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A brief history of phytochromes

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Abstract

Photosensory proteins enable living things to detect the quantity and quality of their light environment and to transduce that physical signal into biochemical outputs which entrain their metabolism with the ambient light environment. Phytochromes, which photoconvert between red-absorbing Pr and far-red-absorbing Pfr states, have been the most extensively studied of these interesting proteins. Critical regulators of a number of key adaptive processes in higher plants, including photomorphogenesis and shade avoidance, phytochromes are widespread in photosynthetic and nonphotosynthetic bacteria and even in fungi. Cyanobacterial genomes also possess a plethora of more distant relatives of phytochromes known as cyanobacteriochromes (CBCRs). Biochemical characterization of representative CBCRs has demonstrated that this class of photosensors exhibit a broad range of wavelength sensitivities, spanning the entire visible spectrum. Distinct protein-bilin interactions are responsible for this astonishing array of wavelength sensitivities. Despite this spectral diversity, all members of the extended family of phytochrome photosensors appear to share a common photochemical mechanism for light sensing: photoisomerization of the 15/16 double bond of the bilin chromophore.

Keywords

phytochrome; cyanobacteriochrome; bilin; Cph2 sensor; photoreceptor

Introduction

All living things can sense and respond to light. Such behavior is critical for photosynthetic organisms, so it is unsurprising that such organisms have proven to be fertile grounds for the discovery of proteins involved in photosensory pathways. While essentially all proteins absorb light in the near-UV region due to the presence of aromatic amino acids, proteins that sense visible light possess chromophore cofactors that confer the desired wavelength sensitivity. Aside from visual photoreceptors of animals, the red/far-red regulators of photomorphogenesis in higher plants called phytochromes are the most well known class of visible light sensors. [1,2] Phytochromes share a conserved photosensory protein core comprising a PAS domain, a GAF domain, and a PHY domain (Fig. 1), to which a linear tetrapyrrole chromophore (bilin) such as phycocyanobilin (PCB), phytochromobilin (PΦB), or biliverdin IXα(BV) is covalently attached (Fig. 2). This conserved core sequence has been used to identify phytochrome-related sensors from a broad range of organisms, including unicellular green algae, diatoms,

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Note Added in Proof: During the preparation of the proofs, an NMR analysis of a GAF domain from a R/FR Cph2 sensor from the thermophilic cyanobacterium *Synechococcus* sp. OS B' was reported (Ulijasz, A.T., Cornilescu, G., Cornilescu, C.C., Zhang, J., Rivera, M., Markley, J.L., and Vierstra, R.D. (2010). Structural basis for the photoconversion of a phytochrome to the activated Pfr form. *Nature* 463, 250–254). The unexpected isomerization of the C4-C5 double bond observed by these investigators contrasts with a wealth of evidence that supports C15-C16 double bond isomerization in non-Cph2 phytochrome sensors. Resolution of this controversy remains an exciting area of research.

cyanobacteria, non-oxygenic photosynthetic bacteria, nonphotosynthetic bacteria, and even fungi.[3–9]

X-ray crystallographic studies have established that the PAS and GAF domains of the photosensory core are knotted together, and *in vitro* studies implicate both to be required for proper incorporation of chromophore.[10,11] Tightly nestled within in a conserved cleft in the GAF domain (Fig. 3), the bilin chromophore is covalently attached to a conserved Cys residue in the phytochrome apoprotein via the C3 sidechain of the bilin A-ring. Phytochromes that utilize BV have a conserved Cys N-terminal to the knot and PAS domain, while those that utilize PCB or PΦB have a conserved Cys in the GAF domain.[11–15] Photoswitching arises due to photoisomerization of the bilin chromophore about its 15/16 double bond (Fig. 2), followed by protein-chromophore relaxation processes that further shift the absorbance spectrum. In most phytochromes, the photoproduct state is metastable and can slowly revert to the thermally stable dark state in a process known as dark reversion. The metastable P_{fr} state is the active signaling state of plant phytochromes, so dark reversion is physiologically significant.[16] The structural changes in the chromophore upon photoisomerization alter chromophore-protein interactions, which trigger signal transduction pathways that lead to changes in various biological outputs. Phytochromes typically have C-terminal histidine kinase domains, and light-regulated protein kinase activity was first reported for the cyanobacterial phytochrome Cph1 from *Synechocystis*. [17] Since this groundbreaking finding, such activity has been reported for several other phytochromes, and the purple photosynthetic bacterium *Rhodospseudomonas palustris* even contains a photosensory pathway in which two such proteins regulate a common response regulator.[18]

Biosynthesis and reactivity of bilin chromophores

The photosensory properties of phytochromes are dependent on the availability of the bilin cofactor. Biosynthesis of bilins begins with the oxidative degradation of heme by heme oxygenase to generate BV.[19] BV is widely utilized as the chromophore precursor for bacterial phytochromes (BphPs, Fig. 1) and for fungal phytochromes (Fphs).[4,6,14,20,21] Primary sequence data suggests that this will also hold true for phytochromes from diatoms (Dphs), although this has not been experimentally verified. BphPs and Fphs form a covalent linkage between the Cys sidechain and the C3² carbon of the BV C3 sidechain (Fig. 2).[10,22] In most oxygenic photosynthetic organisms, such as cyanobacteria, certain algae, and plants, BV is further metabolized by ferredoxin-dependent bilin reductases (FDBRs) to yield PCB or PΦB, both of which contain a reduced A-ring with an ethylidene on the C3 sidechain.[19] Michael addition of the GAF Cys residue at the C3¹ carbon results in spontaneous formation of the final adduct (Fig. 2), and the GAF domain is sufficient for this assembly reaction.[11]

The biosynthesis of bilins, heme, siroheme, and chlorophyll all rely on a single trunk pathway for biosynthesis of tetrapyrroles, so these pathways must be carefully balanced. This is even more of a challenge for photosynthetic organisms such as cyanobacteria, because such organisms also use bilins in their light-harvesting phycobilisome complexes.[23,24] Moreover, cyanobacteria can produce other protein-bound bilin isomers of PCB such as phycoviolobilin (PVB, Fig. 2), that has a saturated 4/5 bond, phycoerythrobilin (PEB), that has a saturated 15/16 bond, and phycourobilin (PUB), where both 4/5 and 15/16 bonds are saturated.[19] Bilins with more oxidized C12 sidechains have also been reported from cryptomonads.[25] Many of the enzymes responsible for this bilin structural diversity still remain uncharacterized and except for PEB, these novel bilin species are only observed as biliprotein adducts.

Mammals also utilize heme oxygenases to convert heme to BV, and that process is also coupled to a bilin reductase. By contrast with oxygenic photosynthetic organisms which possess FDBRs, mammals utilize an NADPH-dependent biliverdin reductase (BVR) that acts at C10

to generate bilirubin IX α (BR, Fig. 2).[26,27] A cyanobacterial enzyme BvdR with a similar catalytic activity also has been described [28]. Interestingly, mammalian BVR can reduce PCB, P Φ B, or PEB to the corresponding rubins, while these bilins are poor substrates for BvdR. [28,29] In neonatal jaundice, accumulation of lipophilic BR due to inefficient efflux in newborns can lead to neurological disorders. Interestingly, a potent therapy for neonatal jaundice exploits photoisomerization of BR at the C15/C16 bond (Fig. 2) to improve clearance of BR from the body via a process strikingly reminiscent of photoconversion in phytochromes. [30–32]

Bilins such as BV and PCB are able to undergo photoisomerization either free in solution or under denaturing conditions, indicating a protein adduct is not required for photoisomerization. However, the *Z* isomer is more stable and can readily be generated photochemically from the *E* isomer under denaturing conditions.[33,34] Formation of the *E* isomer in the absence of protein can also be accomplished through the use of thiol compounds, as these compounds readily add to the bilin at C10 to generate rubinoid adducts which then can be photoisomerized to the *E* form in a manner akin to neonatal phototherapy (Fig. 2).[35,36] Isomerization about the 9/10 and 10/11 bonds is rarely observed, because the bilin C10 methine bridge has considerable aromatic character.[37]

Bilin chromophores also exhibit diverse chemical reactions. C10 is notably electrophilic, reacting with thiols, cyanide, and even methanol (Fig. 2).[35,36,38,39] C4 is also susceptible to nucleophilic attack,[40] although it is more sterically hindered than the C10 position. By contrast, C5 and C15 preferentially react with electrophilic reagents, e.g. to introduce nitro substituents.[37] The proton attached to C5 also exhibits slow exchange with solvent protons when examined by NMR.[41] C5 and C15 are thus electron-rich, possessing mildly nucleophilic character. Such chemical reactivity propensities are expected to remain the same for bilins in proteins, although the local environment can definitely constrain the possible chemical reaction pathways. For example, no known A-ring linked biliprotein has been shown to exhibit photochemistry about the C4/C5 double bond, even though this can be seen for bilins in solution.[37] Thus, proteins can use specific protein-cofactor interactions to reduce the possible reactions available to the bilin cofactor and thereby favor a specific product, but that outcome must be compatible with the intrinsic chemistry of the bilin cofactor. It is not surprising that many of these well-established chemical reactions of bilins are now being observed in the more diverged GAF scaffolds of the extended phytochrome family.

Nomenclature of photosensory bilin-binding GAF domains

Perhaps due to the broad range of organisms in which phytochromes and related proteins are found, the nomenclature of this field is arcane. Classical phytochromes have been defined as bilin-binding red/farred photosensors possessing the PAS/GAF/PHY core module found in plant phytochromes, but those using BV have been named bacteriophytochromes (BphPs) if found in bacteria or named fungal phytochromes (Fphs) or diatom phytochromes (Dphs) in those eukaryotes.[7,20,21,42] Moreover, cyanobacteria contain more diverse biliprotein photosensors with minimal GAF/PHY or even GAF-only photosensory modules, which are respectively known as Cph2s and cyanobacteriochromes (CBCRs).[11,16,43] The designation ‘cyanochrome’ has also been recently proposed for CBCRs.[42] For the purposes of this discussion, we will defer to the original CBCR nomenclature and adopt two guiding principles.

1. *We define three photosensory protein families on the basis of the domain architecture of the photosensory cores.* Therein, phytochromes comprise a three-domain, knotted PAS/GAF/PHY architecture, Cph2 sensors employ an unknotted GAF/PHY bi-domain architecture, and CBCRs possess stand-alone GAF domains that are sufficient to support both covalent attachment of bilin and photochemistry. We do not adopt the proposal that a conserved RIT motif can be used to characterize the Cph2 family,

[44] as this motif is absent in the original *Synechocystis* Cph2 itself with no apparent consequences for photochemistry.[11]

2. We designate the photosensory photocycle by listing the long-wavelength absorbance of the (15Z) form followed by that of the (15E) form. Thus, Cph1 would be described as a R/FR (“red/far-red”) sensor,[17,45] while CBCR Tlr0924 would be described as a B/G (“blue/green”) sensor.[46] This usage easily distinguishes the potentially confusing behavior of the CBCRs SyCcaS and AnPixJ:[47,48] in both cases, the bound PCB chromophore switches between forms that absorb red and green light, but the (Z)/(E) isomerization is reversed. We thus define SyCcaS as a G/R sensor and AnPixJ as a R/G sensor.

The R/FR cycles of phytochromes

Plant phytochromes and Cph1 have undergone extensive spectroscopic characterization. These studies have revealed several key points.[16] First, the reaction pathway for photoconversion of P_r to P_{fr} is distinct from that for conversion of P_{fr} to P_r , with different intermediates and timescales. In Cph1 and plant phytochromes, NMR spectroscopy has provided convincing evidence for protonation of all four nitrogens in both P_r and P_{fr} states.[49,50] NMR, vibrational, and absorbance spectroscopies all support the conclusion that photoisomerization occurs at the 15/16 bond.[41,51–53] Recent breakthroughs in crystallographic studies of the phytochrome photosensory core have now determined the configuration of the chromophore unambiguously for certain phytochromes.

Crystal structures have been reported for three phytochromes in the P_r state (Fig. 3):[10,22,54,55] knotted PAS/GAF fragments of DrBphP (BV chromophore, R/FR photocycle) and RpBphP3 (BV, atypical R/NR), and the PAS/GAF/PHY photosensory core of Cph1 (PCB, R/FR). In all cases, the P_r chromophore adopts a (5Z)-syn (10Z)-syn (15Z)-anti configuration. The chromophore is tightly packed about C10 and covalently attached to the protein via the C3 sidechain, but the D-ring is less tightly packed. This arrangement clearly allows more freedom of motion about the C15 methine bridge than about the C5 or C10 methine bridges, perhaps providing a means for the protein to control photochemistry.[56]

To date, crystallographic information about the P_{fr} state comes only from studies of PaBphP, [57,58] – a so-called bathyphytochrome which converts from the (15Z) P_r state to the (15E) P_{fr} state in the absence of light.[59] The PaBphP structures, which include the entire PAS/GAF/PHY core, reveal a (5Z)-syn (10Z)-syn (15E)-anti configuration for the P_{fr} chromophore. In both Cph1 and PaBphP, a long “tongue” extending from the PHY domain folds back over the bilin-binding pocket (Fig. 3), perhaps explaining the importance of the PHY domain for efficient photochemistry.[11] The location of the domains relative to each other is slightly different for Cph1 and PaBphP,[54,57,58] but it is not yet known whether this has implications for the structural changes that must occur for alterations in signaling state.

Taken together, the structures of DrBphP in the P_r state and PaBphP in the P_{fr} state reveal the structural changes occurring within the bilin-binding pocket upon photoconversion. While some conserved amino acid residues lie in different positions in the P_r and P_{fr} crystal structures, it is noteworthy that many conserved amino acids within 5 Å of the chromophore do not exhibit large changes, including conserved Asp and His residues immediately adjacent to the bilin (Fig. 4). In addition to rotation of the D-ring, the P_{fr} bilin shifts within the pocket, approximately within the plane of the B- and C-rings, and the 12-propionate sidechain rotates to form a hydrogen bond with the sidechain of Tyr176 (DrBphP numbering), which also adopts a different rotamer in the PaBphP structure.[22,57] These findings lead to the question of whether similar changes occur in Cph1 and plant phytochromes. However, such an

extrapolation relies on the assumption that the reaction pathways and P_{fr} states of BphPs are equivalent to those of Cph1 and plant phytochrome.

Three lines of evidence argue that this assumption is not valid. First, mutagenesis of a conserved Tyr residue (Tyr176 in Cph1 and *DrBphP*) results in almost complete loss of photochemistry in Cph1 and plant phytochromes, but only very modest effects in multiple BphP proteins. [57,60–62] By contrast, mutagenesis of a second conserved Tyr residue (Tyr263 in Cph1 and *DrBphP*) results in more profound effects in BphPs than in Cph1 (King, A., N. C. R., and J. C. L., unpublished data). [58,60–62] These results demonstrate that the amino acids required for efficient photoconversion in Cph1 and plant phytochrome *differ* from those required in BphPs. Second, incorporation of bilin 12-monoamides into *DrBphP* and Cph1 produced different effects on photoconversion. [63] This experiment demonstrates that the structural features in the bilin required for efficient formation of P_{fr} also differ for these two classes of phytochromes. Finally, the CD spectra of the bilin transitions in Cph1 and plant phytochromes in the P_{fr} state exhibit the opposite sign from those of BphP proteins in the P_{fr} state. [63–66] We have proposed that the facial disposition of the D-ring relative to the plane defined by the B- and C-rings is critical for the sign of the observed bilin CD spectra, [46] although other interpretations for the CD sign inversion have been proposed. [65] Cph1, plant phytochromes, and BphP proteins share a common CD signature in the P_r state, consistent with the common geometry observed in P_r crystal structures. The conservation of CD sign during photoconversion in BphP proteins is also consistent with the conserved facial disposition of the BV chromophore in P_r and P_{fr} crystal structures of BphP proteins. [22,57] Taken together, these results support the conclusion that two distinct R/FR photocycles exist within the phytochrome family.

Cph2 sensors: unknottting the R/FR photocycle

The importance of the conserved knotted PAS/GAF architecture to phytochrome function remains an interesting question. Atomic force microscopy experiments have demonstrated that the knot in *DrBphP* does not confer a dramatic increase in stability. [67] The advent of the genome-sequencing era has revealed the existence of cyanobacterial proteins with a conserved GAF-PHY architecture, but no PAS domain and hence no knot (Fig. 1).

The first such protein to be described, Cph2 from *Synechocystis*, was shown to exhibit a R/FR photocycle. [11] This protein also has novel output domains implicated in metabolism of the bacterial second messenger cyclic-di-GMP rather than the more common histidine kinase effector domain (Fig. 1). Indeed, members of the Cph2 family often contain multiple GAF domains associated with a histidine kinase output module. [68] While normal R/FR photochemistry of Cph2 required a GAF-PHY domain pair [11], two novel Cph2 sensors from thermophilic cyanobacteria were recently described that retain residual photochemistry even after deletion of the associated PHY domains. [44] This permitted determination of the solution NMR structure of the GAF domain for one of these Cph2 sensors in the P_r state. [69] The chromophore was found to exhibit a (*5Z*)-*syn* (*10Z*)-*syn* (*15Z*)-*anti* geometry similar to those seen in the P_r crystal structures of phytochromes, albeit with a rather twisted D-ring geometry. Interestingly, the covalent linkage between the Cys sulfur atom and the C3¹ atom of the PCB chromophore differs from that of Cph1 and plant phytochrome by adopting the *S* configuration at C3¹ rather than *R*. [41,54,69] Cph2 sensors have interesting implications for the evolution and engineering of phytochromes, because they demonstrate that several features conserved in phytochromes are nonetheless dispensable for photoconversion.

Cyanobacteriochromes: a Photosensor for Every Color

Cyanobacterial genomes contain a bewildering array of predicted proteins containing one or more GAF domains evolutionarily related to phytochrome GAF domains. Most of these lack

the immediately adjacent, conserved PAS and/or PHY domains of phytochromes and Cph2 sensors (Fig. 5). Such GAF-containing proteins or domains have been named cyanobacteriochromes,[43] here abbreviated CBCRs. CBCR GAF domains are also found in Cph2 sensors, including *Synechocystis* Cph2 itself (Fig. 1), in which it was found to bind PCB to produce a blue-absorbing dark state.[11] The significance of CBCR domains in Cph2 sensors is largely unknown, although *Synechocystis* Cph2 has been implicated in a phototactic response to blue light.[70] CBCR domains from a number of other proteins have now been studied *in vitro*, and the emerging picture is one of an extremely diverse family of photosensory proteins. The first CBCR to be identified was RcaE from *Fremyella diplosiphon*, which plays a key role in complementary chromatic adaptation (CCA).[71,72] The other known photobiological process in which CBCRs are implicated is phototaxis, with the TaxD1/PixJ1 CBCR thought to function as a regulator of type IV pilus-dependent motility in response to light.[43,73]

In CCA, cyanobacterial cells regulate the relative accumulation of red-absorbing (green colored) phycocyanin and green-absorbing (red colored) phycoerythrin in response to the ratio of red and green light [74]. RcaE is known to be essential for this process in *Fremyella*, and the homologous protein SyCcaS has been shown to have the properties of a CCA sensor: it exhibits a G/R photocycle which modulates its kinase activity.[47,72] Denaturation analysis of SyCcaS has confirmed the presence of PCB chromophore and photoisomerization at the 15/16 double bond.[47] This group of CBCRs are thus G/R photoswitches.

A second group of CBCRs to be characterized exhibits a B/G photocycle.[75–77] This group includes the thermophilic proteins TePixJ and Tlr0924, two of the five CBCRs present in *Thermosynechococcus elongatus*. [46,75] Denaturation analysis of TePixJ provided evidence that its chromophore is predominantly PVB rather than PCB (Fig. 2).[78] These studies also established that, similar to other phytochromes, TePixJ photochemistry occurs at the 15/16 bond. For native Tlr0924, CD spectroscopy indicated that the blue-absorbing form of this CBCR contains bound PCB.[46] This apparent contradiction could be resolved were such B/G proteins to contain a mix of PVB and PCB; were PVB the majority population, the denaturation analysis would detect PVB, but CD would still detect the residual PCB. We have proposed that these proteins contain a second conserved Cys residue that participates in a second covalent linkage to C10, because mutation of this residue results in loss of blue absorbance and formation of a red-absorbing, PCB-containing holoprotein.[42,46] Such a linkage at C10 would result in a rubinoid species consistent with the observed blue absorbance. [36,46] We further established that photoisomerization of this species at reduced temperature resulted in a slight shift of the blue absorbance maximum,[46] followed by generation of a more conjugated tetrapyrrole chromophore that absorbs green light.

Alternatively, it has been proposed that the “second Cys” instead forms a stable second linkage at C4 or C5,[42] thus accounting for the apparent presence of PVB in the photoproduct upon denaturation. However, formation of a thiol adduct at C5 seems unlikely, because this atom is not electrophilic.[37] While C4 is an electrophilic center, it is sterically hindered; to date, the only known case of nucleophilic attack at C4 involves intramolecular lactone formation that reduces the steric problems while also providing an energetic driving force.[40] In this scenario, such a C4 or C5 adduct would have to rotate the B- or D-ring out of conjugation in the 15Z state to yield a blue-absorbing species, which would imply that the blue-absorbing state is a high-energy species. This is at odds with the thermal stability of this type of CBCR and with the formation of a stable (15E) blue-absorbing intermediate at lower temperatures.[46] We thus favor a reversible C10 adduct for this class of CBCRs, with the chromophore incompletely converting from PCB to PVB after adduct formation.

In summary, this second group of CBCRs can be defined by the presence of a second Cys residue in a conserved sequence motif, with this second Cys being critical for their function as

B/G sensors.[42,46] Interestingly, the SyCikA protein from *Synechocystis* also possesses this second Cys, but this CBCR exhibits peak absorbance at even shorter wavelengths.[79] Photoconversion of this protein results in formation of a species absorbing at longer wavelengths than those of TePixJ and Tlr0924, but this species appears photochemically inert.[79] In contrast to Tlr0924 and TePixJ, SyCikA may function as a “one-way” photosensor, with the photoproduct exhibiting very slow dark reversion.

In addition to the G/R and B/G sensors, a third major group of CBCR has been detected by database searches and protein sequence alignments.[46,48] Three representative members of this group have been characterized, and all are found in the protein AnPixJ.[48] Interestingly, two of the three were able to bind PCB efficiently; however, only one of these exhibited substantial photochemistry, with a novel R/G photocycle. Denaturation analysis for this R/G GAF domain confirmed the presence of PCB chromophore and of 15/16 photoisomerization, similar to phytochrome. In summary, although research has only just begun on GAF domains distributed in the CBCR family, there are already three new distinct photocycles, all of which are based on photoisomerization at C15 (Fig. 6). We expect that other types of photocycles will be uncovered as the survey of this interesting family of sensors is expanded.

Outlook

The phytochromes and CBCRs pose a fascinating challenge for structure/function studies and for protein engineering. It should be possible to engineer existing photosensory proteins to exhibit new spectral sensitivities, fluence responses, or thermal behaviors. It may also be possible to engineer photosensory pathways by replacing photosensory CBCR GAF domains with others of distinct photochemical behavior. Well-established approaches such as X-ray crystallography and site-directed mutagenesis have contributed much to our understanding of these proteins and will contribute still more. Additionally, in common with other photosensory protein superfamilies, phytochromes and CBCRs are amenable to a very broad array of spectroscopic techniques spanning an enormous range of energies and timescales. Another approach that will be key to understanding and engineering structure/function relationships in these proteins over the next few years is the need to expand the number of sequences that have been characterized *in vitro*, so that current models for predicting photochemistry based on primary amino acid sequence can be refined in light of the actual photochemical behavior of more than a few pilot sequences.

One key question that remains unanswered relates to the structural basis for the far-red absorbance of the P_{fr} states of Cph1 and plant phytochromes. The chromophore geometry for the P_{fr} state of BphPs revealed by the crystal structure for PaBphP is fully consistent with far-red absorbance, but this cannot explain the structurally distinct P_{fr} states of Cph1 and plant phytochromes. The extremely diverse photochemistry observed in CBCRs is even less well understood, although it seems plausible that differences in protonation states will play a role in tuning the absorbance spectra, as will photoreversible covalent adducts that can disrupt the conjugated π systems of bilin chromophores. In spite of this astonishing spectral diversity, a common theme has emerged from such studies: photoswitching by all members of this important class of proteins utilizes photoisomerization of the 15/16 bond as the primary photochemical event. Phytochrome-related GAF domains thus appear to have retained the common ability to direct the energy of bilin photoexcitation into a conserved photochemical event. This structural conservation makes it plausible that there are similarly conserved structural mechanisms for transducing the photochemical event into changes in biochemically relevant signaling pathways that ultimately lead to photobiological responses.

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Figure 1.

Domain structure and chromophore configuration of phytochromes and Cph2 sensors. R/FR phytochromes have a conserved PAS/GAF/PHY tridomain photosensory core. Phytochromes from different organisms have characteristic variations of this architecture. Plant and some algal phytochromes have an additional pair of PAS domains C-terminal to the core and lack a recognizable His residue that serves as a phosphoacceptor in *bona fide* His kinases. Bacteriophytochromes (BphPs) and related proteins from fungi (Fph) and diatoms (Dph) differ from plant and cyanobacterial phytochromes in the location of the conserved Cys residue that forms the covalent linkage to the bilin chromophore. Both plant phytochromes and Fph/Dph proteins have N-terminal extensions, while Fph/Dph proteins have C-terminal response

regulator receiver domains. Cph2 sensors retain the GAF and PHY domains but lack the PAS domain and PAS/GAF knot. The family is named after *Synechocystis* Cph2, which has an additional GAF domain that has the hallmarks of a blue/green CBCR. Most Cph2 sensors have His kinase output domains, as is shown for two representatives from thermophilic *Synechococcus* species.[44]

Figure 2.

Bilin cofactor adducts of phytochromes and related photosensors. Phytochromes utilize linear tetrapyrrole (bilin) precursors to form a variety of covalent adducts with different spectral properties. Such adducts are no longer equivalent to the parent named bilin; for example, the covalent adduct formed upon Michael addition of Cys to the C3² atom of BV is formally a (2,3)-dihydrobiliverdin. Plant and cyanobacterial phytochromes incorporate the reduced phytobilins phycocyanobilin (PCB) or phytychromobilin (PΦB) to form adducts shown at top left. A conserved Cys in the GAF domain becomes linked to the C3¹ carbon. This bilin adduct is shown in the (5*Z*)-*syn* (10*Z*)-*syn* (15*Z*)-*anti* configuration of the P_r state, and PCB and PΦB vary in the substituent at C18. By contrast, BphPs and Fphs utilize biliverdin IX_α (BV) as chromophore precursor to form the adduct shown at top right. A conserved Cys N-terminal to the PAS/GAF knot becomes linked to the C3² carbon. This bilin is shown in the (5*Z*)-*syn* (10*Z*)-*syn* (15*E*)-*anti* configuration of the P_{fr} state. A subset of CBCRs and phycobiliproteins are able to isomerize PCB to PVB (bottom left), which has a saturated 4/5 bond. It is not known whether CBCRs carry out this isomerization before or after covalent attachment of the PCB precursor. This bilin is shown in the same configuration as the P_r state of phytochromes. BV can also be reduced to bilirubin IX_α, which has a saturated C10 atom. The (4*Z*, 15*E*) configuration of BR is generated during phototherapy for neonatal jaundice.[30–32] C10 adducts (bottom right, X = nucleophiles) of bilins disrupt the conjugation of the π system and generate blue-absorbing “rubinoid” pigments. Combining such rubinoid C10 adducts with covalent linkage to CBCR Cys residues, as illustrated, could provide an explanation for the large blueshifts observed in some CBCRs.

Figure 3. Structures of the phytochrome photosensory core module. Top, the PAS/GAF fragment of *DrBphP* in the P_r state (PDB accession 2O9C)[22] is shown with the PAS domain in blue and the GAF domain in purple. Middle, the PAS/GAF/PHY photosensory core of *Cph1* in the P_r state (PDB accession 2VEA)[54] is shown with the PAS and GAF domains colored as for *DrBphP* and with the PHY domain in silver. Bottom, the PAS/GAF/PHY core of *PaBphP* in the P_{fr} state (chain A, PDB accession 3C2W)[57] is shown colored as for *Cph1*. In all cases, the knotted PAS/GAF fold is conserved. The two PAS/GAF/PHY structures exhibit a conserved, flexible 'tongue' reaching from the PHY domain and interacting with the chromophore-binding pocket of the GAF domain.

Figure 4.

Stereo views of the *DrBphP* structure in the P_r state (top: PDB accession 2O9C)[22] and the *PaBphP* structure in the P_{fr} state (bottom: PDB accession 3C2W)[57] reveal structural changes associated with photoconversion. In *DrBphP*, the BV chromophore adopts a (5*Z*)-*syn* (10*Z*)-*syn* (15*Z*)-*anti* configuration. His290 is hydrogen bonded to the D-ring carbonyl oxygen (dashed line), and a water molecule is closely associated with the A-, B-, and C-rings. The sidechains of Asp207 and Tyr176 are not in close contact with chromophore. In *PaBphP* the BV chromophore adopts a (5*Z*)-*syn* (10*Z*)-*syn* (15*E*)-*anti* configuration. The D-ring is a-facial in both structures, consistent with CD spectroscopy.[63] The water molecule is similarly located. His277 (equivalent to His290 in *DrBphP*) remains in approximately the same place but no longer interacts with O19. Asp194 (equivalent to Asp207) is in approximately the same place but is now positioned to interact with the D-ring amide NH. Tyr163 (equivalent to Tyr176) adopts a different sidechain rotamer and is hydrogen bonded to the 12-propionate sidechain of BV (dashed line).

Figure 5.

Domain structure of representative cyanobacteriochromes (CBCRs). CBCRs contain isolated bilin-binding GAF domains without PHY domains or PAS/GAF knots. Phylogenetic analyses suggest the existence of several classes of CBCR, and biochemical analyses demonstrate the existence of at least three different photoswitching behaviors.[47,48,76]

Cyanobacteriochromes exhibit much more diverse domain organization than phytochromes or Cph2 sensors.

Figure 6.

Schematic view of phytochrome and CBCR photochemistry. In phytochromes and CBCRs examined to date, the common element is photoisomerization of the bilin chromophore about the 15/16 double bond. In the cyanobacterial phytochrome Cph1 (red), the $(15Z)$ P_r form converts to a $(15E)$ lumi-R photoproduct which is then thermally converted to the $(15E)$ P_{fr} form in a process requiring the 12-propionate side chain.[63] P_{fr} is converted to the $(15Z)$ lumi-R photoproduct, which is then thermally converted to P_r to complete the photocycle. In the B/G photocycle of CBCR Tlr0924 (blue), we propose formation of a second covalent linkage between Cys499 and C10 of the chromophore in the $(15Z)$ state.[46] Such a linkage at C10 would generate a species similar to bilirubin IX α or phycocyanorubin,[29,36] explaining the blue absorbance of P_b^S . The $(15Z)$ P_b^S form can be photoconverted to a $(15E)$ P_b^L photoproduct which is in thermal equilibrium with the P_g form.[46] P_g is generated from P_b^L by cleavage of the second linkage, generating a free thiol side chain at Cys499. Photoisomerization of P_g generates an as-yet unidentified $(15Z)$ photoproduct which regenerates P_b^S by reformation of the second linkage at C10. This B/G cycle can occur in either PCB or PVB chromophores, because it does not involve the 4/5 bond which varies between these two bilins. Thermal relaxation of the $(15E)$ form to the $(15Z)$ form (dark reversion) is known in plant phytochrome,

Cph1, and some BphPs. Thermal conversion of (*15Z*) to (*15E*) is also known in a subset of BphP proteins termed bathyphytochromes, including *PaBphP* (purple).