Cyanobacteriochromes: a new superfamily of tetrapyrrole-binding photoreceptors in cyanobacteria†

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A new group of photoreceptors has been experimentally revealed in cyanobacteria. They are phototaxis regulator SyPixJ1, TePixJ and AnPixJ, chromatic acclimation regulator SyCcaS, circadian input kinase homolog SyCikA and many other candidates, which have been found only in cyanobacteria to date. These new photoreceptors are now proposed to be "cyanobacteriochromes". They are characterized by the presence of a chromophore-binding GAF domain that is homologous to the tetrapyrrole-binding GAF domain of the phytochrome. Here, we summarized unique features of those representatives: (1) only the GAF domain is sufficient for full photoconversion, (2) the GAF domain is homologous to but distinct from the phytochrome GAF, (3) the GAF domain binds a linear tetrapyrrole pigment such as phycoviolobilin or phycocyanobilin, (4) spectral properties are very diverse from near ultra-violet to red region. We also discussed the functionality of the other candidate GAFs, structure and evolution.

Introduction

Phytochromes are the expanding photoreceptor family that originated from the classic plant phytochromes.[1] When the first cyanobacterial genome was determined in 1996, it was revealed that *Synechocystis* sp. PCC 6803 harbors a potential phytochrome homolog gene, *cph1*.[2,3] The prediction was experimentally confirmed by evidence that photoactive Cph1 was successfully reconstituted *in vitro* from the apoprotein and a putative chromophore, phycocyanobilin (PCB).[4,5] Since then, increasing genome projects and screening of homologous sequences have revealed that many but not all cyanobacteria carry one to several phytochrome genes, although some variations are present.[6,7] Further discovery of bacteriophytochromes in some eubacteria[8] and fungal phytochromes[9,10] has been expanding the phytochrome family.

It was even more surprising to know that the cyanobacterial genome harbors phytochrome-like genes, which are homologous to but distinct from the phytochromes. The first implication was provided in the study of complementary chromatic acclimation (also called complementary chromatic adaptation) by Kehoe and Grossman.[11] A filamentous cyanobacterium *Fremyella diplosiphon* exhibits differential expression of the phycobiliproteins, phycoerythrin or phycocyanin, in response to green or red light illumination, respectively, and *FdrcaE* was identified as a putative photoreceptor gene. Interestingly, only the GAF domain is significantly homologous to the chromophore-binding domain of the phytochrome. GAF is the acronym of cGMP phosphodiesterase, adenylyl cyclase and FhlA protein, which represent a common domain of similar 3D fold in a wide variety of signaling proteins including phytochromes.[12] Since then, many

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putative photoreceptor genes, which code for similar domains, have been detected in cyanobacterial genomes[13,14] and some of them have been suggested to be involved in unique light responses. Namely, *sll1124* (*SyplpA*) was implicated to be involved in blue light-induced photomixotrophic growth in *Synechocystis*.[15] *sll0041* (*SypixJ1*, also called *pisJ1* or *taxD1*) was shown to be essential for positive phototaxis toward the light source again in *Synechocystis*.[16,17] *cikA* was initially suggested to be a photoreceptor, which resets the circadian clock in light-induced entraining,[18] although it was later shown to be a redox sensor but not a photoreceptor in a unicellular cyanobacterium *Synechococcus elongatus* PCC 7942.[19,20] SyCcaS was shown to serve as a green light receptor that regulates expression of a phycobilisome linker variant gene *cpcG2*.[21,22] These gene products are predicted to carry the phytochrome-like GAF domain for photoperception. These findings strongly suggest that the cyanobacteria are unique in developing a wide variety of light responsive systems.[23,24]

Very recently, some of them (SyPixJ1/TePixJ, AnPixJ, SyCcaS, and SyCikA) are demonstrated to bind a linear tetrapyrrole to form a photoactive holoprotein[22,25–30] and now we propose to call them "cyanobacteriochrome", although yet a number of such GAF domains remain to be confirmed experimentally. Here, we summarize the current view of this emerging class of photoreceptors.

What is the cyanobacteriochrome?

There are several unique features in the cyanobacteriochromes. First, only the chromophore-binding GAF domain is sufficient for photoperception in cyanobacteriochromes. This contrasts with the phytochrome that requires a series of PAS, GAF and "Phytochrome (PHY)" domains for the complete photocycles (Fig. 1).[31] Second, the chromophore-binding GAF domains of cyanobacteriochromes are significantly homologous to the phytochrome GAF domains but are classified into a distinct family according to the sequence comparison (Fig. 2). The GAF domain was originally defined as distinctive but rather diverged motif consisting of approximately 150 amino acid residues to make a common fold of α-helices and intervening β-sheet.[12] They are divided into many subfamilies but functional studies mostly remain to be done. Notably, some but not all cyanobacterial genomes are enriched in various GAF domains including photoreceptors. For example, *Synechocystis* sp. PCC 6803 and *Anabaena* sp. PCC 7120 harbors 33 GAF domains in 28 proteins and 87 GAF domains in 62 proteins, respectively, while the typical strain of *Escherichia coli* has only 8 GAF domain containing proteins.[13] Fig. 2 shows phylogenetic tree of all the GAF domains of *Synechocystis*, which include two phytochromes and 9 cyanobacteriochrome-type ones. Third, as a chromophore, cyanobacteriochromes bind phycoviolobilin (PVB) or phycocyanobilin (PCB), while phytochromes bind phytochromobilin (PΦB), PCB or biliverdin (BV) (Fig. 3). These linear tetrapyrrole molecules are covalently ligated to a cysteine (Cys) residue in the GAF domain, which is conserved between cyanobacteriochromes and phytochromes except BV-binding bacteriophytochromes. Finally, the cyanobacteriochromes studied so far show very diverse spectral properties in contrast with more or less conservative phytochromes that mostly exhibit reversible photoconversion between red absorbing Pr and far-red absorbing

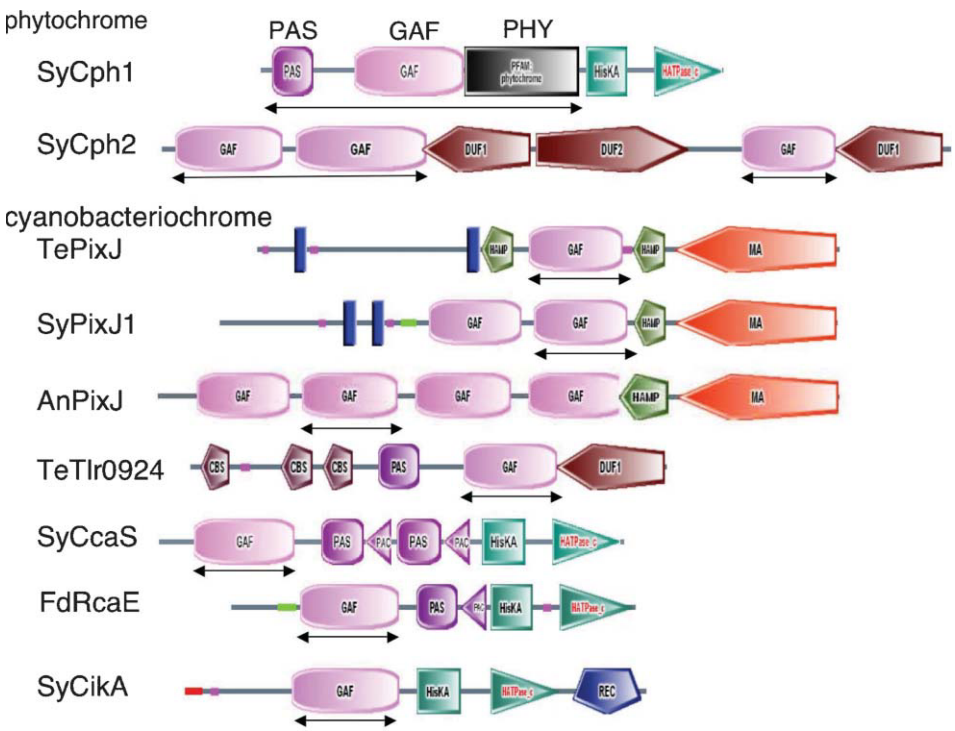

Fig. 1 Domain architecture of cyanobacteriochromes and phytochromes. Domain search was done on SMART (http://smart.embl-heidelberg.de/).[66] Photosensory domains of phytochrome and cyanobacteriochrome are underlined. Other signaling domains are PAS (PAS + PAC), HAMP, methyl-accepting chemotaxis protein (MA), cystathionine β-synthase (CBS), GGDEF motif (DUF1), EAL motif (DUF2), histidine kinase (HisKA + HATPAse_c) and response regulator receiver (REC) domains.

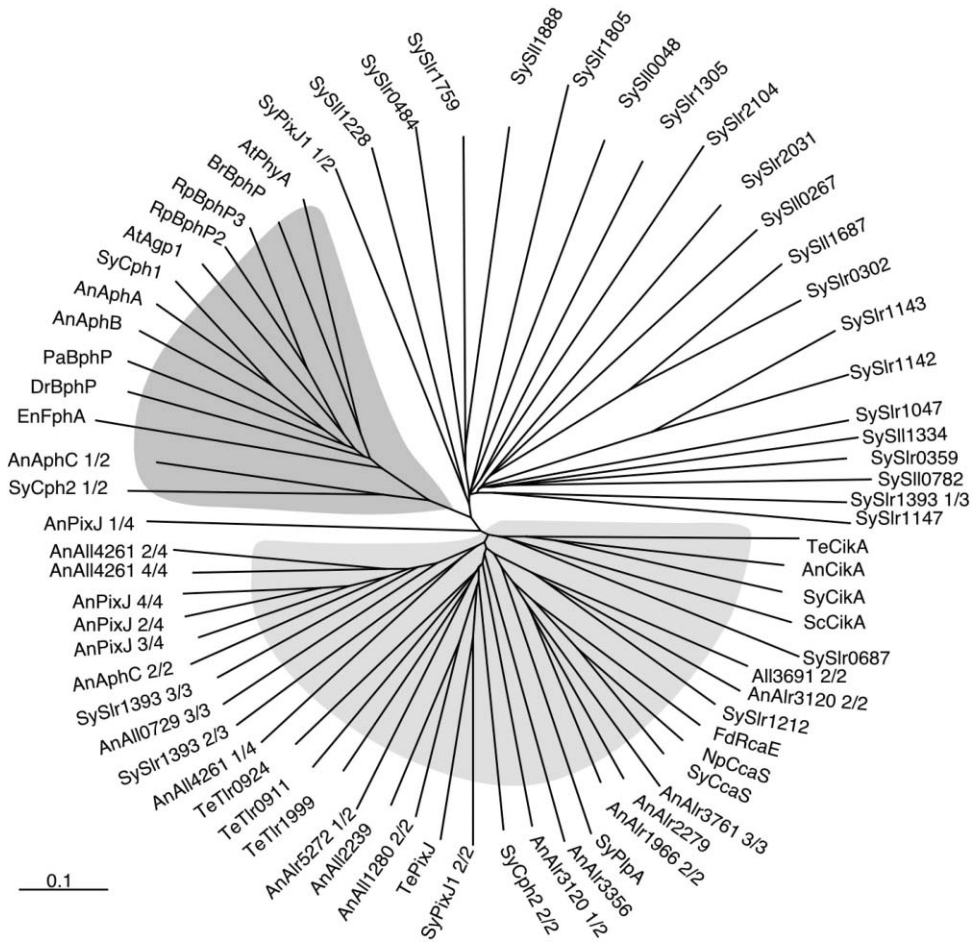

Fig. 2 Phylogenetic tree of GAF domains of cyanobacteriochromes (light shade), phytochromes (dark shade) and other categories. All the GAF domains of *Synechocystis* sp. PCC 6803 were analyzed together with other cyanobacteriochromes, plant phytochromes and bacterial phytochromes by neighbor-joining method of Clustal-W program.[67] Species names are abbreviated as follows: Sy, *Synechocystis* sp. PCC 6803; An, *Anabaena* sp. PCC 7120, Te, *Thermosynechococcus elongatus*; Sc, *Synechococcus elongatus* PCC 7942, Fd, *Fremyella diplosiphon*; At, *Arabidopsis thaliana*; Br, *Bradyrhizobium* sp. ORS278; Rp, *Rhodopseudomonas palustris*; At, *Agrobacterium tumefaciens*; Pa, *Pseudomonas aeruginosa*; Dr, *Deinococcus radiodurans*; En, *Emericella nidulans.* Genes of *Synechocystis*, *Anabaena* and *Thermosynechococcus* are mostly given as gene ID number.

Pfr forms. Fourth, at present, the cyanobacteriochrome-type GAF domains have been found only within the cyanobacterial world (Fig. 2).

The chromophore

Since only a few examples of photoactive cyanobacteriochromes have been demonstrated to date, it may be too early to discuss the variation of its chromophore species. Here we just summarized the chromophore species in cyanobacteriochromes and phytochromes in Table 1. Both AnPixJ and SyCcaS bind PCB. Although PCB is initially incorporated, the final chromophore is PVB in TePixJ and possibly SyPixJ1. However, we must keep in mind that cyanobacteria are known to possess many varieties in bilin pigments as photosynthetic antenna. Further, BV reductase gene has been detected in some cyanobacterial genome. The gene product was experimentally demonstrated to produce bilirubin, even though bilirubin has never been detected in cyanobacteria.[32] Thus, we must be careful in identification of the chromophore. Especially, homologous expression in cyanobacteria would be useful if the natural chromophore is not known. Phytochromes have been mostly expressed in *E. coli* producing various pigments or reconstituted *in vitro*. But expression in cyanobacterial cells demonstrated that Cph1 and FdCphB bound PCB and BV as a natural chromophore, respectively.[33,34]

3D Structure

The crystal structure of PAS-GAF region of bacteriophytochromes DrBphP and RpBphP3 has been determined.[35–37] The chromophore BV is mostly embedded in the GAF domain, while a Cys residue, which precedes the PAS domain, ligates the pyrrole ring A of BV covalently. Instead, another Cys residue in the GAF domain that is conserved and ligates the ring A of PΦB in plant phytochromes is not conserved in the bacteriophytochromes. In cyanobacteriochromes, the latter Cys residue is conserved in the GAF domain and site-directed mutagenesis confirmed that it is essential for the chromophore ligation.[25] Interestingly, the phytochrome GAF has an additional "lasso" sequence that is

Table 1 Summary of properties of cyanobacteriochromes and phytochromes

	Chromophore	Photoconversion	physiology
SyPixJ1_GAF2	PVB	Pb(430–5 nm,*ZZZ*)⇄Pg(535 nm,*ZZE*)	Phototaxis
TePixJ_GAF	PVB	Pb(433 nm,*ZZZ*)⇄Pg(531 nm,*ZZE*)	Phototaxis
AnPixJ_GAF2	PCB	Pr(648 nm,*ZZZ*)⇄Pg(543 nm,*ZZE*)	Phototaxis?
SyScaS_GAF	PCB	Pg(535 nm,*ZZZ*)⇄Pr(672 nm,*ZZE*)	Induction of antenna
FdRcaE_GAF	?	?	Induction of antenna
SyCikA	?	Pv(400 nm)⇄Py(565 nm)	Circadian rhythm?
Cph2_GAF2	?	?	Phototaxis
AtPhyA	PΦB	Pr(*ZZZ*)⇄Pfr(*ZZE*)	Germination, shade avoidance, *etc.*
SyCph1	PCB	Pr(*ZZZ*)⇄Pfr(*ZZE*)	?
DrBphP	BV	Pr(*ZZZ*)⇄Pfr(*ZZE*)	Carotenoid accumulation
RpBphP3	BV	Pr(705 nm,*ZZZ*)⇄Pnr(650 nm,*ZZE*)	Induction of antenna

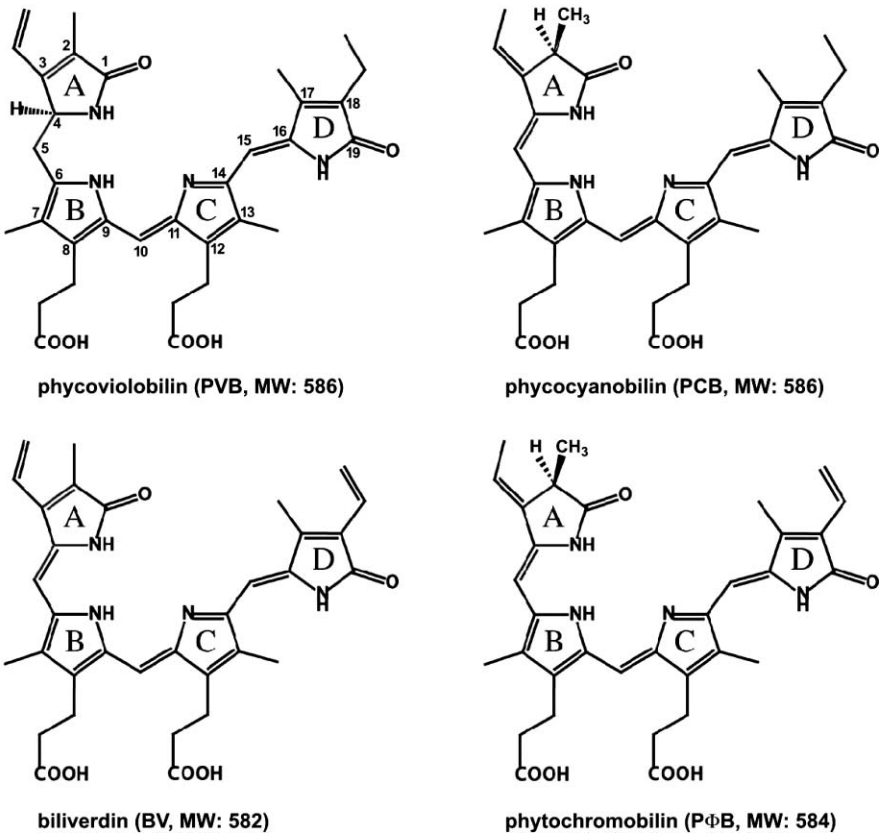

Fig. 3 Chromophore structure. Molecules are shown in a form before covalent ligation to the Cys residue at ring A. The chromophores except PVB are illustrated in C5-*Z,syn*/C10-*Z,syn*/C15-*Z,anti* configuration.

absent in all cyanobacteriochrome GAF. This lasso sequence is a part of the unique figure-of-eight knot, which connects PAS and GAF domains in phytochrome.[36] The absence of lasso sequence may be consistent with the absence of the preceding PAS domain. The 3D structure of DrBphP and RpBphP3 also revealed that three pyrrole rings A, B and C of BV are connected by hydrogen bond *via* a specific water molecule and highly conserved aspartate (Asp) residue, which is again absent in all cyanobacteriochromes. Co-planar structure of conjugated double bonds in the three rings may be critical for absorption of the phytochrome in the red light region and some distortion of the structure might be responsible for wide variation in spectral properties of cyanobacteriochromes.

Phototaxis regulator PixJ1/PixJ of *Synechocystis* and *Thermosynechococcus*

As mentioned above, *pixJ1* (*SypixJ1*) is essential for positive phototaxis in *Synechocystis* sp. PCC 6803.[16,17] The predicted PixJ1 protein consists of two GAF domains and MCP (methyl-accepting chemotaxis protein) domain (Fig. 1) and *pixJ1* gene is flanked with genes related to *cheY*, *cheW*, and *cheA*, which are essential for regulation of flagella-dependent chemotaxis of bacteria.[38] It is thus implicated that PixJ1 perceives light, transduces the signal to CheY-like PixG/PixH *via* CheA-like PixL and finally switches retraction and extension of a certain pili for phototaxis. Motility of *Synechocystis* cells has been established as

pili-dependent twitching one.[39] Mutational studies have identified many *pil* genes that are involved in the assembly of type IV-like pili[39–41] and some phototaxis genes that are involved in regulation of positive phototaxis.[40,42] Complex regulatory mechanism of phototactic motility has been summarized in several reviews.[43,44]

SyPixJ1 protein with an N-terminal polyhistidine tag was isolated from a membrane-fraction of *Synechocystis* cells after detergent solubilization.[25] The preparation thus obtained revealed covalent-binding of a linear tetrapyrrole as shown by Zn^{2+}-induced fluorescence after SDS-PAGE. Surprisingly, the native holoprotein showed clear reversible photoconversion between ~430–5 nm peak (blue absorbing form, Pb) and ~535 nm peak (green absorbing form, Pg) upon irradiation of blue-violet light and green light, respectively. It was obvious that either form does not absorb in red or far-red region in contrast with phytochromes, although the preparation was contaminated with chlorophyll and other pigments. The complete absorption spectra of Pb and Pg forms were later obtained from TePixJ_GAF of *Thermosynechococcus elongatus* that was heterologously expressed in *Synechocystis* (Fig. 4).[27] The chromophore-binding site was identified as a conserved Cys-His motif in the second GAF domain of SyPixJ1 by site-directed mutagenesis.[25] This motif is also conserved in the GAF domain of plant phytochromes and cyanobacterial phytochrome Cph1 but not in bacteriophytochromes. When the GAF domain of SyPixJ1 was expressed in *E. coli*, which co-expresses *ho1* (heme oxygenase) and *pcyA* (phycocyanobilin: ferredoxin reductase) to produce PCB,[45] the holoprotein thus assembled showed reversible photoconversion, which is very similar to the genuine holoprotein.[26] Mass spectrometry analysis revealed that a linear tetrapyrrole of molecular mass 586 (equivalent to PCB) is ligated to the Cys residue *via* a thioether bond. Mass fragmentation of the chromopeptide suggested that the tetrapyrrole is ligated to the Cys residue at the ring A. However,

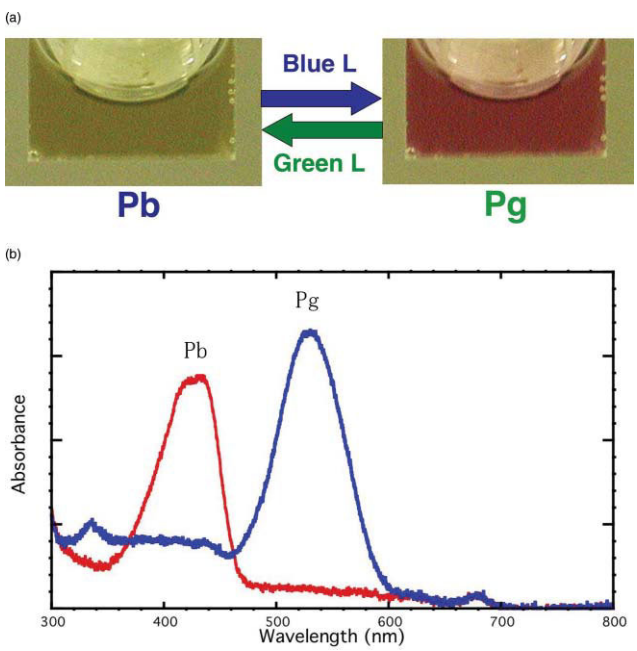

Fig. 4 Two spectral forms of TePixJ_GAF. Photographs of solution (A) and absorption spectra (B).

these results raised another question how PCB-like chromophore exhibits such anomalous blue-shifted absorption at 430 nm or 530 nm.

Further examination of TePixJ_GAF by denaturation with acidic urea revealed that the natural chromophore is not PCB but its isomer PVB.[46] So far, PVB has been found only in α-phycoerythrocyanin of some filamentous cyanobacteria.[47] No PVB biosynthesis enzyme has been identified yet. PecE/F proteins of *Mastigocladus laminosus* have been identified to catalyze ligation-coupled isomerization of PCB to PVB in the process of chromophorylation of α-phycoerythrocyanin *in vitro*.[48–50] For TePixJ_GAF, PCB is also a likely pigment to be incorporated based on *in vivo* and *in vitro* assembly experiments. When the apoprotein of TePixJ1_GAF was expressed in PCB-producing *E. coli*, the chromophore of the photoactive complex was a mixture of PCB and PVB (T. Ishizuka, Y. Ochiai, T. Kohchi, and M. Ikeuchi, unpublished observation). More clearly, *in vitro* reconstitution using synthetic PCB demonstrated that PCB was slowly converted to PVB on the apoprotein, whereas covalent chromophorylation and assembly of a photoactive complex was completed within a few minutes. These results indicate that the GAF domain of TePixJ possesses triple function: auto-lyase, auto-isomerase and photoisomerization, although these activities may require additional factor(s) in cyanobacteria. Surprisingly, this transient PCB-binding complex already acquired partially reversible photoconversion with anomalous blue-shifted properties, which are analogous but not identical to those of the final holocomplex. (Ishizuka, T., Kamiya, A., Narikawa, R., Inomata, K., and Ikeuchi, M., manuscript in preparation). This implies that yet unknown fast reaction underlies the initial chromophorylation to produce the intermediary photoactive complex and isomerization proceeds slowly afterwards to assemble the fully photoactive native holocomplex. The anomalous blue shift of PCB in the intermediary complex may suggest that the conjugated double bonds of PCB are strongly distorted when incorporated into the apoprotein. It may be further speculated that the distortion may drive the next isomerization step.

In this context, it is of note that a certain group of cyanobacteriochromes including TePixJ and SyPixJ1 possess a second Cys residue at the position of conserved Asp residue in phytochromes, which are connected to the three pyrrole rings *via* a specific water molecule (see the above section, "3D structure"). Recently, Lagarias and coworkers reported that another Pb/Pg-type cyanobacteriochrome Tlr0924 of *T. elongatus* requires this conserved Cys residue for assembly of its spectral properties.[51] When the mutant Tlr0924 lacking the Cys residue was expressed together with PCB, it assembled a red-absorbing photochemically inert biliprotein. Combined with theoretical calculation, they proposed that this Cys residue forms a second covalent linkage to the chromophore PCB possibly at C10 to form a blue-absorbing form. We also observed that a second Cys residue (Cys494) as well as the canonical Cys residue in Cys-His motif (Cys522) is essential for efficient assembly of the photoactive TePixJ complex (T. Ishizuka, R. Narikawa, and M. Ikeuchi, unpublished observation). The site-directed mutation of either Cys residue did not affect much the covalent chromophorylation in PCB-producing *E. coli* but abolished formation of the photoactive blue-shifted complex. In contrast, simultaneous mutation of both residues completely abolished the covalent binding. These observations may suggest

that the second Cys residue is also involved in assembly of the blue-shifted photoactive complex, although it is difficult to distinguish the second covalent binding in the native complex at the moment.

Other cyanobacteriochromes

AnPixJ

Many cyanobacterial genomes harbor *pixJ* homologs, although most culture strains seem to have lost cellular motility or phototaxis. PixJ homologs consist of a chromophore-binding GAF domain(s) and MCP domain, but the GAF domains of *Anabaena* and *Nostoc* PixJ are grouped into another clade distinct from that of TePixJ/SyPixJ1. The PixJ homolog of *Anabaena* sp. PCC 7120 (AnPixJ) possesses four contiguous GAF domains and the second one (AnPixJ_GAF2) has been experimentally proved to show reversible photoconversion between red absorbing form (λ_{max} = 648 nm, Pr[AnPixJ]) and green absorbing form (λ_{max} = 543 nm, Pg[AnPixJ]) upon irradiation with green and red light.[29] Acidic denaturation analysis revealed that Pr possesses *ZZZ*-PCB, while Pg possesses *ZZE*-PCB in configuration. *Anabaena* sp. PCC 7120 does not retain capability to develop motile hormogonia, whereas related *Anabaena variabilis* develops hormogonia (or motile trichomes), which exhibits phototaxis toward light of 590–670 nm.[52,53] The hormogonia glide along the multicellular filament toward or away from the light source. PixJ may be involved in switching of positive and negative phototaxis like in *Synechocystis*.

SyCcaS

Many cyanobacteria exhibit chromatic acclimation to modify their photosynthetic apparatus in response to changes in light quality. In *Synechocystis* sp. PCC 6803, which possesses phycocyanin but not phycoerythrin, the phycocyanin content is chromatically regulated. Namely, cells grown under green-orange light accumulate less phycocyanin than the cells grown under red light. The former conditions induce expression of a gene for a unique phycocyanin linker protein called CpcG2.[54] Mutational analysis revealed a putative photoreceptor CcaS and a cognate transcriptional regulator CcaR that is specifically required for induction of *cpcG2* gene.[21] CcaS has a putative chromophore-binding GAF domain and a flavin-binding PAS domain. Isolation of CcaS_GAF domain from *Synechocystis* and PCB-producing *E. coli* demonstrated that it shows reversible photoconversion between a red absorbing form (λ_{max} = 672 nm, Pr[SyCcaS]) and a green absorbing form (λ_{max} = 535 nm, Pg[SyCcaS]).[22] These features appear to be homologous to those of AnPixJ. However, the chromophore configuration was opposite: *ZZZ*-PCB for Pg[SyCcaS] and *ZZE*-PCB for Pr[SyCcaS] (Table 1). Such relationship between the chromophore configuration and absorption is very unusual and would be useful for thorough understanding of the tetrapyrrole-binding photoreceptors. Autophosphorylation of CcaS and phosphotransfer from CcaS to CcaR were biochemically demonstrated and the Pr form showed higher phosphorylation and phosphotransfer activities than the Pg form. This indicates that CcaS is the green light receptor.

FdRcaE

RcaE of *F. diplosiphon* has a GAF domain that is closely related to SyCcaS_GAF (Fig. 2). It binds a tetrapyrrole but spectral properties or photoresponsiveness has not yet been elucidated.[28] Genetic studies suggested that FdRcaE is the red light receptor to induce expression of phycocyanin operon *cpc2*,[55] in contrast with the green light-perception of SyCcaS. FdRcaE possesses domain architecture (GAF-PAS-His kinase), which is similar to SyCcaS (GAF-PAS-PAS-His kinase, see Fig. 1). Indeed, the GAF domain of FdRcaE is highly homologous to that of SyCcaS, whereas the PAS and His kinase domains are classified into groups that are distantly related to those of SyCcaS. These mosaic features might account for the difference in the active form between FdRcaE and SyCcaS, even if both GAF domains might assemble similar Pr and Pg forms. In *F. diplosiphon*, a second signaling pathway to recognize green light has been hypothesized for induction of genes or posttranslational regulation of phycoerythrin and its linker polypeptides,[55–57] which could be regulated by yet unknown cyanobacteriochrome-type photoreceptor. Current genome sequencing project would reveal the genetic background of the whole complementary chromatic acclimation process.

SyCikA

CikA that harbors a "chromophore-binding" GAF domain was initially reported to be essential for resetting the circadian rhythms in *Synechococcus elongatus* sp. PCC 7942.[18] However, it lacks the chromophore-anchoring Cys residue and the similarity to other CikA homologs is not so high. Consistently, CikA isolated from *Synechococcus* did not bind a linear tetrapyrrole chromophore, although *in vitro* reconstitution with excess tetrapyrroles allowed some covalent ligation.[19] Specific interaction of the C-terminal pseudo-receiver domain with a quinone is crucial for the phase resetting of the rhythms.[20] On the other hand, the GAF domain of CikA homolog (SyCikA_GAF) in *Synechocystis* sp. PCC 6803, which was expressed in *Synechocystis* or PCB-producing *E. coli*, exhibited the identical absorption spectrum peaking at 324 and 402 nm.[30] This violet absorbing form was irreversibly photoconverted to 565 nm-absorbing form, which was slowly dark-reverted to the violet absorbing form. These properties of SyCikA are very unusual but seem to be compatible with the nature of circadian rhythms.

Candidate cyanobacteriochromes

Cph2 of *Synechocystis* sp. PCC 6803 is a unique photoreceptor that possesses phytochrome-type GAF in the N-terminus and cyanobacteriochrome-type GAF in the C-terminus of a single protein (Fig. 1). *In vitro* reconstitution of the former GAF with PCB or PΦB formed phytochrome-like photoactive complex showing typical red and far-red reversible conversion, while the latter gave inefficient assembly of blue light absorbing complex.[58,59] Physiologically, *cph2* has been reported to be involved in light regulation of positive and negative phototaxis of *Synechocystis*.[60,61] A related gene *aphC*, which codes for a composite photoreceptor of phytochrome- and cyanobacteriochrome-type GAF, was proposed to regulate red light-inducible activation of adenylyl cyclase

CyaC in *Anabaena* sp. PCC 7120.[62] These cyanobacteriochrome-type GAF domains must be carefully studied by more direct methods such as isolation of the protein from cyanobacterial cells. For example, we observed full assembly of the cyanobacteriochrome TePixJ_GAF in *Synechocystis*, but only partial assembly in PCB-producing *E. coli.*

There are many candidates of cyanobacteriochromes in various cyanobacterial genomes (Fig. 2). They are grouped into several distinct clades and some of them seem to share several sequence motifs. We have expressed some of them (Slr1212 and Alr1966) in PCB-producing *E. coli* and found that they bind a tetrapyrrole and exhibit photoreversible conversion (Narikawa, R. and Ikeuchi, M., unpublished observation). More comprehensive studies on various cyanobacteriochromes with mutagenesis, spectrometry and crystallization will be needed to understand the whole view of the cyanobacteriochrome.

Two to seven tandem repeats of the cyanobacteriochrome GAF domains are popular in the PixJ homologs except for the single GAF in TePixJ. The Anabaena PixJ homolog AnPixJ has four cyanobacterial-type GAF domains: the first GAF does not possess the conserved Cys residue, while the other three possess the Cys residue. We have examined all of them (Fig. 1) by separate expression in *E. coli*, which co-expresses PCB.[29] The second GAF domain assembled fully photoactive holoprotein as mentioned above, while the first GAF domain did not bind any chromophore at all. The third and fourth ones bound PCB but did not show any photoconversion. Thus, it is obvious that the sequence prediction of the cyanobacteriochrome is useful but not sufficient and should be experimentally confirmed.

Evolution of phytochromes and cyanobacteriochromes

Phytochromes are widely distributed to eukaryotes (fungi, plants and algae), and bacteria (proteobacteria, cyanobacteria, Deinococci, and actinobacteria).[31,63] Generally, phytochromes consist of photoactive PAS-GAF-PHY composite domain with the figure-of-eight knot between the PAS and GAF. On the other hand, the simple GAF domain is sufficient for assembly of photoactive cyanobacteriochromes. As noted above, the knot is not needed and as a consequence the lasso sequence within GAF is absent in cyanobacteriochrome. Nonetheless, overall sequence of the GAF domain is significantly homologous between phytochromes and cyanobacteriochromes, although many other GAF domains in other categories have been detected in eukaryotes, bacteria and archaea. This is illustrated in Fig. 2, which only includes GAF domains of some representative phytochromes, cyanobacteriochromes and all the GAF domains of *Synechocystis.* The chromophore-binding GAF domains of phytochromes and cyanobacteriochromes belong to a GAF/PAS superfamily that has very ancient origin.[64,65] Perhaps, judging from the phylogenetic tree, phytochromes and cyanobacteriochrome share a single origin in the GAF/PAS superfamily. Since the cyanobacteriochromes have been found only in cyanobacteria, they may have been derived from phytochromes in cyanobacteria. Variation in the chromophore-binding site between bacteriophytochromes and other phytochromes also suggested the ancient origin of the BV-binding type.[7] Alternatively, simple GAF domain of the cyanobacteriochrome might be an ancestor of the composite the PAS-GAF-PHY composite domain of the phytochromes. In this case, an ancestral phytochrome gene was created from the cyanobacteriochrome GAF domain in cyanobacteria and then horizontally transferred to bacteria, fungi and algae. In either case, it is not clear at the moment why flexible cyanobacteriochromes, which cover wider range of wavelength than phytochromes, were not transferred to other organisms especially in algae and plants.

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