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 photochromic 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 photocycle (Fig. 1A): photoisomerization of the bilin 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 (Fig. 1D), and probe the behavior of output domains and downstream signal transduction pathways. Despite similar chromophores, photoschemistry, and self-assembly, Phys and CBCRs differ in several striking ways. Most phycobacteriochromes 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 photocycle (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 photoschemistry 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. 1D), 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).

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 *Thermosynechococcus 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 surface of the sheet (Fig. 1B). An AnPixJ 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. 1D). In phytochromes, the 15Z configuration is associated with the red-absorbing Pr state, and the 15E configuration 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.
seen in the present structures. Exquisitely, 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 PHD 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 photoprodut of AnPixJ and related proteins remains to be elucidated (10, 13, 14). The case is reversed for TePixJ, in which the green-absorbing photoprodut was crystallized and the chromophore-binding domain of phytochrome. AnPixJ and related photoreceptors for use in optogenetics, and the present structures will provide a structural rationale.

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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 a helices of conserved length (Figs. 1 B and C).

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.

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).