cys thiol group and bond formation, the strict planarity of the A ring is lost (Wagner et al., 2007) (Figure 3). In high-resolution structures, the nonplanarity of the chromophore A-ring due to the chiral C2 is clear (Wagner et al., 2007; Yang et al., 2007). The B and C-rings are co-planar with the A-ring tipped slightly, while the D-ring is rotated ~30 (in Cph1) to ~45° (in BphPs) out of the plane (Wagner et al., 2005; Yang et al., 2007; Essen et al., 2008) (Figure 3). For BphPs, the bilin lyase ligation at $C3^2$ converts BV to a PϕB-like dihydroBV. The protein environment seems to affect the equilibrium racemic ratio of the resulting 2(R), 3(E)-PϕB (Wagner et al., 2007) and 2(S), 3(E)-PϕB (Yang et al., 2007). The chromophore in both the Pr and Pfr forms of phytochromes is fully protonated (Kneip et al., 1999), a state that is achieved in the protein environment prior to ligation of the free bilin (Borucki et al., 2003; Röhmer et al., 2010).

The structures of BphPs in the Pr state have revealed a 5Zsyn, 10Zsyn, 15Zanti (ZZZssa) configuration of the tetrapyrrole (Figure 4A) (Wagner et al., 2005; Wagner et al., 2007; Yang et al., 2007; Essen et al., 2008) in agreement with many previous spectroscopic data including measurements on a bacterial phytochrome coupled to a covalently locked ZZZssa BV derivative (Inomata et al., 2005). The Pr-form of the PCB chromophore in Cph1-PCD likewise adopts a ZZZssa configuration (Essen et al., 2008), also observed in previously reported heteronuclear nuclear magnetic resonance (NMR) analyses (Hahn et al., 2008). In every phytochrome crystal structure to date both with and without the PHY domain, a very well-ordered 'pyrrole water' has been reliably noted. This water molecule is tightly bound between the three pyrrole nitrogens of rings A, B, and C and a nearby His. Also observed in the high-resolution DrBphP Pr structure is a small buried cavity adjacent to the D-ring (Wagner et al., 2007), which increases the freedom of the D-ring to rotate thus corroborating the long-standing view that isomerization of the C15 = C16 double bond is the light-driven step that initiates photoconversion.

The chromophore structure in a subset of the GAF-only CBCR family of phytochromes deserves special discussion. *Synechocystis* PixJ and its *Thermus elongatus* homologues *Te*PixJ and Tlr0924 have Pb/Pg photochemistry (Yoshihara et al., 2004; Ishizuka et al., 2006; Rockwell et al., 2008). Based on spectroscopic studies of the denatured protein, *Te*PixJ was proposed to autoisomerize its PCB chromophore to phycoviolobilin (PVB) after covalent attachment (Ishizuka et al., 2007). PVB is fully saturated at C5 (Figure 1). Interestingly, the Pb form of these PixJ chromoproteins is blue-shifted further than the isolated PVB spectrum would suggest. The molecular nature of this blue-shift was deduced from CD spectra measured for Tlr0924 (Rockwell et al., 2008). Two covalent attachment sites were proposed to explain the observed Pb/Pg photochemistry. The first attachment site at the well-known GAF domain Cys was verified at Cys527 in Tlr0924 (Rockwell et al., 2008), as it had been for Cys592 in *Sy*PixJ1 (Yoshihara et al., 2004). A second attachment site was uncovered in Tlr0924 within the DXCF motif that is found in these CBCRs in place of the conserved phytochrome DIP motif. Tlr0924 Cys499, corresponding to the C in DXCF, was determined to be necessary for wild-type

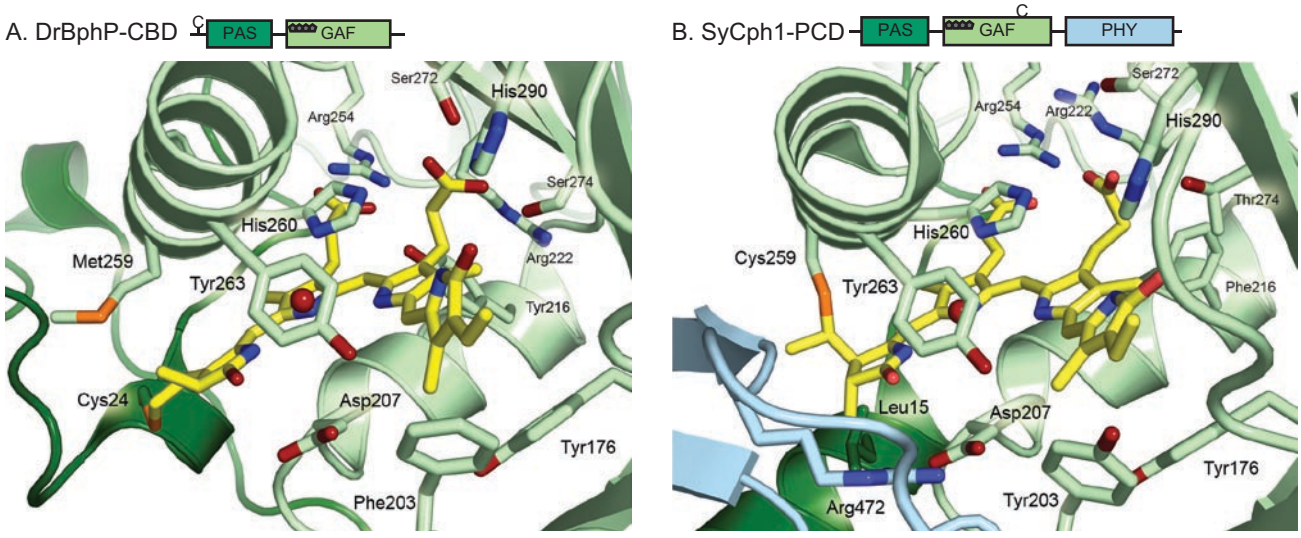


Figure 3. Chromophore-binding domains of representative bacterial and cyanobacterial phytochromes. (A) DrBphP (1.45 Å resolution, pdb code 2O9C). BV chromophore carbons are colored in yellow, residues of note are shown as sticks with the remaining protein backbone shown as ribbons. Colors correspond to domain origin: PAS domain dark green, GAF light green, and PHY blue. The red sphere is the pyrrole water. Schematic shows which domains were included in protein construct used for crystallization. (B) Cph1 (2.21 Å resolution, pdb code 2VEA). Depicted as in 3A with PCB chromophore shown in yellow.

photochemistry and was proposed to serve as the reversible second linkage with PVB at the C10 carbon (Rockwell et al., 2008). Attachment would shorten the π conjugation system further and lead to the blue-shifted Pb form relative to PVB itself. A more recent study of *Te*PixJ was in agreement with a second site linkage, although the chemical structure proposed was different (Ulijasz et al., 2009). Incomplete conversion of the PCB chromophore to PVB in Tlr0924 (Rockwell & Lagarias, personal communication) results in a heterogeneous chromophore population and provides an explanation for otherwise anomalous results including the observation of multiple spectral species in Tlr0924 (Rockwell et al., 2008) and PCB-like behavior of the DXCF to DXAF *Te*PixJ variant (Ulijasz et al., 2009), respectively.

The configuration of the Pfr form of PAS-GAF-PHY phytochromes is of great interest as is the pathway by which this state is achieved. Most data indicate that both BphP and Cph1 phytochromes are ZZEssa in the Pfr configuration, differing from the ZZZssa Pr configuration at

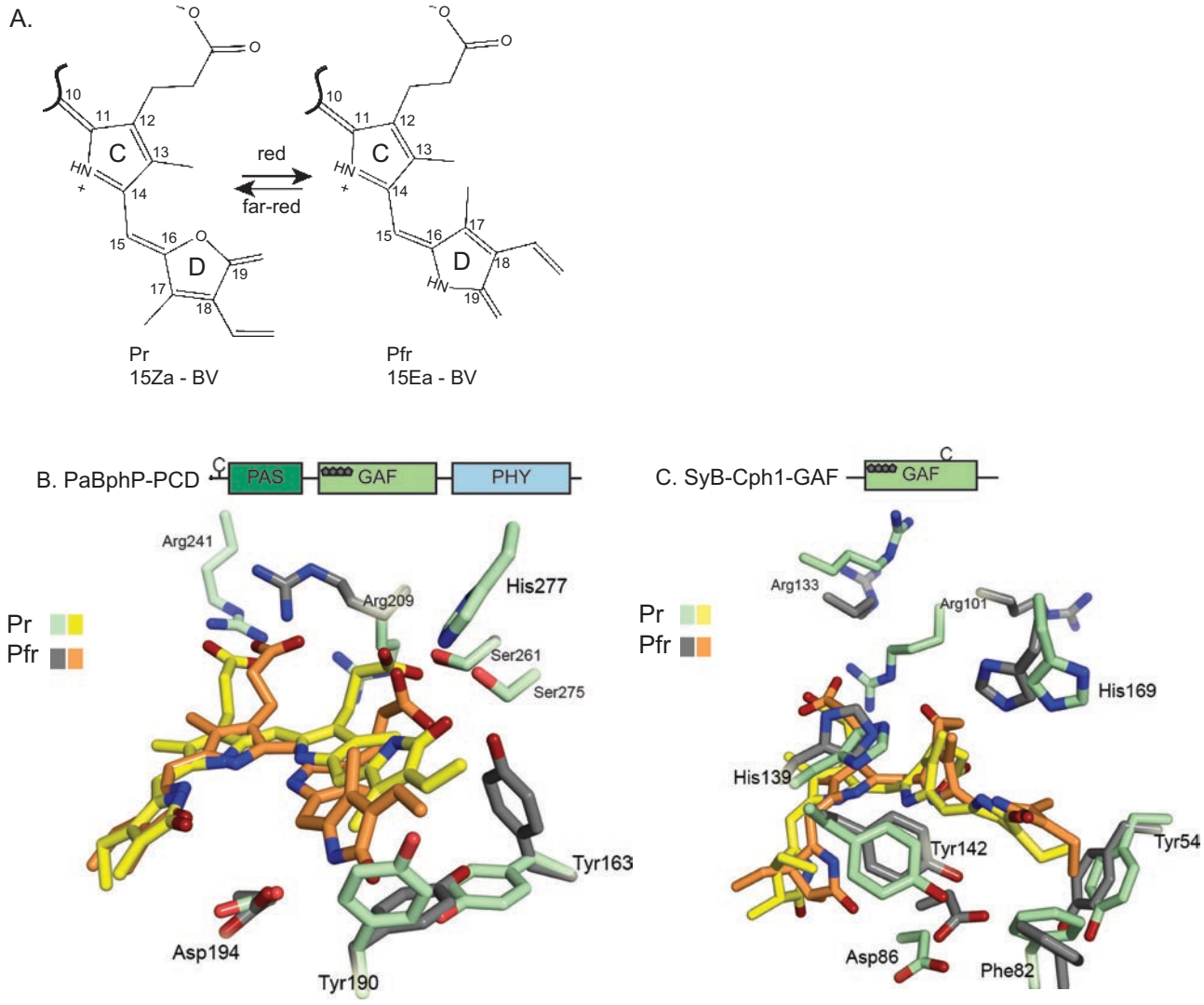


Figure 4. Pr/Pfr Structural Pairs. (A) Chemical configuration of Pr and Pfr BV. Cis (Z)/trans (E) isomerization at the 15–16 double bond as shown in Fig. 4B converts 15Za to 15Ea. Only C and D rings are shown for clarity. (B) Comparison of the Pr (BV—yellow and residues—light green) and Pfr (BV—orange and residues—gray) forms present in a mixed population in a PaBphP-PCD crystal (pdb code 3IBR). Schematic shows which domains were included in protein construct used for crystallization. Residues are shown as sticks, ribbon Cα trace is not shown, A-ring is on the left. Residues shown were found to be important to wild-type chromophore photochemistry. Most residues are structurally preserved while Arg209, Tyr163, and Tyr190 showed changes in the Pr and Pfr forms. (Tyr163, Tyr190, Asp194, Arg209, Arg241, Ser261, Ser275, and His277 are equivalent to DrBphP Tyr176, Phe203, Asp207, Arg222, Arg254, Ser274, Ala288, and His290). (C) Pr and Pfr solution NMR structures of SyB-Cph1 GAF1 domain (pdb codes 2KOI, Pr and 2KLI, Pfr). Coloring as in 4B. Most notable are the dramatic change seen in Arg 101 and the unprecedented A-ring movement in stark comparison with the immobility of the D-ring, the location typically thought to change during Pr to Pfr photoconversion. (Tyr54, Asp86, Phe82, Arg101, Arg133, His139, and Tyr142 are equivalent to DrBphP Tyr176, Asp207, Phe203, Arg222, Arg254, His260, and Tyr263, respectively).

the C15=C16 double bond (Figure 4A) (Inomata et al., 2006; Hahn et al., 2008). Moreover, magic-angle spinning NMR experiments show that the C and D rings experience the greatest chemical shifts upon Pr to Pfr conversion in both Cph1 and oat phyA (Röhmer et al., 2008). In addition, the bathyphytochrome, PaBphP (Pfr ground state) contains its chromophore in the ZZEssa configuration (Yang et al., 2009), although the extent to which this ground state Pfr phytochrome is equivalent to a red-light induced Pfr phytochrome remains to be seen.

Upon excitation with red light, phytochrome goes through several short-lived photointermediates before reaching the Pfr form. The first step of the photoconversion process is the very fast (picoseconds) isomerization of the C15=C16 double bond to form the LumiR intermediate (Matysik et al., 1995; Andel et al., 1996; Andel et al., 2000; Foerstendorf et al., 2000). Rotation of the D ring lengthens the conjugation system and leads to the longer observed wavelength absorption maximum in Pfr vs. Pr. Subsequent transition to MetaR via deprotonation of the D-ring and final relaxation to Pfr via subsequent reprotonation (Remberg et al., 1997; van Thor et al., 2001; Borucki et al., 2005) is on the microsecond scale, while rotation of the C14-C15 single bond is on the millisecond time scale. Illumination with far-red light begins the light-driven conversion back to Pr. The pathway is not the reverse of the forward cycle. In the backward light driven reaction, C15=C16 double bond isomerization is also the light-driven first step, but subsequent rearrangements differ in their order (Röhmer et al., 2010). Dark reversion is the thermal relaxation of the chromophore to the ground state, by a mechanism which differs from the Pfr -> Pr photocycle and which furthermore differs among phytochromes (Rockwell et al., 2006).

In the forward Pr → Pfr phototransition, in addition to D-ring motions, further chromophore adjustments during the photocycle have been reported. These have been interpreted at least in part as relaxation of the chromophore because major rotation around the C-D methine linker would be hindered by the protein environment (Kneip et al., 1999). Recent NMR data of Cph1 indicate that the Pr to Pfr conversion also results in alterations in the C-ring environment, an observation that may have implications in signal transmission from the chromophore to the surrounding protein (Röhmer et al., 2008). The Pr/Pfr mixed crystal structures of Yang et al. indicate a rigid-body rotation of the entire chromophore relative to the surrounding protein chain following the C15=C16 isomerization (Figure 4B) (Yang et al., 2009). Quantum mechanical/molecular mechanics (QM/MM) calculations of Kaminski and colleagues suggest slight motions of the A-ring (Kaminski et al., 2009). Moreover, doubly locked chromophores attached to the Agp1 phytochrome led to the conclusion that the C5 methine bridge twists during Pr to Pfr conversion (Inomata et al., 2009). Most dramatically, NMR structures of dark adapted and illuminated Cph2-class phytochrome, SyB, derived from measurements on Pr and a mixed population of Pr/Pfr forms suggested a light-driven isomerization at the C4-C5 single bond and a substantial twisting of the thioether linkage (Ulijasz et al., 2010) (Figure 4C).

A unifying view of the Pr to Pfr photocycle is that the C15=C16 bond isomerizes upon photon absorption and is accompanied by changes in D-ring and to some extent C-ring interactions with the protein, with smaller additional motions of other rings occurring as the protein and chromophore relax around the new BV conformation (Lagarias & Rapoport, 1980; Rüdiger et al., 1983; Mroginski et al., 2007; Röhmer et al., 2008; Mroginski et al., 2009). A-ring motions have been observed and the possibility that different phytochromes have somewhat different Pfr chromophore states is likely (Inomata et al., 2009). Where SyB and other related proteins fit in this model is not clear; one intriguing possibility is that the chromophore motion is different in PAS-less (unknotted) phytochromes than in canonical phytochromes.

Chromophore (re)arrangement is guided by protein

The solution behavior of linear tetrapyrroles is marshaled by their interactions with phytochrome proteins both in setting the Pr and Pfr configurations and in steering the trajectory from one to the other. The photochemistry of the chromophore is highly influenced by the surrounding protein, such that a simple swap of attachment site and chromophore (i.e., switching the N-terminal Cys for the GAF Cys as well as BV chromophore for PCB) does not recapitulate the spectral quality seen in a wild-type protein:chromophore pair (Lamparter et al., 2003; Wagner et al., 2008; Borucki et al., 2009).

In all phytochromes, chromophore contacts are primarily with the defining GAF bilin lyase domain (Figure 3A and 3B). When comparing the Pfr-PaBphP three-dimensional structure with that of Pr structures, the most striking changes are in the orientation of two aromatic residues (Tyr163 and Tyr190 corresponding to DrBphP Tyr176 and Phe203) surrounding the D-ring (Yang et al., 2008; Yang et al., 2009) (Figure 4B). Nonetheless, static structures of the Pr and Pfr forms of phytochrome do not lead to an unambiguous description of the structural trajectory of bilins. Aromatic residues corresponding to PaBphP Tyr163 and Tyr190 are highly conserved among phytochromes. Underscoring its importance, substitution of Cph1 Tyr176 (equivalent to PaBphP Tyr163) or the equivalent position in *Arabidopsis thaliana* phyB confers a nonphotoconvertible, fluorescent phytochrome. Strangely this is not the case for the equivalent PaBphP or DrBphP variant (Fischer & Lagarias, 2004; Fischer et al., 2005; Wagner et al., 2008; Yang et al., 2009). These and other differences in photochemical phenotypes between

the Cph1-like and BphP-like phytochromes provide the framework for the hypothesis that the initial direction of rotation of the D-ring is not the same in the two sets of proteins. This model is supported by CD measurements (Borucki & Lamparter, 2009; Rockwell et al., 2009) and structure calculations, which suggest the D-ring rotates clockwise in BphPs and counter-clockwise in Cph1s (relative to that pictured in Figure 3).

An invariant Asp, which is a component of the highly conserved DIP motif, sits just below the bilin chromophore. It is key to the photochemical properties of phytochromes and plays a role in both Pr -> Pfr and Pfr -> Pr conversion (Röhmer et al., 2010; Piwowarski et al., 2010). This Asp interacts with an Arg in the PHY extension in both the Cph1 Pr structure (Figure 3B) (Essen et al., 2008) and the PaBph1 Pfr/Pr structure. In the latter, the Asp also forms a hydrogen bond to the D-ring nitrogen (Figure 4B). Asp207 in Cph1 and in DrBphP-CBD is also within hydrogen-bonding distance to Tyr263, a residue that takes part in the formation of a hydrophobic pocket surrounding ring D (Essen et al., 2008; Wagner et al, 2007) (Figure 3A and 3B). Substantiating its importance to the photocycle, a DrBphP-Asp207His variant is unable to photoconvert and is fluorescent (Wagner et al., 2008), a photochemical property not seen in the equivalent Cph1 variant (Ulijasz et al., 2009).

Strong ionic interactions of the B- and C-ring propionate side chains anchor the chromophore in the pocket (Figure 3A and 3B). The charged propionate side chains are notably sequestered from solvent, in contrast to many other bilin:protein complexes such as cyanobacterial PCB:FDBR PcyA or the *Prochlorococcus* PSSM-2 phage-encoded PEB:FDBR PebS and in contrast to heme-bound proteins such as heme oxygenases or myoglobin (Watson, 1969; Sugishima et al., 2005; Hagiwara et al., 2006; Dammeyer et al., 2008). In both BphP and Cph1-type GAF domain pockets, the B-ring propionate carboxylate oxygens pair with Arg254 and makes a hydrogen-bond to the backbone nitrogen of residue 257, while the C-ring propionate interacts with His260, Ser272, and Ser/Thr274 (amino acid numbering is identical in DrBphP and Cph1 GAF domains) (Figure 3). Differences between propionate side chain and protein interactions are seen between these two classes of phytochromes. For example, the B-ring propionate interacts with Tyr216 in DrBphP but in Cph1 this interaction cannot take place because this position is a phenylalanine (Figure 3). Differential affects of substitutions of the C-ring propionate group provide additional evidence for different Pr -> Pfr pathways in BphP vs. Cph1-type phytochromes and suggest the interaction between Cph1 Tyr176 and the C-ring propionate is indispensable (Rockwell et al., 2009).

Other conserved residues are present in alternative conformations in the PaBphP Pr vs. Pfr structures. Arg209 (Arg222 in DrBphP and Cph1) shifts (Figure 4B). The importance of this side chain to the photocycle is evident in the Arg209Ala substitution, which results in a Pfr/Pr mixed-state independent of illumination (Yang et al., 2009). The arrangement of Arg222 in Cph1 Pr is unlike that seen in DrBphP Pr (Figure 3A and 3B) but whether this difference is physiologically meaningful is unknown. The corresponding residue in SyB (Arg101) also moves in the SyB Pr vs. Pfr solution structures (Ulijasz et al., 2010). However, while the SyB Pfr Arg101 points toward the helix connecting the GAF and PHY domains, the PaBphP Pfr Arg209 points away from the equivalent helix (Yang et al., 2009). (In Figure 4B and 4C, the aforementioned helix would be on the right). This different arrangement may have consequences for light-activated signaling as this helix is not only important for dimerization but has been implicated in propagating the light signal sensed by the bilin-binding GAF domain through the PHY domain to the output domain (see concluding comments for additional discussion).

Several other residues exhibit changes in photochemistry when changed to Ala in PaBphP even though no structural changes were observed at these locations when comparing the Pfr and Pr crystal structures (Asp194, Arg241, Ser261, Ser275, His277, and Ser459) (Figure 4B). Of note is His277Ala (His290 in DrBphP), which shows the Pr/Pfr photochemistry of a canonical Pr ground state phytochrome. Ser459Ala within the PHY extension and led to a Pr ground state configuration (Yang et al., 2009). Based on the dark reversion rates of Ala variants, Yang et al. proposed that Arg209, Ser275, and His277 are important for maintaining a Pfr state while Ser261 and Arg241 are important for Pr stability.

A final notable change is seen in the solution structures of SyB Pr vs. Pfr (Ulijasz et al., 2010). The imidazole ring of SyB His139 (equivalent to DrBphP His260) rotates (Figures 3A and 4C). This residue is conserved in all structurally characterized phytochromes and takes part in coordinating the pyrrole water (Figure 3– red sphere). In the SyB Pfr structure, the His side chain is rotated so that hydrogen bond formation between it and the pyrrole water is disrupted.

Phytochrome is a dimer

Plant phytochrome has long been known to dimerize, but was thought to do so via the C-terminal histidine kinase (HK)-like domain (Edgerton & Jones, 1992; Romanowski & Song, 1992; Park et al., 2000c). Dispelling this idea, dimers were present in the crystal structures of DrBphP-CBD (Wagner et al., 2007), RpBphP3-CBD (Yang et al., 2007), PaBphP-PCD (Yang et al., 2008), and Cph1-PCD (Essen et al., 2008) despite the lack of a HK-like domain (Figure 2). In DrBphP-CBD, RpBphP3-CBD, and PaBphP–PCD three α-helices from each GAF domain form a six-helix bundle stabilizing a parallel dimer. Dimer

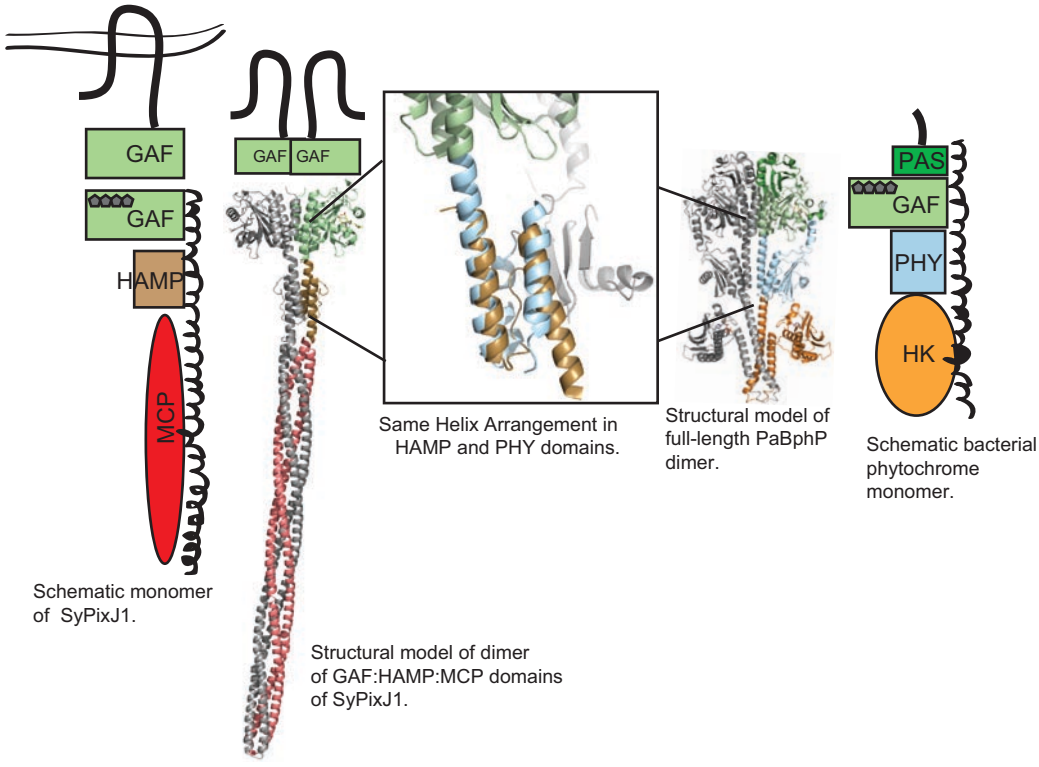


Figure 5. Full-length models of phytochrome family members. (Left) Model of the cyanobacterial phototaxis receptor *Sy*PixJ1. The GAF/PHY domains are derived from DrBphP (pdb code 2O9C) (Wagner et al., 2007), the HAMP domain from pdb code 2ASW, and the MCP signaling domain from 2CH7. (Right) Full-length model of a 'canonical' PAS-GAF-PHY-HK phytochrome. This model is based on the structure of PaBphP (pdb code 3C2W (Yang et al., 2008) and the structure of the HK domain from the *Thermotoga maritima* sensor HK (pdb code 2C2A) (Marina et al., 2005). (Central inset) The HAMP domain helix-loop-helix (pdb code 2ASW) superimposes well upon a subset of the secondary structure elements within the PHY domain.

formation in the longer PaBphP-PCD, is also parallel and is dominated by the α-helical bundles from the GAF and PHY domains. Much of the dimerization interface is contributed by structural elements outside the common β-sheet core of the PAS, GAF, and PHY domains (Figure 2). Supporting the dimerization interface within the PHY domain are opposing Glu and Arg residues (Yang et al., 2008). In Cph1-PCD, an antiparallel dimer (Essen et al., 2008) permits the PHY domain of one monomer to interact with the PAS domain of its dimer partner. The dimer interface of the Cph1-PCD is light dependent (Strauss et al., 2005), and thus it may be reasonable that the parallel dimer that would orient the HK domains across from one another is not seen in the Pr crystal form. No clashes are predicted when monomers of Cph1-PCD are aligned onto the two monomers of the parallel PaBphP dimer (data not shown). These observations suggest that while cyanobacterial (and plant) phytochromes may differ from BphPs in the dimerization dynamics they are competent to adopt the parallel dimer in the activated state.

Although the GAF helical bundle is the conserved dimer interface, subtle differences between the packing orientations of the different dimers captured by crystallography may represent the twisting that the long backbone of the molecule undergoes upon illumination. There are now eight independent dimer interfaces that have been analyzed by protein crystallography for BphP-class phytochromes (one dimer pair each from pdb codes 1ZTU, 2O9C, 3IBR, 2OOL, and four from 3C2W). Alignment of these structures shows that the angular orientation of the bilin lyase domain to its dimer partner varies by up to 30° (Yang et al., 2008). The malleable dimer demonstrates the potential for this interface to respond dynamically to light signaling.

Full-length models

Although no high-resolution structures of a full-length phytochrome have been obtained, several studies have begun to address overall shape and relative domain orientation. Small-angle x-ray scattering (SAXS) analysis of full-length RpBphP2 showed that its asymmetric structure is that of the letter Y (Evans et al., 2006), a design also reported in pea phytochrome (Nadasako et al., 1990). The x-ray crystal structures of DrBphP-CBD (Wagner et al., 2005) and an HK domain (Marina et al., 2005) were fit into the RpBphP2 SAXS envelope. The SAXS model proposed each CBD was contained within

one arm of the Y, with the PHY domains continuing through to the base of the arms, and the HK domains crossing one another to form the trunk (Evans et al., 2006). In this model, the HK phosphoacceptor histidine is facing the ATP binding site of the dimer partner, in line with *in trans* phosphorylation (Yang & Inouye, 1991; Swanson et al., 1993).

Using the PaBphP-PCD x-ray structure (PAS, GAF, and PHY domains) and the above noted HK structure (Marina et al., 2005), Yang et al. proposed a full-length structure that takes into account the dimerization interface between both the PCD and the HK domains, such that the overall architecture takes on less of a Y-shaped structure (Yang et al., 2008) (recreated in Figure 5). Of note, the continuation of the α-helical spine beginning with the connection between the GAF and PHY domains extends into the HK domain. To create such a full-length phytochrome model, the HK dimer can be appended at the C-termini of the phytochrome PHY domains without invoking substantial 3D structural changes in either protein (Figure 5). Some quaternary adjustment is needed, as the HK N-termini are only ~6 Å apart and must be untwisted to accommodate the PHY domain C-termini, which are approximately 20 Å apart in a ground state Pfr PaBphP structure (Yang et al., 2008). This repositioning would affect the HK:HK interface, and speaks to the dynamic nature of phytochrome.

Recently published electron microscopy reconstructions of full-length DrBphP confirmed the 2-fold symmetry axis as well as the parallel dimerization interface between two monomers that was observed in DrBphP-CBD, PaBphP-PCD, and RpBphP3-CBD x-ray structures. The predicted continuation of the dimer interface was shown, although there is a gap in the density due to the single α-helix spanning the CBD-PHY connection. The complete HK domain is not visible, presumably due to the known flexibility within the HK domain (Li et al., 2010). The twisting nature of the HK domains at the neck of the full-length model (Figure 5) was postulated to fit electron microscopy data as well (Li et al., 2010).

The extensive contacts between phytochrome monomers, both observed and hypothesized, lead to an emerging picture of how absorption of a photon by the bilin chromophore results in propagation of a signal. Upon excitation with the appropriate wavelength of light, the chromophore isomerizes with a concomitant alteration in the immediate surrounding residue conformations. Possible sites include C-ring propionate interactions, His290 (Wagner et al., 2005; Wagner et al., 2007; Yang et al., 2007; Essen et al., 2008; Röhmer et al., 2008; Ulijasz et al., 2010), and Asp207 interactions with PHY domain and /or D-ring (Yang et al., 2008; Röhmer et al., 2010). Signal transmission from the pocket to the GAF dimerization interface follows. Because the GAF, PHY, and HK domains are tethered to one another through the central helices, the conformational change presented at the GAF domain helical bundle is reverberated through the PHY to the HK domain.

Physiological function

Output domains

Many of the phytochromes described thus far in this review contain histidine kinase modules as their output domains. In response to light, a typical two-component phosphorelay system begins with the transfer of a phosphate from ATP to a histidine residue within the HK domain of phytochrome. The phosphate is then transferred to an invariant aspartate residue on a response regulator (RR). The binding of the RR to its DNA or protein target is affected by its phosphorylation state and leads to a functional response, such as transcriptional activation (Bijlsma & Groisman, 2003). Examples of HK-containing phytochromes with RRs contained within the same polypeptide (Table 1) or existing as a separate protein have both been identified. Cph1 was the first phytochrome to which HK activity was experimentally attributed (Yeh et al., 1997). Compared to the Pfr form, the Pr form of Cph1 has increased autophosphorylation and phosphotransferase activities toward its RR, Rcp1, though this is not the case for all phytochromes. Cph1 (Yeh et al., 1997), RpBphP2, 3, and 6 (Giraud et al., 2004; Giraud et al., 2005), Agp1 (Lamparter et al., 2002; Karniol & Vierstra, 2003), CphA, and CphB (Hübschmann et al., 2001) exhibit more efficient activity in the Pr form, while PsBphP (Bhoo et al., 2001) and Agp2 (Karniol & Vierstra, 2003) display higher Pfr-dependent enzyme activity. Interestingly, variation is found within species. For example, *Agrobacterium tumefaciens* maintains two phytochromes, Agp1 and 2. Agp2 is a bathyphytochrome with Pfr/Pr photochemistry, an atypical HK domain and an RR domain within one polypeptide. Agp1 has Pr/Pfr photochemistry and consists of the classical PAS-GAF-PHY-HK domain architecture (Lamparter et al., 2002; Karniol & Vierstra, 2003). The utility of maintaining disparate phytochromes is presumed to expand the ability of an organism to sense and respond to its environment.

Output strategies other than the HK domain have been observed (Table 1). Both *Rhodopseudomonas palustris* BphP1 and *Bradyrhizobium* ORS278 BphP1 terminate with a second PAS domain (Giraud et al., 2002) and BrBphP3 consists only of PAS-GAF-PHY domains (Jaubert et al., 2007). These phytochromes must propagate the light-induced signal via interaction with as yet unidentified downstream targets.

Several phytochromes have two domains typically found in signaling systems controlling chemotaxis. MCP (methyl-accepting chemotaxis protein) signaling

domains and HAMP (histidine kinases, adenylyl cyclases, methyl binding proteins, phosphatases) domains are found in *Sy*PixJ1 (Bhaya, 2004; Yoshihara et al., 2004; Ikeuchi & Ishizuka, 2008), *Te*PixJ (Ishizuka et al., 2006; Ishizuka et al., 2007; Ulijasz et al., 2009) and *An*PixJ (Narikawa et al., 2008a). MCP and HAMP domains were historically characterized in the transmembrane chemoreceptors controlling flagellar motility (reviewed in Kirby, 2009). MCP signaling domains are long coiled coils that are reversibly methylated to control signaling. HAMP domains are short helical motifs (Hulko et al., 2006) that are thought to regulate the phosphorylation or methylation state of these dimeric receptors (Aravind & Ponting, 1999). In the case of *Sy*PixJ, light induces motility driven by the Type IV pilus retraction motor (Bhaya et al., 2000).

Microbial phytochromes with GGDEF domains alone, or in conjunction with EAL domains have both been identified (Table 1). GGDEF and EAL domains are named after the residues required for the activity of diguanylate cyclases (DGCs) and phosphodiesterases (PDEs), respectively, which in turn synthesize and degrade cyclic-dimeric-GMP (c-di-GMP) (reviewed in Römling et al., 2005 and Schirmer & Jenal, 2009). Two phytochromes from *R. sphaeroides*, BphG1 and BphG2, contain GGDEF and EAL domains. RsBphG1 conserves a Cys upstream of its PAS domain, binds BV and possesses Pr/Pfr photochemistry. Initial tests using full-length protein showed that RsBphG1 lacked DGC activity but possessed light-dependent PDE activity. Subsequent analysis of a truncated RsBphG1 lacking the C-terminal EAL domain uncovered DGC activity enhanced by red light exposure (Tarutina et al., 2006). A number of physiological responses have been attributed to cellular levels of c-di-GMP, including control of biofilm formation (Römling et al., 2005). The physiological role of BphG1 and BphG2 in *R. sphaeroides* as well as the functional significance of a cryptic DGC activity and PDE activity dissociated from signal perception is an active area of investigation.

Downstream physiology

Many bacterial and cyanobacterial phytochromes have yet to be associated with a physiological function. However, the polycistronic organization of bacterial mRNA has provided useful clues. Often, the phosphate-receiving RRs are encoded within the same operon as the HK-containing phytochrome. In other cases, the phytochrome gene is located near the genes that it regulates. This convenient genomic organization has led to identification of downstream targets and physiological functions. For example, the RR DrBphR is located downstream of DrBphP and has been implicated together with DrBphP in the upregulation of carotenoid synthesis in response to red light (Davis et al., 1999). Below we describe several examples in which a phytochrome signaling network in a bacterial system has been assigned to a physiological function.

Complementary chromatic adaptation

Complementary chromatic adaptation (CCA) is the well-studied process of phycobilisome (PBS) remodeling in the freshwater filamentous cyanobacteria *F. diplosiphon*. PBSs are the light harvesting systems that capture solar energy and funnel high-energy photons to photosynthetic reaction center II (see Kehoe & Gutu, 2006 for a comprehensive CCA review). *F. diplosiphon* changes color in response to light quality. This differential pigmentation is a result of changes in biliprotein content of the PBS. Phycoerythrin (PE) is a biliprotein that typically binds PEB as its pigment and therefore absorbs green light maximally, while phycocyanin (PC) binds PCB and absorbs red light maximally. PE is preferentially produced under green light growth conditions, and PC is preferentially produced under red light growth conditions. Because of the different absorption characteristics of PE and PC, cells that accumulate PE appear red in color and those that accumulate PC are blue–green in color. In addition to alterations in pigmentation, *F. diplosiphon* cell shape changes from long and cylindrical under green light to shorter and more round under red light. This phenotype has been linked with the periplasmic protein TonB (Stowe-Evans et al., 2004; Pattanaik & Montgomery, 2010a; Pattanaik & Montgomery, 2010b).

CCA is controlled in part by the Regulator of chromatic adaptation (Rca) system (Kehoe & Gutu, 2006). Rca is a phosphorelay signal transduction system that leads to changes in the PC to PE ratio within PBSs, thus customizing biliprotein content to light spectral quality. RcaE is the photoreceptor at the apex of this response and is distinguished from other phytochromes in that it controls a green/red photoreversible process (Kehoe & Grossman, 1996; Terauchi et al., 2004). Bilin lyase activity of RcaE is not well defined and to date its naturally occurring chromophore remains unknown. RcaE holophytochrome, whose bilin binding is detectable by Zn^{2+}-dependent fluorescence, is obtainable *in vivo*, although *in vitro* bilin lyase activity occurs to a much lesser extent. In addition, co-expression of RcaE with bilin biosynthetic enzymes for PCB failed to yield photoactive holoRcaE. These data have led to the hypothesis that RcaE may not possess intrinsic lyase activity in its GAF domain and may instead require an as yet uncharacterized lyase for covalent chromophore attachment (Terauchi et al., 2004).

Cells deficient in RcaE exhibit a rounded morphology, a perturbation in their light regulated biliprotein content, and a black pigmentation phenotype independent of light due to intermediate levels of PE and PC (Kehoe & Grossman, 1996; Terauchi et al., 2004; Bordowitz &

Montgomery, 2008). The RRs RcaF and RcaC have been placed downstream of RcaE and confer a red-pigmented phenotype when mutated due to the lack of PC accumulation (Chiang et al., 1992; Kehoe & Grossman, 1997). It is thought that RcaE is an active kinase in red-light but may act as a phosphatase in green light as evidenced by the red phenotype of cells containing RcaE variants with mutated HK domains seen under both green and red light (Kehoe & Grossman, 1997). A second as yet uncharacterized pathway, control of green light induction or Cgi, is thought to work in conjunction with the Rca system in regulating the CCA response in *F. diplosiphon* (Kehoe & Gutu, 2006).

An additional photoreceptor/RR pair was shown to regulate CCA in *Synechocystis* (Hirose et al., 2008). CcaS is a Pg/Pr photorevertible phytochrome that binds PCB as its chromophore. CcaR is an RR that is more efficiently phosphorylated by the Pr form of CcaS and thus differs from the Rca system by being a predominantly green-light sensing system (Hirose et al., 2008). In *Nostoc punctiforme*, CcaS and CcaR are both located within a gene cluster encoding for PBS proteins and were found to be responsible for controlling the appropriate PE content under green light conditions (Hirose et al., 2010).

CcaS shares several key features with RcaE. Like RcaE, CcaS lacks the N-terminal PAS domain and the PHY domain and is an unknotted phytochrome of the CBCR class (Table 1). Key residues surrounding the chromophore differ in CcaS and RcaE in comparison with other CBCRs. The Asp in the highly conserved DIP motif is crucial for a classic phytochrome photocycle and is replaced by a valine in these CBCRs. In addition, the histidine immediately adjacent to the GAF domain chromophore binding Cys, which along with the chromophore ring nitrogens coordinates the pyrrole water, is replaced with a Leu in CcaS and RcaE (Hirose et al., 2008). These changes may allow the CCA phytochromes to be sensors of bilins as well as sensors of light.

Photosystem regulation in Bradyrhizobiaceae

The photosynthetic apparatus in purple bacteria consists of light-harvesting (LH) complexes surrounding a reaction center. In order to optimize the energy obtained, these anoxygenic photosynthetic bacteria carefully regulate the expression of photosystem genes in response to light and redox potential (Bauer et al., 2003). Regulation of photosystem expression by a bacteriophytochrome was shown in the symbiotic N_2-fixing bacterium, *Bradyrhizobium* ORS278. The action spectrum of photosystem synthesis was found to closely match that of BrBphP1-Pfr absorption spectra and wavelength dependent expression of key photosystem proteins was seen. Furthermore, a BrBphP1 null mutant lacked the ability to regulate its photosystem in a light-dependent manner. The transcription factor PpsR was found in close genomic proximity to BrBphP1 and is likely involved in the signaling pathway beginning with BrBphP1, as a PpsR null shows constitutive photosystem activity (Giraud et al., 2002). PpsR is a PAS domain containing transcriptional repressor first identified in *R. sphaeroides*, where it is sensitive to the redox state of the cell through intermolecular disulfide bond formation (Penfold & Pemberton, 1994; Ponnampalam et al., 1995; Masuda & Bauer, 2002; Masuda et al., 2002). In *R. sphaeroides*, regulation by PpsR is complex (reviewed in Kovacs et al., 2005 and Elsen et al., 2005), as it is itself repressed by AppA, a BLUF (sensor of blue light using flavin adenine dinucleotide) domain-containing protein that also senses oxygen (Moskvin et al., 2007). Two PpsR genes were found in *Bradyrhizobium* ORS278. One of these, PpsR2, contains no cysteines and is therefore not redox sensitive. PpsR1 acts as an activator under reducing conditions; it contains a single cysteine that upon oxidation forms intermolecular disulfide bonds leading to higher order oligomers, which render the protein inactive (Jaubert et al., 2004). Regulation by BrBphP must occur by a means unlike the more typical phosphorelay signaling system, as it does not contain a HK domain (Giraud et al., 2002).

A somewhat more complex photosystem regulatory mechanism was proposed in the closely related *Rps. palustris* (Giraud et al., 2002). The *Rps. palustris* genome includes six BphP genes (Giraud & Vermeglio, 2008). RpBphP1 is encoded in the photosynthesis gene cluster. In conjunction with the transcriptional regulator PpsR2, RpBphP1 controls expression of photosystem genes and leads to a decrease in respiratory activity (Kojadinovic et al., 2008). RpBphP2 and 3 are organized sequentially in the genome near genes for LH4. RpBphP2 shows normal Pr/Pfr photochemistry while RpBphP3 has a Pr ground state but upon illumination with red light forms a Pnr (near-red) conformational state, which is fully reversible. Both RpBphP2 and 3 contain HK domains and in their Pr forms can autophosphorylate and are capable of phosphotransfer to Rpa3017, an RR encoded downstream. The unusual phytochemistry of RpBphP3 in concert with RpBphP2 is hypothesized to maximize the synthesis of LH4 relative to other light harvesting complexes in order to harvest light from an environment also occupied by competing phytoplankton (Giraud et al., 2005).

RpBphP4 represents an unusual phytochrome in that the chromophore attachment Cys is not conserved across isolates. Instead of sensing light, RpBphP4 from *Rps. palustris* CGA009 is sensitive to redox state. Two Cys residues located in the PHY and HK domains are essential to redox sensitivity, with HK activation and LH2 induction occurring under reducing conditions. Sequences with high similarity to RpBphP4 have been identified in other *Rps. palustris* strains, but these do conserve the Cys attachment site and act as light-regulated HKs (Vuillet et al., 2007).

Phototaxis

All organisms, including bacteria, require the ability to position themselves within their environments where nutrients and light are optimal. Photosynthetic bacteria such as cyanobacteria must carefully balance light quality with nutrient availability as too much light during periods of slow growth produces damaging free radicals (Bhaya, 2004). Phototaxis is directional movement in response to light, which in *Synechocystis* sp. PCC6803 is reliant on type IV pili (Bhaya et al., 1999). TaxD1, a protein with homology to phytochromes, and TaxAY1, a HK with a CheY-like C-terminal RR domain, were identified in a population of motility-defective mutants. Insertions within *taxD1* or the 3′ end of *taxAY1* were found to confer an inverted or negative phototaxis response. A model in which the CheY-domain of TaxAY1 undergoes autophosphorylation induced by an interaction with TaxD1 was proposed. This signal would then be transduced through PilT2, a pilus motor component, and lead to positive phototaxis output. In support of this model, null mutants of *taxD1*, the *cheY* domain of *taxAY1* or *pilT2* (Bhaya et al., 2000) have the same inverted phenotype. TaxAY1 may be multifunctional as a null mutation confers a nonmotile phenotype (Bhaya et al., 2001).

TaxD1 was independently identified as PixJ1 and found within a locus required for positive phototaxis (Yoshihara et al., 2000). TaxD1/PixJ1 (referred to from here forward as *Sy*PixJ1) is a CBCR with Pb/Pg reversible photochemistry (Yoshihara et al., 2004), whose chromophore arrangement was discussed earlier (see Chromophore Configuration). *Sy*PixJ1 contains two predicted transmembrane segments, a nonphytochrome GAF domain, a conserved bilin lyase GAF domain that binds the chromophore, a HAMP domain and a C-terminal MCP signaling domain (Bhaya et al., 2001; Yoshihara et al., 2004; Yoshihara et al., 2006; Ikeuchi & Ishizuka, 2008) (Figure 5).

Phototaxis has also been tied with Cph2 in *Synechocystis*. Cph2 is a PAS-less phytochrome. In addition to an N-terminal GAF domain that binds PCB and possesses Pr/Pfr photochemistry (Park et al., 2000b; Wu & Lagarias, 2000), Cph2 contains a C-terminal GAF domain of the CBCR class (Table 1). This second GAF domain is competent to covalently bind PCB *in vitro*, although ligation is substantially less efficient than for the N-terminal domain and the spectral properties are not robust (Wu & Lagarias, 2000; Park et al., 2000b). Photoreversibility has not been demonstrated. Deletion of *cph2* in *Synechocystis* PCC6803 resulted in positive phototaxis in response to directional blue light, in contrast to wild-type cells, which were nonmotile under the same conditions. These results suggest that Cph2 is able to sense blue light and inhibit a phototactic response. No changes in red, white, or green light taxis were observed in *cph2* mutants. Substitution of the Cys in either of the GAF domains conferred the same positive phototactic phenotype as deletion of the gene (Fiedler et al., 2005). The blue-light taxis of *cph2* mutants was eliminated by the addition of DCMU, an inhibitor of the photosynthetic electron transport chain (Wilde et al., 2002), suggesting that Cph2 is also sensitive to the redox status of the cell. Perhaps, the CBCR domain of Cph2 utilizes a nonstandard chromophore, requires an additional protein for lyase activity *in vivo*, and/or takes advantage of reversible bilin binding in its regulation of blue light phototaxis.

Circadian rhythm

Despite their short generation time, bacteria preserve a circadian clock with periodicity of approximately 24 h. Central to clock maintenance are the oscillator proteins KaiA, KaiB, and KaiC and the Circadian Input Kinase A (CikA) (Mackey & Golden, 2007). CikA mutants have a shortened cycle, altered gene expression profile and are unable to reset their clocks following periods of darkness, the latter of which suggested an input role for CikA. *Synechococcus elongatus* CikA is a CBCR; its domain architecture consists of an N-terminal bilin lyase GAF domain without a conserved Cys for chromophore attachment, a HK domain, and a C-terminal pseudoRR similar in sequence to the *bona fide* RR PhoB but lacking the conserved phosphoacceptor Asp (Schmitz et al., 2000). Although CikA does not covalently bind a bilin chromophore, intriguingly it does bind a quinone in its pseudoRR domain (Mutsuda et al., 2003; Ivleva et al., 2005; Ivleva et al., 2006).

A large protein complex called the periodosome makes up the cyanobacterial clock. Periodosome proteins include KaiA, KaiB, KaiC, and a kinase, SasA (Golden, 2004). CikA (Schmitz et al., 2000) and LdpA (light dependent period), an Fe_4S_4-cluster containing protein that is responsible for responding to light intensity (Katayama et al., 2003) are also involved in adjustment of the circadian period. Both CikA and LdpA are unstable in the presence of DBMIB, a quinone analog that mimics a high light environment by causing a reduction of the plastoquinone pool subsequently leading to a shorter period length. The sensitivity of CikA to DBMIB is not solely dependent on the presence of LdpA (Ivleva et al., 2005), indicating that CikA senses redox status independent of LpdA. The pseudoRR domain of CikA is required for its sensitivity to DBMIB and was shown by 2D-NMR to directly bind to it. Thus, the periodosome in part receives information about the light environment indirectly through the redox sensing capabilities of CikA's pseudoRR domain. Removal of the pseudoRR domain enhanced CikA autophosphorylation, whereas GAF domain deletion resulted in no phosphorylation, indicating that these domains jointly regulate the kinase activity

of CikA (Mutsuda et al., 2003). The input received from CikA, LpdA, and Pex (period extender, another protein involved in the input pathway) is transmitted to the Kai oscillator proteins. Furthermore, the KaiC phosphorylation state plays a major role in maintaining a functioning clock (Mackey & Golden, 2007).

In contrast to *Se*CikA, a CikA homolog in *Synechocystis* conserves a Cys within its GAF domain, binds PCB as its chromophore and absorbs in the violet region of the spectrum. The Pv form of SyCikA converts to a yellow absorbing form when irradiated with violet light; however, the Py form does not revert to Pv with yellow irradiation, and dark reverts poorly (Narikawa et al., 2008b). This altered photochemistry may be due to the fact that, like *Sy*CcaS (Hirose et al., 2008) and FdRcaE (Kehoe & Grossman, 1996; Terauchi et al., 2004), *Sy*CikA contains a Leu at a position that is typically a His responsible for coordinating the pyrrole water. Whether *Sy*CikA is an input protein for a circadian clock is unknown (Narikawa et al., 2008b). The lifestyle of *Synechocystis* as a facultative heterotroph differs from that of *Synechococcus* so this regulation may not be needed. Many cyanobacterial species contain CikA homologs; however, the *Se*CikA style architecture is typically found only in those species that also contain the KaiABC circadian system (Baca et al., 2010).

Phytochrome as a signaling model and biotech tool

To date, phytochromes from five microbial species have been structurally characterized to varying extents (PDB codes: 1ZTU, 2O9B, 2O9C, 3C2W, 3IBR, 3G6O, 2OOL, 2VEA, 2K2N, and 2KOI). These include members of the BphP, Cph1 and Cph2 families. Although no CBCR structure is known, a crystallization report for *An*PixJ suggests this situation may soon be reversed (Narikawa et al., 2009). Much focus surrounding these structures has been placed on the chromophore-containing bilin lyase GAF domains to address questions about chromophore attachment, arrangement and the role of neighboring residues. The PAS-GAF-PHY structures of Cph1 (Essen et al., 2008) and PaBphP (Yang et al., 2008; Yang et al., 2009) extend our view and allow for initial hypotheses directed at understanding the mechanism of signal transduction following a photochemical event. The long α-helix traversing the GAF and PHY domains is a construction seen in a variety of signaling proteins. The 'signaling helix' coined by Anantharaman et al. is a coiled-coil crucial to the signal transmission mechanism in histidine kinases, diguanylate cyclases, and PP2C phosphatases (Anantharaman et al., 2006). The phytochrome GAF-PHY α-helix does not conform to a coiled-coil in its strictest definition but it does pack tightly with its dimer partner to locate hydrophobic residues toward its center and opposing Glu and Arg residues at its periphery. At the carboxy end of the PHY domain, leading into the HK domain, is a parallel four-helix bundle formed by the dimer pair. Each outer α-helix directly connects to the HK to form another long centrally located α-helix (Figure 5). In response to light-driven conformational changes in the GAF bilin lyase domain, these long helices must reorient relative to each other in a twisting and/or translational movement that positions the output domains in an active state.

Downstream of GAF domains in *An*PixJ, *Sy*PixJ1, and *Te*PixJ is an ~50 amino acid HAMP signaling domain. In addition to serving as the connection between the light-sensing core and the output module, this HAMP domain may serve as a structural replacement for the PHY domain. The HAMP fold (pdb code 2ASW) superimposes well upon a subset of the secondary structure elements within the much larger PHY domain (Figure 5, inset). The short parallel two-helix motif dimerizes to form a 4-helix bundle in an analogous manner to the PHY domain (Figure 5, *Sy*PixJ1 model). The small HAMP domain cannot affect spectral properties of phytochromes in the way the PHY domain does, yet its helix pair provides an essential signaling capacity. Signaling by HAMP domains is proposed to rely on alterations in helix packing due to conformational changes in the upstream signal receptor domains (Parkinson, 2010). We predict the same is true of HAMP domain-containing phytochromes (Figure 5).

The modularity of phytochromes is not a new observation (Pepper, 1998; Park et al., 2000a; Rockwell et al., 2006; Krall & Reed, 2000) and the continued discovery of microbial phytochromes through genome sequencing projects regularly adds building blocks to the sets of known spectral inputs and functional output domains. Because of the distinct nature of their component parts, photoreceptors have become the raw materials for the rational design of several biotechnology tools (Shimizu-Sato et al., 2002; Levskaya et al., 2005; Strickland et al., 2008; Levskaya et al., 2009; Möglich et al., 2009; Wu et al., 2009; Möglich et al., 2010a). Thoughtful engineering of a light-regulated HK led to the identification of a coiled-coil whose degree of rotation varied among constructs and resulted in a range of output activities (Möglich et al., 2009). A similar helical rotation may explain the enhanced kinase activity seen in either the Pr or the Pfr forms of several phytochromes (Yeh et al., 1997; Bhoo et al., 2001; Hübschmann et al., 2001; Lamparter et al., 2002; Karniol & Vierstra, 2003; Giraud et al., 2004; Giraud et al., 2005).

Another biotechnological advantage of phytochromes is their inherent ability to fluoresce. Near infrared excitation maxima of red fluorescent phytochromes are especially useful for imaging through tissue. Increased fluorescence has been found in a number of phytochromes and engineered variants with application to cell biology and *in vivo* imaging (Fischer & Lagarias, 2004; Fischer et al., 2005; Wagner et al., 2008; Shu et al., 2009; Toh et al., 2010; Zienicke et al., 2010; Zhang et al.,

2010). Fluorescent phytochromes are an explosive area of current research and will have an enormous impact on biological imaging strategies.

Bacteria sense their environment through a variety of mechanisms. Phytochromes offer a versatile system by which organisms can employ the tetrapyrrole-binding GAF domains as antennae. Early observations of the similarity of the ethylene receptor Etr1 to FdRcaE (Kehoe & Grossman, 1997) and more recently to *Sy*PixJ1 (Ulijasz et al., 2009) are indicators that nature uses the generalized architecture seen in phytochromes broadly in sensory systems and signal transmitters. Phytochromes gather information about the type and concentration of bilins, assess redox status, and gauge the availability and wavelength of light. This information is propogated from the sensor domain through the polypeptide chain to diverse output domains to elicit appropriate cellular responses.

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