Molecules and Cells

Minireview

Color Sensing and Signal Transmission Diversity of Cyanobacterial Phytochromes and Cyanobacteriochromes

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https://doi.org/10.14348/molcells.2020.0077
www.molcells.org

To perceive fluctuations in light quality, quantity, and timing, higher plants have evolved diverse photoreceptors including UVR8 (a UV-B photoreceptor), cryptochromes, phototropins, and phytochromes (Phys). In contrast to plants, prokaryotic oxygen-evolving photosynthetic organisms, cyanobacteria, rely mostly on bilin-based photoreceptors, namely, cyanobacterial phytochromes (Cphs) and cyanobacteriochromes (CBCRs), which exhibit structural and functional differences compared with plant Phys. CBCRs comprise varying numbers of light sensing domains with diverse color-tuning mechanisms and signal transmission pathways, allowing cyanobacteria to respond to UV-A, visible, and far-red lights. Recent genomic surveys of filamentous cyanobacteria revealed novel CBCRs with broader chromophore-binding specificity and photocycle protochromicity. Furthermore, a novel Cph lineage has been identified that absorbs blue-violet/yellow-orange light. In this minireview, we briefly discuss the diversity in color sensing and signal transmission mechanisms of Cphs and CBCRs, along with their potential utility in the field of optogenetics.

Keywords: color sensing, cyanobacteria, cyanobacterial phytochromes, cyanobacteriochromes, signal transmission

INTRODUCTION

Most organisms have evolved a number of receptors that can sense environmental fluctuations, which is a critical advantage for survival. Bilin-based photoreceptors are one of the best studied light sensing photoswitches in plants, algae, cyanobacteria, and other bacterial species. Unlike higher plants that employ blue light photoreceptors, cryptochromes, phototropins, and FLAVIN-BINDING, KELCH REPEAT, F-BOX 1 (FKF1) and ZEITLUPE (ZTL) as additional players in various photobiological responses, cyanobacteria mostly utilize bilin phytochromes (Cphs) and cyanobacteriochromes (CBCRs) in various photobiological responses (Hwang et al., 2019; Rockwell and Lagarias, 2020).

Cphs and canonical plant phytochromes (Phys) are modular in structure, with an N-terminal photosensory core module (PCM) and a C-terminal output regulatory module. PAS (Period/Arnt/Single-minded), GAF (cGMP phosphodiesterase/Adenylyl cyclase/FhlA), and PHY (phytochrome-specific) domains form the PCM. While the GAF domain is necessary for forming the bilin adduct, PAS and PHY domains are involved in bilin lyase activity and the stabilization of 15*E* lit photostate, respectively (Rockwell and Lagarias, 2017). In addition to Cphs, another group of bilin-based photoreceptors known as CBCRs are widespread among cyanobacteria. In contrast to canonical Cphs, CBCRs sense a wide range of light wavelengths including near-ultraviolet (UV), visible, and far-red (FR) spectra, and this sensitivity is enabled only by the GAF domain since CBCRs lack the PAS and PHY domains (Fushimi and Narikawa, 2019). Such absorption of spectral difference

Received 24 March, 2020; revised 28 April, 2020; accepted 28 April, 2020; published online 22 May, 2020

eISSN: 0219-1032

is called color (spectral) tuning, which is attributable to varied effective π conjugation length of the bilin chromophore that originates from either the chromophore-binding plasticity of the apoprotein or chromophore-binding geometry of the pocket and ionic environments. The mechanisms underlying such color tunings involve bilin chromophore species and cysteine (Cys) residues in the GAF domain, which are further regulated by different types of residues surrounding the bilin-binding pocket that affect the tilted geometry and protonation status of bilins. Recently, phylogenetic analysis, transient absorption spectroscopy, crystallography, and molecular genetics revealed novel CBCRs and Cphs that exhibit broader chromophore-binding specificity and protochromicity of the lit state. For instance, in a newly established tandem cysteine cyanobacterial phytochrome (TCCP) lineage, a second Cys present within the GAF domain is responsible for blue-violet/yellow-orange photoconversion (Rockwell et al., 2011; Song et al., 2020). In this review, we summarize the biochemical properties and photobiological functions of some Cphs and CBCRs, and based on their properties, we propose future perspectives for the application of Cphs and CBCRs in various fields including optogenetics.

CYANOBACTERIAL PHOTOBIOLOGICAL RESPONSES

Cyanobacteria represent the first organisms on primitive Earth that utilized solar radiation and evolved oxygen to acquire chemical energy (ATP) and the reducing power (NADPH) necessary for cellular processes. The ability of cyanobacteria to harness solar energy exceeds their photosynthetic capability. Cyanobacteria exploit the quality, quantity, and photoperiodicity of solar radiation to perform various photobiological responses including cell growth, chromatic acclimation, phototactic movements, hormogonia development, circadian rhythms, biofilm formation, and UV-absorbing compound production (Table 1 and references therein). These responses are directly or indirectly related to optimal photosynthesis but avoid either limited or excess light environments.

Growth of the unicellular cyanobacterium *Synechocystis* PCC 6803 (hereafter referred to as *Synechocystis*) in red (R) and FR light is controlled by Cph1 and Cph2 in an antagonistic manner. Disruption of Cph1 decreased the growth of *Synechocystis* under FR light, while disruption of Cph2 decreased its growth under R light (Fiedler et al., 2007). Mutation of Cph2 altered growth rate and biofilm formation in response to trophic condition changes, implying that Cph2 is involved in the regulation of the primary energy metabolism (Schwarzkopf et al., 2014). Similar to other photosynthetic organisms, *Synechocystis* exhibits differential growth under different light wavelengths, leading to an unbalanced excitation of photosystems, thus hampering the photosynthetic utilization of solar radiation in an unbalanced and inefficient manner. Under such conditions, bilin composition of cyanobacterial light-harvesting antenna phycobilisomes is adjusted, allowing cyanobacteria to balance the proportion of light absorption between two photosystems. This type of response is known as chromatic acclimation (CA). To date, six types of CA have been reported (Sanfilippo et al., 2019; Wiltbank and Kehoe, 2019). Various components of CA include RcaE and DpxA in *Fremyella diplosiphon*, CcaS in *Synechocystis* and *Nostoc punctiforme,* and the R/FR knotless Cph, RfpA in *Leptolyngbya* sp. JSC-1; all of these four proteins harbor a histidine (His) kinase domain, which acts as the sensor kinase in a signal transduction cascade.

Cyanobacteria lack flagella but exhibit phototaxis in response to variation in light quantity and quality. However, *Synechocystis* exhibits type IV pili-dependent twitching motility (Bhaya, 2004), while the filamentous cyanobacterium *N. punctiforme* exhibits gliding motility (Khayatan et al., 2015) to relocate to another location for optimal photosynthesis. In *Synechocystis*, positive (Yoshihara and Ikeuchi, 2004) and negative (Song et al., 2011) phototaxes are mediated by two different CBCRs, namely, PixJ and UirS, respectively. By contrast, in *Synechococcus elongatus*, a single 5-GAF domain photoreceptor, $PixJ_{se}$, senses the direction of illumination by wavelengths that induce both positive and negative phototactic movements (Yang et al., 2018b). Orthologs of bacterial methyl-accepting chemotaxis proteins such as SyPixJ (Yoshihara et al., 2004), TePixJ (Ishizuka et al., 2006), and AnPixJ (Narikawa et al., 2008) are mostly involved in such taxis.

Like other microorganisms, cyanobacteria form biofilms, where cells are mostly attached to and grow on a surface and produce extracellular polymers. In *Synechocystis*, these surfaces include exopolysaccharides, the S-layer and pili (Allen et al., 2019). In filamentous *Leptolyngbya* and *Scytonema*, biofilm formation involves hormogonia, whose sticky ends are adhered to a surface (Maldener et al., 2014). Formation of biofilm in *Thermosynechococcus* is mediated by the bacterial second messenger, cyclic diguanosine monophosphate (c-di-GMP) (Agostoni et al., 2016). Three CBCRs, SesA/B/C, in the blue/green light (ON/OFF)-c-di-GMP switch control sessility and motility in planktonic communities (Enomoto et al., 2014; 2015), where blue/green light penetration is restricted to upper layers (Enomoto and Ikeuchi, 2020).

In addition, cyanobacteria sense diurnal photoperiod to adjust photosynthetic and respiratory activities. In *S. elongatus* PCC 7942, the circadian clock regulates genes at dusk and dawn in a promoter-dependent manner. Regulation of promoters is period-specific, which in turn leads to diurnal regulation of energy metabolism, cellular division, and chromosome architecture modification. KaiA, the first component of the *S. elongatus* oscillator (KaiABC), and CikA (circadian input kinase A) PsR domains act as environmental sensors, detecting the redox state of the quinone pool during the transition from day to night (Cohen and Golden, 2015).

Most heterocystous or non-heterocystous filamentous cyanobacteria form hormogonia, i.e., motile short filaments formed during asexual reproduction from vegetative cells or trichomes (Marsac, 1994). Hormogonium differentiation in the cyanobacterium *Calothrix* sp. PCC 7601 is stimulated by R light and inhibited by green light (Damerval et al., 2007). In *N. punctiforme*, a methyl-accepting chemotaxis protein (MCP)-like photoreceptor, PtxD, is involved in the phototaxis of hormogonia (Campbell et al., 2015).

Photoinhibitory light environments trigger the production of sunscreen pigments in some cyanobacteria. For instance, in response to high light or UV irradiance, mycosporine-like amino acids and scytonemins (Rastogi et al., 2014) are pro-

Table 1. Domain structure and biological functions of cyanobacterial photoreceptors

Photoreceptor[a]		Signal transmission[b]	Response	Organism (reference)
AtPhy	Single-Cys/GAF (PΦB)	Phosphorelay	Growth and development	*Arabidopsis thaliana* (Franklin and Quail, 2010)
BphP	Single-Cys/PAS (BV)	Phosphorelay	LH4 synthesis	*R. palustri* (Evans et al., 2005)
Cph1	Single-Cys (PCB)	Phosphorelay	Growth	*Synechocystis* (Fiedler et al., 2007)
ToTCCP	Dual-Cys (PCB)	n.a.	n.a.	*Tolypothrix* PCC 7910 (Song et al., 2020)
Cph2	Single-Cys (PCB)	2nd messenger	Growth, phototaxis	*Synechocystis* (Fiedler et al., 2007; Wilde et al., 2002)
RcaE	Single-Cys (PCB)	Phosphorelay	Chromatic acclimation	*F. diplosiphon* (Hirose et al., 2013)
SyCcaS	Single-Cys (PCB)	Phosphorelay	Chromatic acclimation	*Synechocystis* (Hirose et al., 2013)
Slr1393-RGS	Single-Cys (PCB)	Phosphorelay	n.a.	*Synechocystis* (Chen et al., 2012)
TePixJ	Dual-Cys (PVB)	Phosphorelay	Phototaxis	*T. elongatus* BP-1 (Ishizuka et al., 2007; 2011)
UirS	Dual-Cys (PVB)	Phosphorelay	Phototaxis	*Synechocystis* (Song et al., 2011)
SesA	Dual-Cys (PVB)	2nd messenger	Cell aggregation	*T. elongatus* (Enomoto and Ikeuchi, 2020; Enomoto et al., 2014)
UGS1	Dual-Cys (PCB)	n.a.	n.a.	*Microcoleus* IPPAS B353 (Cho et al., 2015)
UGS2	Dual-Cys (PCB)	n.a.	n.a.	*Microcoleus* IPPAS B353 (Cho et al., 2015)
Anacy_4718	Single-Cys (PCB, BV)	n.a.	n.a.	*A. cylindrica* PCC 7122 (Rockwell et al., 2016)
n.a.		n.a.	UV protectant synthesis	*Fischerella* PCC 9339 (Yang et al., 2018a)

PΦB, phytochromobilin; BV, biliverdin; PCB, phycocyanobilin; PVB, phycoviolobilin; , GAF; , histidine (His) kinases, adenylyl cyclases, methyl-accepting proteins, and phosphatases (HAMP); , phytochrome-specific (PHY); , Period/Arnt/Single-minded (PAS)/the C-terminal end of PAS; , His kinase; , diguanylate phosphodiesterase; , ATPase domain of His kinase; , methyl-accepting domain; , response regulator; , cystathionine β-synthase; , receiver domain; , diguanylate cyclase; , PAS; n.a., not available.

[a]Photocycles are represented as color-coded 15*Z*/15*E* states; gray color indicates the absence of photocycle.

[b]Experimentally verified signal transmission pathways such as phosphorelay and 2nd messenger are shown.

duced and accumulated in the outer cell wall spaces in halophilic cyanobacteria such as *Euhalothece* sp. and *Microcoleus* sp., several *Nodularia* species, and *Scytonema hofmanii* (Sinha and Häder, 2008; Yang et al., 2020). Sensing and signaling components of these photobiological responses are likely mediated by bilin photoreceptors, i.e., Cphs and CBCRs, although further investigation is needed.

DIVERSE COLOR SENSING ABILITY OF Cphs AND CBCRs

Since the first cyanobacterial genome of *Synechocystis* was reported, the discovery of diverse photoreceptors and their color-tuning mechanisms has largely depended on the identification of a vast number of cyanobacterial proteins containing PAS-GAF-PHY domains (Yeh et al., 1997). Whole-genome sequencing of cyanobacterial species including *Microcoleus* IPAS B373 (Cho et al., 2015), *Euhalothece* Z-M001 (Yang et al., 2020), and *Tolypothrix* PCC7910 (Song et al., 2020) revealed the absence of the *HY2* gene, which encodes phytochromobilin (PΦB) synthase; therefore, PΦB, a bilin chromophore covalently bound to canonical Phys in higher plants, is absent in these cyanobacteria. Instead, these cyanobacteria harbor the *pcyA* gene, which encodes a phycocyanobilin (PCB):ferredoxin oxidoreductase that catalyzes four-electron reduction of biliverdin IXα (BV) to PCB, a major

cofactor of Cphs and CBCRs (Fujita et al., 2015; Fushimi and Narikawa, 2019). The number of Cphs and CBCRs varies among cyanobacteria, with three in *Euhalothece*, eight in *Synechocystis*, nine in *Microcoleus* IPAS B353, 12 in the chlorophyll *d*-containing cyanobacterium *Acaryochloris marina*, 18 in *N. punctiforme*, and 36 in *Tolypothrix* PCC 7910. The total number of bilin photoreceptors in a cyanobacterium is roughly proportional to its genome size (Cho et al., 2015). Additionally, in cyanobacteria, CBCRs are more abundant than Cphs, and the abundance ratio of blue CBCRs to red CBCRs is likely related to light conditions in the natural habitat. For instance, *Microcoleus* IPAS B353 is enriched in near-UV and violet CBCRs but lacks red/green and green/red CBCRs, suggesting that the enrichment of short wavelength-absorbing CBCRs is critical for acclimation of cyanobacteria to high light environments in their natural habitat (Cho et al., 2015).

The PCB-bound holo-Cphs make spectral difference with PΦB- or BV-bound Cphs because of differences in the number of π electrons; PCB has two- or four-electrons less than PΦB and BV. Consequently, the spectra of Cph1- and knotless Cph2-bound PCB chromophores show a slight blue shift compared with PΦB-bound PhyB and BV-bound BphP (Rockwell and Lagarias, 2010). However, unlike canonical Phys or bacterial-type Phys BphP, the newly identified TCCPs shift their light sensing maximum into the violet spectral region (Rockwell et al., 2011; Song et al., 2020). The color sensing diversity of TCCPs seems to be related to the protonated 15*E* bilin chromophores with different ionization state of the non-canonical 'second' Cys sulfhydryl group (Fig. 1; Song et al., 2020). This expanded range of light absorption by Cphs is also observed in some eukaryotic algal species including *Cyanophora paradoxa* (Song et al., 2020).

Unlike canonical Phys and Cphs, CBCRs only require the GAF domain(s) to bind to a bilin and then to exhibit photocycle encompassing near-UV to FR light (Ikeuchi and Ishizuka, 2008; Table 1, Fig. 1). Like Cphs, CBCRs are categorized into two subgroups, based on the number of Cys residues in the GAF domain that form covalent adducts to apoproteins

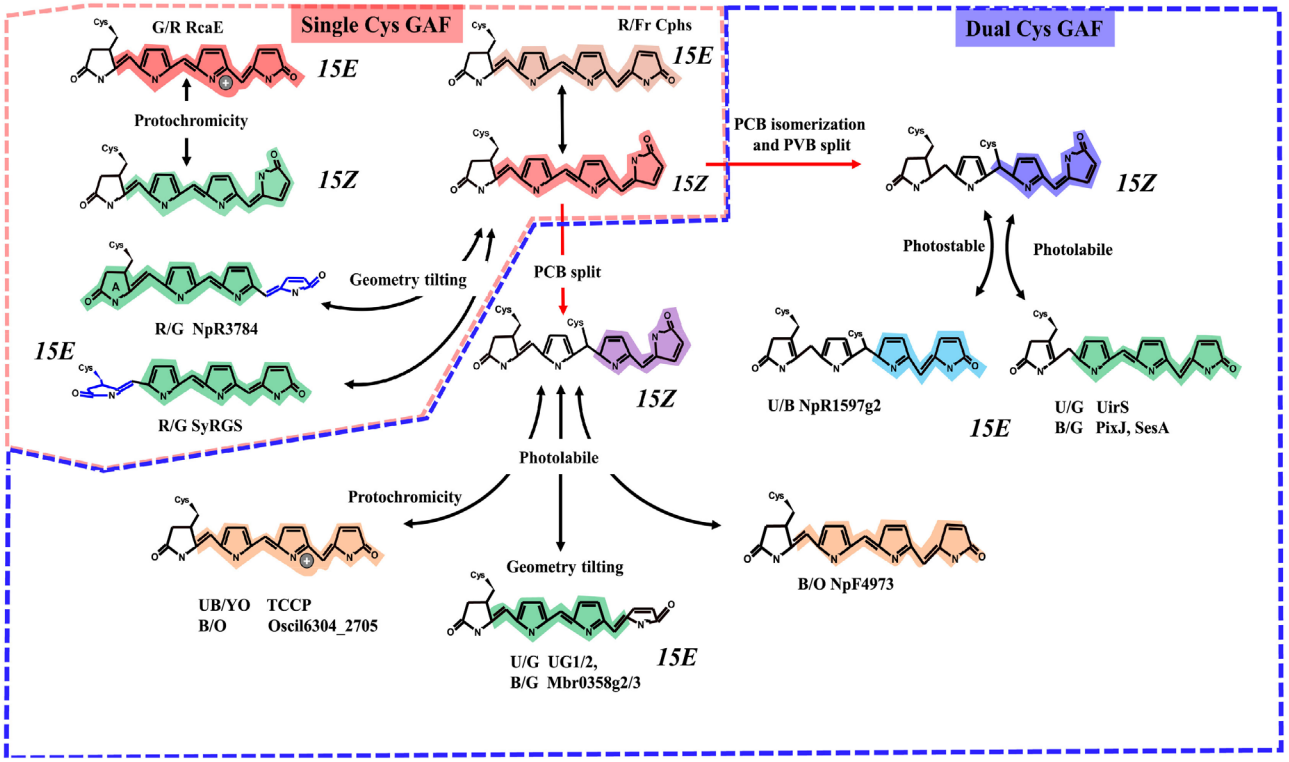

Fig. 1. Color-tuning mechanisms adopted by cyanobacterial phytochromes (Cphs) and cyanobacteriochromes (CBCRs). Cphs and CBCRs exhibit reversible photoisomerization at C15-C16 (R/FR photocycle). Single-cysteine (Cys) red/green or green/red CBCRs bind to phycocyanobilin (PCB) in the dark state, exhibiting red and green absorption maxima. Upon red or green illumination, green or red light-absorbing forms are formed by lowering effective π-conjugation length via protochromicity of bilin (RcaE) or geometry tilting by the A-ring (SyRGS) or D-ring (NpR3784). A second Cys residue located at the DXCF or CXXR/K motif forms a second thioether linkage at C10, yielding a blue light-absorbing dark state. Some dual-Cys CBCRs isomerize PCB to phycoviolobilin (PVB), forming violet or blue light-absorbing dark state. These adducts are either photolabile or photostable upon photoisomerization, yielding green or blue-absorbing lit states. Such variation is further enhanced by bilin protochromicity or tilted geometry, yielding violet, teal, orange and red -absorbing photoproducts. In dual-Cys cyanobacterial phytochromes, tandem cysteine cyanobacterial phytochrome (TCCP)-specific Cys is responsible for the bilin split, and lit state tuning is related to the protonated lit form with different ionization state of the Cys thiol group. Black double arrows and red forward arrow represent reversible photoisomerization around the C15=C16 double bond of a bilin and second thioether linkage formation with or without bilin isomerization, respectively.

(Fushimi and Narikawa, 2019; Rockwell and Lagarias, 2017; Rockwell et al., 2015). Singly- or doubly-bound Cys residues of GAF domains form thioether linkages with the chromophore because of their auto-lyase activity. The canonical Cys residue in the CH motif of the GAF domain binds to the $C3^1$ (PCB and PVB) or $C3^2$ (BV) atom of bilin (Fushimi and Narikawa, 2019), forming a red/green photocycle, unlike the canonical R/FR photocycle, mostly because of the conserved phenylalanine (Phe) and aspartic acid (Asp) residues that partially deconjugate the D-ring (Rockwell et al., 2014) or A-ring in Slr1393G3 (SyRGS) CBCR (Buhrke et al., 2020), respectively, by trapping bilin in a twisted geometry (Lim et al., 2018; Xu et al., 2020). By contrast, protochromicity of bilin chromophores determine green/red (Hirose et al., 2013) or blue/orange (Sato et al., 2019) photocycles. For instance, the green light-absorbing dark state contains deprotonated chromophore, which is protonated in the R light-absorbing lit state via the involvement of protochromic triad residues (Hirose et al., 2013).

Some CBCRs contain additional Cys residues located in the highly conserved DXCF motif or the weakly conserved CXXR/K motif in the insertion loop (insert-Cys) that forms the second thioether linkage via the C10 atom of their dark states (Cho et al., 2017; Rockwell et al., 2011; 2014). In many dual-Cys CBCRs, this second thioether linkage is unstable and light-labile, forming additional reversible thioether adducts. Accordingly, these CBCRs can sense blue or violet light in the dark state and teal, green, yellow, or orange light in the lit state. In the case of teal-DXCF CBCRs, tilting of the D-ring by Phe residues leads to teal-absorbing photoproducts (Rockwell et al., 2014). Similarly, insert-Cys CBCRs adopt both tuning mechanisms, namely, photolabile second thioether linage and tilted geometry (Cho et al., 2017). By contrast, blue/orange CBCR Oscil6304_2705 combines protochromic and two-Cys photocycles in separate time scales (Sato et al., 2019). Unlike CBCRs above mentioned, the second thioether linkage is formed upon illumination of the dark state, yielding red/blue photocycle (Narikawa et al., 2014).

Like Cphs, cyanobacterial CBCRs mainly bind to PCB. However, some CBCRs also bind to BV (Fushimi et al., 2016; Narikawa et al., 2015), phycoviolobilin (PVB) (Ishizuka et al., 2007; Song et al., 2011; Cho et al., 2015), and PΦB (Rockwell et al., 2016). PVB adduct is formed by the isomerization of a covalent PCB adduct, shortening the π-conjugated system in the dark (Ishizuka et al., 2011; Rockwell et al., 2012). This conversion of PCB into PVB is DXCF-CBCRs subfamily specific (Rockwell et al., 2012) although residue(s) involved have not been characterized yet. In several cases, some CBCRs and Phys covalently bind to porphyrin compounds, which are considered as contaminants when expressed in a heterologous system (Fischer et al., 2005; Rockwell et al., 2016; Wagner et al., 2008). Despite the characterization of such tuning mechanisms, some tuning mechanisms remain unknown among the newly discovered CBCRs such as Fr/O (Rockwell et al., 2016).

Signal transmission diversity

Despite the development of molecular genetic tools in a few model species such as *F. diplosiphon*, *Synechocystis*, *S. elongatus* PCC 7942, and *Thermosynechococcus vulcanus*, the physiological functions of most CBCRs remain elusive (Wendt and Pakrasi, 2019). Nonetheless, several cyanobacterial photoreceptors have been studied in detail, indicating that signal transmission is accompanied mostly by phosphotransfer or c-di-GMP-initiated downstream pathways (Table 1). Phosphorelay is observed in several signal transmission pathways involved in the autophosphorylation of His residues by His kinases, followed by phosphotransfer to cognate response regulators. For instance, the CBCR UirS is an atypical membrane-bound His kinase, and its cognate AraC family response regulator, UirR, functions as a UV-A-sensing two-component signaling system in *Synechocystis* (Song et al., 2011). The DNA-binding transcriptional regulator, UirR, targets the adjacent PatA family response regulator, LsiR, which functions as a signal output regulator to reverse the twitching orientation of *Synechocystis* cells from positive to negative under unidirectional UV-A illumination. Activation of UirR by UirS is mediated by phosphorelay upon the UV-A-dependent activation of UirS (Song et al., 2011). In addition, phototactic motility in *Synechocystis*, controlled by the blue/green MCP domain-containing SyPixJ (previously known as TaxD; Yoshihara and Ikeuchi, 2004), is subjected to a light-dependent phosphorelay cascade. CA signal transmission is also regulated by phosphorelay between sensors and response regulators. Upon green light illumination, SyCcaS is autophosphorylated, and the phosphate is transferred to its cognate response regulator CcaR (Castillo-Hair et al., 2019; Hirose et al., 2008; 2010).

SesA CBCR induces cell aggregation in *T. vulcanus* via c-di-GMP signaling (Enomoto et al., 2014; 2015). Blue light-activated SesA activates the GGDEF domain, leading to the generation of c-di-GMP (Enomoto et al., 2014), although the activation mechanism remains elusive. Similarly, the GAF domain of Cph2 activates c-di-GMP signaling involved in blue light-dependent inhibition of positive phototaxis (Wilde et al., 2002). The N-terminal domain of SesA contains two tandem repeats of the cystathionine beta-synthase (CBS) domain that binds to nucleotides such as ATP, ADP, and AMP, which possibly regulate SesA activity at the chromophorylation step. The CBB domain-containing Slr2111 allosterically regulates chromophorylation of the red/green sensor (RGS) Slr1393. ATP-bound CBS-Slr2111 dissociates from RGS, allowing the chromophorylation of Slr1393. By contrast, AMP-bound Slr2111 maintains a strong interaction with Slr1393, hindering chromophorylation of Slr1393 (He et al., 2018). Thus, CBCRs with or without CBS domains seem to be post-translationally activated by cellular energy charge (ATP/[ADP + AMP] ratio), although this hypothesis needs further verification.

PERSPECTIVES

Color and signal transmission diversity of Cphs and CBCRs arise from the presence of light sensing and signal transmitting domain(s) in various combinations. Such a variety of signal input and output domains provides advantages in regulating *in situ* cellular functions such as photosynthesis, growth, development, and circadian rhythm. Despite the

well-characterized color-tuning and signal-relaying mechanisms, the role of multiple GAFs in a single CBCR is largely unknown. Genomes of cyanobacteria such as *N. punctiforme* and *Tolypothrix* PCC 7910 encode several multiple-GAF CBCRs. This either represents redundancy for signal addition and amplification, or plays a regulatory role, for instance, in controlling the direction of movement (Yang et al., 2018b). Comparative X-ray crystallography or cryogenic electron microscopy (cryo-EM) image analysis of the whole structure of wild-type and mutant (domain-less) CBCRs in the dark, intermediate, and lit states, and development of genetic transformation toolboxes for naturally untransformed cyanobacterial strains, could help elucidate the unknown biological roles of previously characterized cyanobacterial photoreceptors. Additionally, CBCRs stand out as ideal sources for optogenetic toolboxes (Castillo-Hair et al., 2019; Fushimi et al., 2019; Ramakrishnan and Tabor, 2016), considering the pros and cons of Phys (Rockwell et al., 2016). The broad range of light inputs of CBCRs and signal transmitting pathways seem advantageous in the field of fluorescence probes (Chernov et al., 2017; Oliinyk et al., 2019; Ong et al., 2018) and light-controlled subcellular functions (Milias-Argeitis et al., 2016; Tandar et al., 2019) including protein–protein interactions, gene expression and c-di-GMP/-AMP-dependent signaling cascades (Blain-Hartung et al., 2018; Klausen et al., 2019). The recently characterized porphyrin-binding and pH-sensitive CBCRs are particularly appealing for application in porphyrin sequestration and as fluorescence reporters for diagnosing abnormal cells with varied intra- and inter-cellular pH values.

ACKNOWLEDGMENTS

This work was supported by grants from the Next-Generation BioGreen 21 Program, Rural Development Administration (PJ013118), the KIST Open Research Program (2E30642-20-152), and the Collaborative Genome Program funded by the Ministry of Oceans and Fisheries (20180430), Korea.

AUTHOR CONTRIBUTIONS

Y.V., H.W.Y., and Y.I.P. wrote the manuscript. Y.I.P. secured fundings.

CONFLICT OF INTEREST

The authors have no potential conflicts of interest to disclose.

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