Cyanobacteriochromes in full color and three dimensions

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Sensory photoreceptors occur in all kingdoms of life, eliciting diverse organismal adaptations in response to incident light. The recently identified cyanobacteriochromes (CBCRs) mediate photochromatic and phototactic responses in cyanobacteria (1–3). Great strides toward a molecular understanding of photoreception and signal transduction in this spectrally diverse and exciting photoreceptor family have now been taken by Narikawa et al. (4), who report high-resolution structures of two CBCR photosensor modules in PNAS.

CBCRs are related to plant and bacterial phytochromes (Phys), with which they share the intrinsic ability to form thioether linkages to linear tetrapyrrole (bilin) chromophores via conserved cysteine residues. Moreover, these photoreceptor families use a unifying photochemical mechanism (Fig. 1*A*): photoisomerization of the chromophore between *15Z* and *15E* configurations with concomitant rotation of the terminal bilin D-ring (5–7). The *15Z* and *15E* states differ in their absorption properties (photochromism) and modulate the behavior of output domains and downstream signal transduction pathways. Despite similar chromophores, photochemistry, and self-assembly, Phys and CBCRs differ in several striking ways. Most phytochromes require a three-domain PAS-GAF-PHY architecture [GAF domain, cGMP-phosphodiesterase/adenylate cyclase/FhlA (8); PAS domain, Per/ARNT/Sim; PHY domain, phytochrome-specific] for reversible photoconversion (3, 5, 7, 9). CBCRs instead achieve fully reversible photochemistry with a lone chromophore-binding GAF domain. Multiple CBCRs often occur in tandem within a single protein, allowing integration of multiple light signals at a single C-terminal output domain (10). Whereas Phys predominantly respond to the red/far-red spectral region, CBCRs display a rich variety of photocycles spanning the entire visible and near-UV spectrum (2, 11–13). At least four subfamilies of CBCRs can be distinguished on the basis of their underlying photochemistry and primary structure.

Curiously, two of these subfamilies feature opposite photocycles: green/red CBCRs have a green-absorbing *15Z* dark (ground) state and red-absorbing *15E* photoproduct (2), but red/green CBCRs instead have a red-absorbing *15Z* dark state and green-absorbing *15E* photoproduct (14). The other two subfamilies, insert-Cys and DXCF CBCRs, both make use of additional conserved cysteine residues and typically exhibit a *15Z* dark state sensitive to shorter wavelengths (near-UV to blue) and a *15E* photoproduct absorbing at longer wavelengths from blue to orange (3, 11, 12, 15). DXCF CBCRs can autocatalytically isomerize the phycocyanobilin (PCB) chromophore of CBCRs into phycoviolobilin (PVB) (Fig. 1*A*), thereby tuning photoproduct absorbance between teal and orange light (12, 15, 16). Two of these subfamilies and both photostates are represented in the structures described by Narikawa et al. (4).

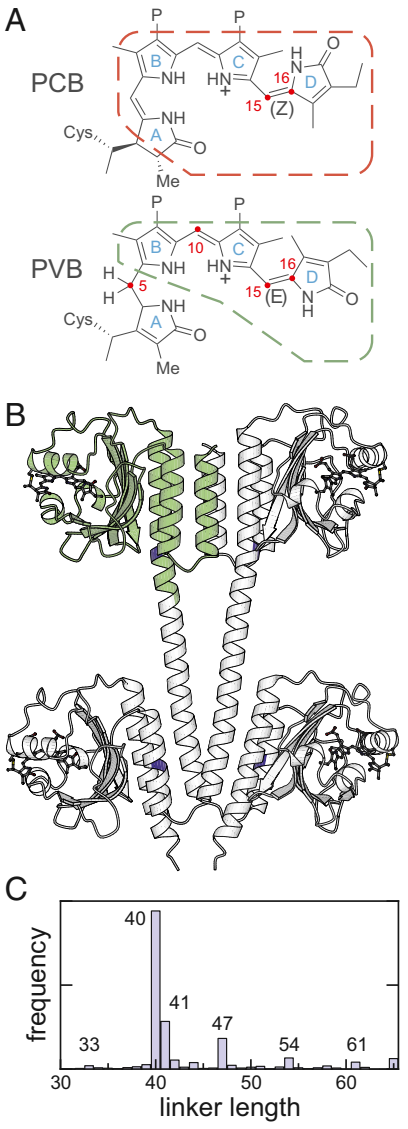

Fig. 1. Cyanobacteriochrome structure and function. (*A*) Bilin chromophores and photochemistry of CBCRs. PCB and PVB are shown with their 15,16-double bonds in the configurations revealed by the work of Narikawa et al. (4). Conjugated π systems are outlined, propionate sidechains are indicated by "P," and selected carbon atoms are labeled by red numbers. (*B*) Structure of the AnPixJ CBCR dimer showing the six-helix bundle formed by the distal helices. CBCR domains frequently occur in tandem, modeled in white by the duplicate structure. (*C*) The interdomain linker in tandem-GAF proteins, as measured between the positions highlighted in blue in *B*, shows a strong preference for discrete lengths, hinting at conserved mechanisms of signal transduction and integration.

Using X-ray diffraction, Narikawa et al. (4) have determined 1.8-Å and 2.0-Å resolution structures of two CBCR photosensor modules from the cyanobacteria *Nostoc* sp. PCC 7120 (AnPixJ) and *Thermosynecchococcus elongatus* BP-1 (TePixJ). Both CBCRs adopt the canonical GAF fold and bind their bilin chromophores in a cleft formed by a six-stranded antiparallel β sheet and three proximal α helices; three distal helices are situated on the opposite face of the sheet (Fig. 1*B*). AnPixJ is a red/green CBCR using PCB as chromophore (14), and it was crystallized in the red-absorbing *15Z* dark state. TePixJ is a DXCF CBCR containing a mix of PCB and PVB (15), with only the PVB population represented in the crystal structure of the green-absorbing *15E* photoproduct (Fig. 1*A*). In phytochromes, the *15Z* configuration is associated with the red-absorbing Pr state, and the *15E* conformation is associated with the far-red-absorbing Pfr state (3, 6, 7, 9). A comparison of the CBCR structures to those of bacterial Phys thus grants unprecedented molecular insight into photosensory mechanisms inherent to all bilin-based photoreceptors and into specific mechanisms used by individual CBCR subfamilies.

In phytochromes, crystallography and NMR spectroscopy provide robust evidence for *Z*/*E* photoisomerization of the 15,16-double bond (3, 6, 7, 9, 17). A large body of biochemical data implicates the same primary photochemistry in CBCRs (2, 11–16, 18), which is now confirmed by the *15Z* dark state and *15E* photoproduct

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seen in the present structures. Excitingly, key protein–chromophore interactions are also conserved between CBCRs and Phys: a conserved histidine or tyrosine residue forms a hydrogen bond to the carbonyl oxygen of the bilin D-ring in the *15Z* state (4, 5, 9), and the amide nitrogen of the D-ring is hydrogen-bonded to a conserved aspartate residue in the *15E* state (4, 7, 17). Conservation of both primary photochemistry and key chromophore–protein interactions raises the intriguing possibility that transduction of the photochemical signal to the C-terminal output domain will also be conserved.

The CBCR structures also shed light on the diverse panoply of photocycles. CBCRs lack the PAS and PHY domains of Phys, causing the bilin A- and B-rings to be solvent-exposed. In both AnPixJ and the cyanobacterial phytochrome Cph1 (9), the chromophore adopts the *15Z* configuration with overall similar geometry. However, the conserved aspartate plays different roles: in Cph1, it interacts with a conserved residue in the PHY domain, but in AnPixJ it directly interacts with the bilin rings A, B, and C (4). The structural basis for formation of the green-absorbing photoproduct of AnPixJ and related proteins remains to be elucidated (10, 13, 14). The case is reversed for TePixJ, in which the green-absorbing photoproduct was crystallized and the blue-absorbing dark state remains to be characterized. Electron density unambiguously identifies the bilin chromophore as a singly linked PVB adduct (4); the critical DXCF cysteine residue (15) is unattached to the chromophore. In PVB, the C5 methine bridge of PCB is saturated, shortening the conjugated π electron system (Fig. 1*A*). The structure of TePixJ thus elucidates the basis for perception of green light. There are not yet structures for the blue-absorbing dark state, but biochemical and spectroscopic studies provide compelling evidence for a covalent linkage between the DXCF cysteine and the C10 atom of the bilin chromophore in this state (11, 12, 15, 16, 18).

The work by Narikawa et al. now provides a structural backdrop for future spectroscopic and mechanistic studies of CBCRs.

The CBCR structures also offer tantalizing clues about signal propagation from chromophore to output domain. AnPixJ and TePixJ both crystallize as parallel dimers, with the distal α helices of the dimeric partners forming a helical bundle. Highly similar quaternary structural arrangements have been observed for other GAF proteins (8) and phytochromes (5, 7), in which the distal helical bundle has been implicated in the transduction of light signals to downstream output modules (7, 17). On the basis of sequence analysis, Narikawa et al. (4) argue that CBCRs also connect to their output modules via continuous "signaling helices" (19), which propagate the signal toward the C terminus (e.g., via piston, pivot or rotary movements within helical bundles). Interestingly, sequence data further indicate that both tandem CBCR photosensor modules and tandem GAF domains are serially connected by α helices of conserved length (Figs. 1 *B* and *C*). Tandem CBCR photosensor modules might thus integrate multiple light signals via a series of helical movements conserved in GAF and other domains (10, 20), implying a wider relevance for the work of Narikawa et al. (4).

In summary, the work by Narikawa et al. (4) now provides a structural backdrop for future spectroscopic and mechanistic studies of CBCRs. Because of their related photochemistry but simpler domain architecture, CBCRs can serve as powerful paradigms for phytochromes. Finally, given their compact size and their ability to sense various light colors and intensities (13), CBCRs are attractive building blocks in the engineering of photoreceptors for use in optogenetics, and the present structures will provide a structural rationale.

Note Added in Proof. Burgie et al. have recently determined two structures of TePixJ in its blue-absorbing dark state that confirm the presence of a covalent bond between the DXCF cysteine and the C10 atom of the bilin (21).

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