Chapter

Cyanobacterial Phytochromes in Optogenetics

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Abstract

Optogenetics initially used plant photoreceptors to monitor neural circuits, later it has expanded to include engineered plant photoreceptors. Recently photoreceptors from bacteria, algae and cyanobacteria have been used as an optogenetic tool. Bilin-based photoreceptors are common light-sensitive photoswitches in plants, algae, bacteria and cyanobacteria. Here we discuss the photoreceptors from cyanobacteria. Several new photoreceptors have been explored in cyanobacteria which are now proposed as cyanobacteriochrome. The domains in the cyanobacteriochrome, light-induced signaling transduction, photoconversion, are the most attractive features for the optogenetic system. The wider spectral feature of cyanobacteriochrome from UV to visible radiation makes it a light potential sensitive optogenetic tool. Besides, cyanobacterial phytochrome responses to yellow, orange and blue light have more application in optogenetics. This chapter summarizes the photoconversion, phototaxis, cell aggregation, cell signaling mediated by cyanobacteriochrome and cyanophytochrome. As there is a wide range of cyanobacteriochrome and its combination delivers a varied light-sensitive response. Besides coordination among cyanobacteriochromes in cell signaling reduces the engineering of photoreceptors for the optogenetic system.

Keywords: cyanobacteriochrome, cyanophytochrome, photoswitch, photoreceptor, cell signaling transduction

1. Introduction

Photoreceptors in cyanobacteria are diverse in their spectral character from ultraviolet to visible wavelength. Plant photoreceptors were widely used in optogenetics, but their responses to specific wavelengths need more revision. When compared to these photoreceptors cyanobacteriochromes (CBCRs) receive more attention as a versatile optogenetic tool. Several photoreceptors respond to a wide range of light, photoconversion ability and photoswitches for dual light are new approaches and powerful tools for optogenetics [1]. Engineering of these photoreceptors will develop more versatile CBCR to alleviate the conventional methods like mutation and recombination [2]. Optogenetics in mammalian tissue adopted far-red illumination and adjacent infra-red

radiance to visualize and activate responses in the cell. The CBCRs with linear tetrapyrrole is very sensitive to red and far-red light. Utilization of these infra-red sensitive and red light responsive CBCRs raised their application in optogenetics. So far phytochromobilin is used in mammalian cells recently cyanobacterial phytochrome 1 (CPH1) has been applied in mammalian cells proven its benefit in synthetic biology [3].

1.1 Cyanobacteria

Cyanobacteria are evolutionarily ancient phototrophic Gram-negative bacteria widely distributed in terrestrial, freshwater and marine environments. They are oxygenic photosynthesizers having major photosynthetic pigment chlorophyll-a and light-harvesting pigments phycobiliproteins. They survive in many extreme environments, such as hot and cold deserts, hot springs, and hypersaline environments [4].

1.2 Cyanobacteriochrome

Light is an important factor for their nutrition and growth, therefore, it has a multitude photosensory complex that responds to a wide array of illumination. Each chromophore is a response to a particular wavelength based on the incident light it changes the arrangement and composition of pigments in the photon capturing antenna. This rearrangement of pigments to the incident light is the process of complementary chromatic acclimation. Cyanobacteria possess phototaxis movements it means they can move towards or away from specific light. Photoreceptors in cyanobacteria are commonly referred to as CBCRs [5].

1.3 Phytochromes

Generally, Phytochromes are photoreceptors that have been found in plants, algae, and bacteria. These photoreceptors are broadly utilized in biosensors and optogenetics to screen and regulate diverse intracellular cycles like phosphorylation, gene activation, degradation of protein and change of calcium ions [6].

1.4 Phytochromes from cyanobacteria

Phytochromes are photochromic photoreceptors, generally responding to red and far-red radiation in the visible spectrum. Bilin is the most important portion in the chromophore and it is distributed in three different forms. Phytochrome in plants made of phytochromobilin, whereas in cyanobacteria it is in the form of Phycocyanobilin. Further Phytochromes in plants, algae and cyanobacteria constitute linear tetrapyrrole biliverdin [7]. The chromophore part in plant phytochrome has cysteine at the N terminal site of the protein. The phytochrome in plants differs from cyanobacteria by having biliverdin in the chromophore part. Evolutionary development in cyanobacteria brings out cysteine linked with biliverdin in the GAF domain and formed as phycocyanobilin also referred to as phytochromobilin. The transformation of phytochrome into CBCR is due to changes in the molecular level.

1.5 Phytochrome classification

Phytochromes were primarily arranged into three subfamilies dependent on the number of domains in their photosensory core module (PCM). Phytochrome has

three domains in their core structure, for example, PHY - phytochrome-explicit area, PAS - Per-Arnt-Sim, and GAF - cGMP phosphodiesterase-adenylate cyclase-FhlA. Even though the amino acid groupings of these domains have a dissimilar sequence, their structures were similar. Further subfamilies are cyanobacterial phytochromes (Cph), lack an N-terminal PAS area, and CBCRs, which contain a solitary GAF domain [8]. The domain proteins of PAS, GAF and PHY were interconnected to form homo and heterodimers [9].

1.6 Features of cyanobacteriochrome

Phytochrome in plants and algae has the sensitivity to the different light spectrum. Plant phytochromes are sensitive to red radiance furthermore, it performs red and far-red photoreversible photocycle. The phytochrome with bilin photoreceptors in eukaryotic green algae and prokaryotic cyanobacteria are sensitive to the visible spectrum [10–12].

The CBCRs are photoreceptors involved in the regulation of phototaxis. The photoreceptors SyCcaS, SyPixJ1, TePixJ, AnPixJ, SyCikA are now proposed to be CBCRs due to the presence of chromophore binding GAF domain.

- The domain GAF is enough for photoconversion
- chromophore in GAF domain varies from phytochrome GAF
- The GAF domain binds to linear tetrapyrrole pigments like phycoviolobilin or phycocyanobilin
- The chromes are responsive to a wide range of light from ultraviolet to the red region

2. CBCR in cyanobacteria

2.1 AnPixJ

The cyanobacterial genomes of Anabaena and Nostoc harbor *PixJ* homologs, having chromophore-linked GAF domains and domain MCP. The PixJ-GAF domains of *Anabaena* and *Nostoc* were distinct from the blue-shifted complex of CBCR TePixJ and CBCR SyPixJ1 [13]. The four GAF domains of PixJ are continuously arranged in AnPixJ of *Anabaena* sp. PCC 7120 (**Figure 1A**) that possess reversible photoconversion between red (648 nm) Pr[AnPixJ] to green (543 nm) absorbing form Pg (AnPixJ) [14]. Acidic denaturation of AnPixJ in *Anabaena* sp. PCC 7120 affected the gliding motility of hormogonia and phototaxis.

2.2 SyCcaS

Chromatic acclimation is an adaptive mechanism in some cyanobacteria capable of modifying their photosynthetic system reaction to the incident radiance. The phycocyanin content in *Synechocystis* sp. PCC 6803 is chromatically synchronized under red and green-orange light. The cells irradiated with red light produced a higher quantity of phycocyanin [15] than the cells exposed to green-orange light. The red

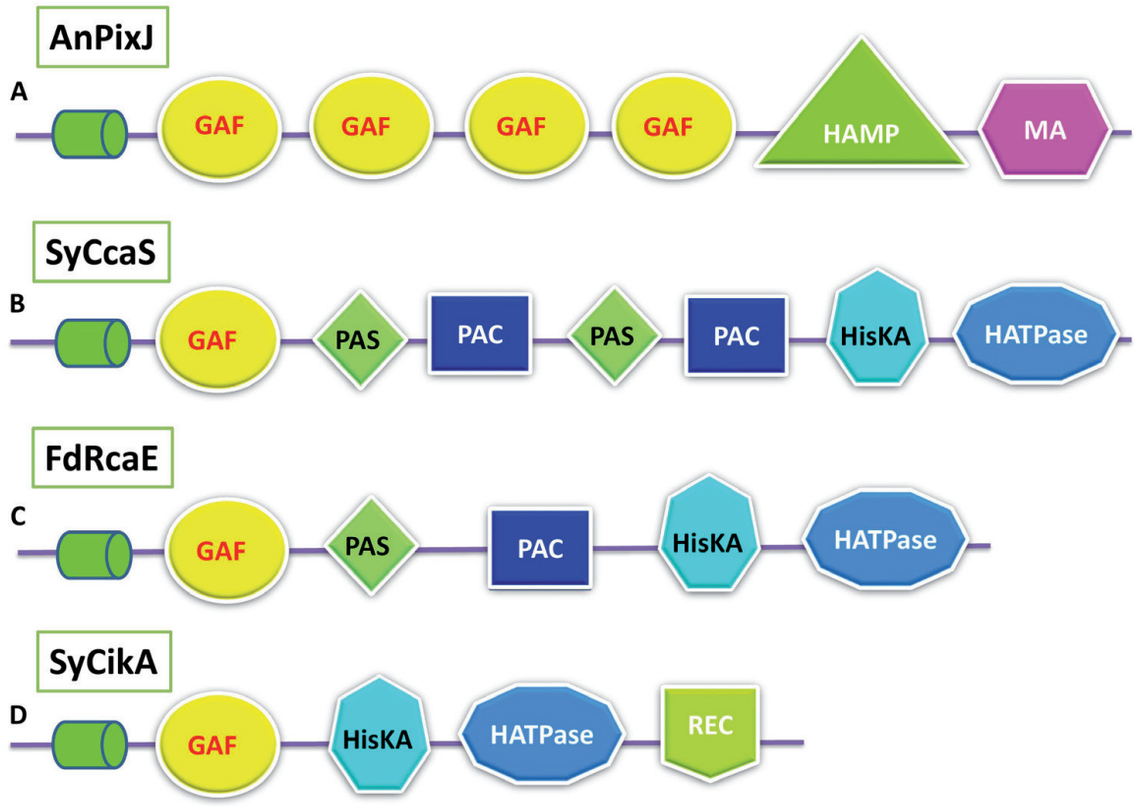

Figure 1.
Domain architecture of common cyanobacteriochromes (A) AnPixJ (B) SyCcaS (C) FdRcaE (D) SyCikA and their specific domains GAF with additional signaling domains are HAMP, methyl accepting chemotaxis protein (MA), PAS (PAS+ PAC- Photoswitchable adenyl cyclase), histidine kinase (HisKA+HATPase) and response regulator receiver domains (REC).

light condition activated the gene cpcG2 which encodes the synthesis of phycocyanin linker protein. Under red light CcaS, photoreceptor and transcriptional regulator CcaR induced the expression of the *cpcG2* gene [16]. It has a single GAF domain followed by PAS and PAC domains (**Figure 1B**).

2.3 FdRcaE

Fremyella diplosiphon harbor photoreceptor RcaE_GAF sector is homologous to SyCcaS_GAF. Genetic studies on FdRcaE revealed that it is a red light receptor, involved in the expression of operon *cpc2* encode synthesis of phycocyanin, [17] FdRcaE domain structure GAF, PAS and His kinase (**Figure 1C**), are parallel to SyCcaS (**Figure 1B**). Though the GAF domain is analogous to FdRcaE_ and SyCcaS, their light response is different in which the SyCcaS is a green light receptor. In *F. diplosiphon*, the green light has been used to activate genes for phycoerythrin post-translational modification and its linker polypeptides through the second signaling pathway by CBCR [18, 19]. The modern genome sequencing project would reveal the genetic background of the whole complementary chromatic acclimation process.

2.4 SyCikA

The chromophore-binding GAF domain of CikA in *Synechococcus elongatus sp. PCC* 7942, (**Figure 1D**) plays a crucial role in resetting the circadian rhythms [20].

Generally, the cyanobacterial chromophore is ligated with cysteine residue but it lacks the chromophore-tied Cys residue is parallel to other CikA homologs. Interconnection between the C-terminal pseudo-receiver domain and quinone is essential for the phase synchronizing of the rhythms [21]. CikA GAF domain of *Synechococcus* is comparable to the SyCikA_GAF of *Synechocystis* sp. PCC 6803. The properties of SyCikA are extremely uncommon however appear to be viable with the idea of circadian rhythms.

3. Functions of CBCR

3.1 Coordination of the cyanobacteriochromes

The photo biochemical properties of SesA holoprotein from the cyanobacterium *Thermosynechococcus vulcanus* have a blue light-responsive DGC (Diguanylate cyclase) activity. The SesB holoprotein isolated from *T. vulcanus* exhibited a reversible photoconversion system. It becomes blue light (417 nm) capturing form to a teal light (498 nm) assimilator. Another homologous CBCR from *T. vulcanus* is SesC which photoconverts a blue light (415 nm) assimilator to a green light (522 nm) absorber. These three CBCR proteins (SesA, SesB, and SesC) have phycoviobilin (PVB) and phycocyanobilin (PCB). These CBCR proteins were genetically expressed in *E. coli* which contains both PVB and phycocyanobilin [22, 23]. The SesA and SesB, perform independent photo conversion in *E. coli* in contrast when it is expressed in cyanobacteria it shows single photoconversion (**Figure 2**). Even though their spectral wavelength is different they coordinate and expressed single photocycle conversion.

SesB has GGDEF- type DGC (Diguanylate cyclase) domain (**Figure 3B**) and SesC has EAL- type PDE domain to deliver the c-di-GMP signal (**Figure 3C**). The SesB

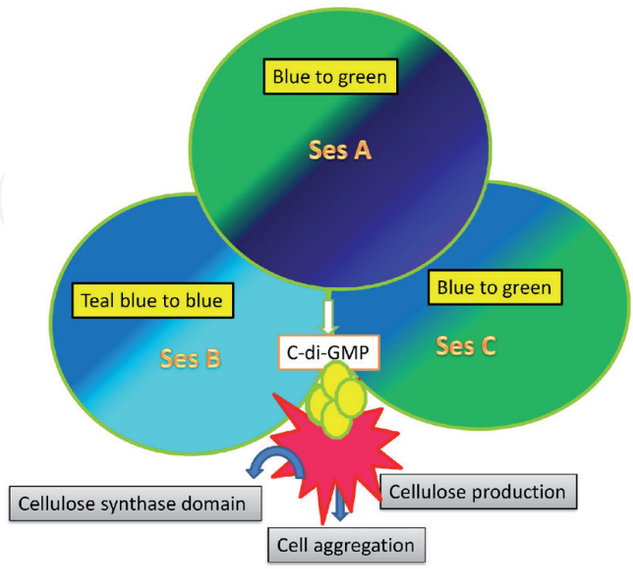

Figure 2.
Three different CBCR individually expressed to reveal different c-di-GMP signals. Ses A-produces c-di-GMP under blue light, Ses B- degrades c-di-GMP under teal light, Ses C- produces c-di-GMP under shorter wavelength and degraded c-di-GMP at the longer wavelength. These CBCR were coexpresses in Thermosynechococcus revealed c-di-GMP signal binds with cellulose synthase domain and promoted cell aggregation.

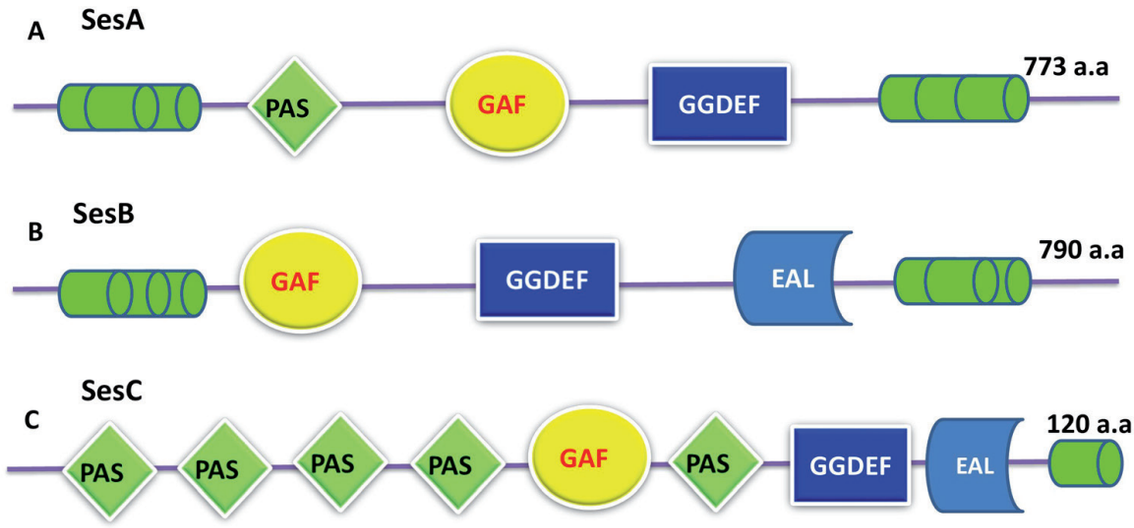

Figure 3.
Domain architecture of cyanobacteriochromes Ses A, Ses B and Ses C-GAF photosensitive domain and cell signaling domain PAS (Per/Arnt/Sim), GGDEF, EAL capped with a.a-amino acids.

DGC for c-di-GMP signal degraded under teal light, in contrast, expressed higher in blue light. In Ses, A c-di-GMP is higher under blue light and lowered in teal blue light. SesC DGF activity is maximum in blue light and minimum in green light. This is a chrome-responsive cyanobacterial c-di-GMP signaling coordination of (Ses –A, B and C) CBCRs.

i. SesA a blue light-responsive DGC

ii. SesB a teal light-responsive and GTP sensitive PDE

iii. SesC, a dual-active CBCR having blue light-sensitive DGC and green light-responsive PDE activity

3.2 Cyanobacteriochrome in cell aggregation

The cell aggregation signaling molecule Cyclic dimeric guanosine monophosphate (c-di-GMP) is unique to cyanobacteria and bacteria [24]. Light is a key factor in controlling c-di-GMP signaling [25, 26]. The domain (GGDEF) for the synthesis and (EAL/HD-GYP) (**Figure 3A** and **B**) destruction of the c-di-GMP is higher in the CBCR GAF structure of freshwater cyanobacterial genomes. The CBCR induces the c-di-GMP signaling pathway. The CBCR—GAF domain of SesA (**Figure 3A**) from the thermophilic cyanobacterium *Thermosynechococcus elongatus* is activated by blue light irradiation, and disordering of *T. vulcanus* SesA inhibited cell aggregation.

Thermosyncechococcus spp., genomes possess five CBCR genes, three homologous CBCRs involved in the clumping of cyanobacterial cells are SesA (Tlr0924), SesB (Tlr1999), and SesC (Tlrtml). This CBCR has a photosensory domain with a c-di-GMP protein production/destruction domain. The CBCR-GAF domain of these three CBCR is involved in the light-controlled cell accumulation. There is a coordinated system of cell accumulation by c-di-GMP signaling via, Ses (A, B and C) CBCR (**Figure 2**) [27, 28].

3.3 Cyanobacterial photobiological responses

Prokaryotic photosynthetic organisms, cyanobacteria, depend on bilin-linked phytochromes (Cphs) and CBCRs, photoreceptors which are structurally and functionally vary from plant photoreceptors. The CBCRs are made of light-absorbing domains with various color-tuning and signal transmission processes, that make cyanobacteria capture a wide wavelength of light from UV–visible to far-red lights. The genome of filamentous cyanobacteria has a different type of CBCRs with wide chromophore-linked selectivity and photocycle protochromicity. The Cph lineage can absorb a wide range of light from blue-violet to yellow-orange light. This chapter also emphasized the color-sensitive diversity [29, 30] and signal transmission process of Cphs and CBCRs, concerning optogenetic.

Bilin-linked phytochrome Cphs and plant phytochromes (Phys) are similar in structure, with an N-terminal photosensory core module (PCM) and a C-terminal output regulatory module. The PCM contains the following domains PAS (Period/Arnt/Single-minded), GAF(C-GMP phosphodiesterase/Adenylylcyclase/FhlA), and PHY (phytochrome-specific). The GAF domain is necessary for forming the bilin cross-linking; PAS and PHY structures are involved in bilin lyase activity [31]. Cyanobacteria have two types of bilin-linked photoreceptors Cphs, and CBCRs. In contrast to Cphs with PAS and PHY domain, CBCRs (lack PAS and PHY) absorb a wide array of light, by the GAF structure [32]. This wide array of light absorption by CBCR is called a color or spectral tuning mechanism.

3.4 CBCR in photobiological responses

Growth of the cyanobacterium *Synechocystis* PCC 6803 in red (R) and far red (FR) light is regulated by Cph1 and Cph2 in an antagonistic method. Modification in Cph1 negatively affects the *Synechocystis* growth in FR light, further destruction of Cph2 hinders its growth in red light [33]. Mutation in Cph2 transformed the growth rate and exopolysaccharide biofilm formation, involved in the control of the principal energy metabolism [34]. Under unusual light environments, the bilin conformation of the cyanobacterial antenna with light-absorbing phycobilisomes rearrangement is known as chromatic acclimation (CA). This process allows cyanobacteria to neutralize the proportion of light absorption between the photosystems [35, 36].

3.5 Dual light system

The CBCR response to two different light systems is mediated by the histidine (His) kinase domain. In *Leptolyngbya* sp. JSC-1, His domain is found in the proteins of Cph, RfpA, whereas CcaS in *Synechocystis* and *Nostoc punctiforme*, RcaE and DpxA in *Fremyella diplosiphon*, act as sensor kinase [35].

3.6 Phototaxis

The non-flagellated cyanobacteria adapt phototaxis in response to light. In *Synechocystis*, move towards light [37] and away from light [38] phototaxes are achieved by PixJ and UirS CBCRs. The CBCR- PixJ-GAF domain in *Synechococcus elongatus*, can respond to the direction of illumination by wavelengths that induce both progressive and refusal phototactic movements [39]. Other similar CBCR viz., SyPixJ [37], TePixJ [40], and AnPixJ [41] are commonly involved in phototaxis.

3.7 Photoinhibition

In some cyanobacteria, photoinhibition light conditions trigger the synthesis of photoprotective pigments. For example, intense radiation or UV radiation, accumulate mycosporine-like amino acids and scytonemins [30, 42]. Cyanobacteria, like *Nodularia* sp., *Euhalothece* sp. *Microcoleus* sp., and *Scytonema hofmanii* [43, 44] possess bilin photoreceptors, Cphs and CBCRs. These photosensitive receptors facilitate photobiological reactions by sensing and delivering signaling compounds.

3.8 Circadian clock

Cyanobacteria are responsive to diurnal photoperiods by adjusting their photosynthesis and respiration. In *S. elongatus* PCC 7942, the circadian clock controls the genes using promoters in light and dark conditions. Control of promoters is time-dependent, which sequentially maintains energy metabolism, cell division, and chromosome structure. Some CBCR domain (KaiABC), CikA (circadian input kinase A) and PsR in the *S. elongatus* oscillator become natural sensors that identify the change from light to dark by detecting the redox condition of the quinone pool [45].

3.9 Biofilm

Cyanobacteria form biofilms, which favor attachment on a surface to grow and produce extracellular polymers. This biofilm development in *Thermosynechococcus* is intervened by the cyclic diguanosine monophosphate (c-di-GMP) a bacterial secondary messenger [46]. Three CBCRs, SesA, B and C, in the blue/green light (ON/OFF) - c-di-GMP switch control non-motile and motile in planktonic networks [26, 47].

4. Photosensitive features of CBCR

4.1 Color sensing by Cphs and CBCRS

Cyanobacterial proteins contain the accompanying regions PAS-GAF-PHY [48]. Entire genome sequencing of cyanobacterial species, for example, *Microcoleus* IPAS B373 [49], *Euhalothece* Z-M001 [44], and *Tolypothrix* PCC7910 [50] are devoid of gene HY2, for phytochromobilin (PΦB) synthase. Further, these cyanobacteria have pcyA gene that encodes phycocyanobilin (PCB): ferredoxin oxidoreductase that catalyzes the conversion of biliverdin (BV) to PCB, a significant cofactor of Cphs and CBCRs [32, 51]. The quantity of Cphs and CBCRs differ among cyanobacteria, *Euhalothece* has 3 numbers, *Synechocystis* (8), *Microcoleus* IPAS B353 (9), *Acaryochloris marina* (12), *N. punctiforme* (18), and *Tolypothrix* PCC 7910 (36). In cyanobacterium, the complete number of bilin photoreceptors relies upon the size of its genome [49]. Besides, CBCRs are more plentiful in cyanobacteria, than Cphs, and the proportion of CBCRs for blue to red is corresponding to the environmental light conditions. For example, *Microcoleus* IPAS B353 grown in UV light developed only violet CBCRs than red/green and green/red CBCRs. Generally, UV light is recommended to develop and improve the quantity of short wavelength responsive CBCR [49].

4.2 Dual cays residues in CBCR for dual photocycle

Some CBCRs with exceptionally unchanged DXCF motif or the feebly rationed CXXR/K motif have extra Cys amino acids in the insertion loop (embed - Cys) via second thioether bond at the C10 atom under dark phase [52]. This sort of double Cys CBCRs, with a second thioether bond, is fragile and light-labile. These CBCRs are extremely responsive to capture violet or blue light in dark phase but it absorbs green, yellow, orange or greenish-blue light in the light phase. The cyanobacterial CBCRs are primarily linked to PCB yet some may link to phycoviolobilin (PVB) like Cphs [53, 54]. The change of PCB into PVB is unique to the DXCF-CBCRs subfamily [22]. The color tuning systems of CBCRs for far-red to orange (Fr/O) remain unidentified [55].

4.3 Signal transmission by CBCR

Cyanobacterial photoreceptors associated with signal transmission through phosphor transfer or c-di-GMP. Phosphorelay is a signal transmission process engaged with the autophosphorylation of His amino acid residue by His kinases, continued by phosphotransfer in association with reaction controllers. A film bound His kinase CBCR-UirS in *Synechocystis* accompanied with the reaction controller AraC family and UirR roles as a UV absorbing two-segment signaling framework [38]. Signaling in the chromophorylation process is regulated by the cystathionine beta-synthase (CBS) in the N-terminal of SesA. This in SesA can bind to ATP, ADP, and AMP which regulate the signaling process in chromophorylation.

4.4 Autolyase and autoisomerase in CBCR

Cyanobacterial photoreceptors are also called CBCRs that are similar to phytochromes [56]. PixJ GAF, from a thermophilic cyanobacterium *Thermosynechococcus elongatus*, regulates phototaxis. The BP-1 bacterial photoreceptors (TePixJ_GAF) reveal reversible photoconversion between a blue light (433 nm) capturer and a green light (531 nm) capturer. TePixJ GAF chromoprotein expressed in *Synechocystis* was denatured using acidic urea (8 M urea/HCl, pH 2.0) and it was compared with the cyanobacterial phytochrome Cphl having chromophore phycocyanobilin (PCB). The PCB is not a chromophore part in TePixJ, but PCB is a part of its isomer, phycoviolobilin (PVB). It confers the autolyase and autoisomerase property of GAF in TePixJ.

The primary CBCR for the phototaxis controller was recognized as PixJ. The CBCR SyPixJl of *Synechocystis* sp. PCC 6803 and TePixJ of *Thermosynechococcus elongatus* BP-1 showed selective reverse photo transfiguration between blue absorber (425-435 nm) Pb to green (531–535 nm) absorber Pg [57, 58]. Genetic modification in the pixJ of SypixJl and SypixD lost progressive phototaxis, these CBCR in original structure perceive blue light and characterize the order of motility as a regulatory switch [59]. The anticipated secondary arrangement of SyPixJl has N-terminal transmembrane helices, two successive GAF domains and a C-terminal methyl-accepting structure [5]. Proteolytic destruction and mass spectrometric investigation of SyPixJ 1_GAF and TePixJ_GAF showed that a straight tetrapyrrole was covalently bound to a peptide connected with phytochrome, a moderated Cys-His motif [5].

At the point when His6-TePixJ_GAF was digested with acidic urea in the dark phase, the Pb peak (433 nm) was changed from native form to a peak at 594 nm with a shoulder at 565 nm. The PVB in TePixJ_GAF captures a shorter wavelength of light

than PCB. In any case, it ought to be noticed that the Pb absorb at 433 nm in native form is extraordinarily smaller than the urea-denatured PVB absorb at 594 nm. PVB is an isomer of PCB with a similar atomic mass, yet conjugated double bonds are detached at the C5 position. PVB with the apoprotein is accountable for the extraordinary blue capturing structure Pb and photoreversible modifications. The PCB transformation to PVB is because of the PecE and PecF proteins which are fundamental for ligation and isomerization.

5. Color-tuning mechanisms of cyanobacteriochromes

The term cyanobacteriochrome was first reported in 2004 by Dr. Ikeuchi in his paper about photoreceptor SypixJ1 [60]. This photoreceptor covalently binds a linear tetrapyrrole chromophore and performed reversible photoconversion between a blue-absorbing form (Pb) and a green-absorbing form (Pg). This protein has a cGMP-phosphodiesterase/adenylate cyclase/FhlA (GAF) similar to those of the Phytochromes. The GAF in cyanobacterial signals transduction proteins is identified as CBCRs. The GAF s of cyanobacteria are covalently linked to tetrapyrrole chromophore. This chromophore can sense different light (UV–visible spectra). In some cyanobacteria, this GAF regulate phototactic motility, chromatic acclimation and light-dependent cell aggregation.

5.1 CBCR structure and diversity

In CBCRs, the GAF is essential for chromophore incorporation and photoconversion [61] CBCRs have N and C terminals in which GAF s are located at the N-terminus. Signal output s viz. (HisKA + HATPase_c), Methyl-accepting (MA), GGDEF, and EAL s located at the C- terminus. His kinase is frequently detected as signal output s.

5.2 Chromophore variation in color-tuning mechanism

Generally, four types of linear tetrapyrrole chromophores, phycocyanobilin (PCB), biliverdin (BV), phytochromobilin (PFB) and phycoviolobilin (PVB), have been identified in the CBCR GAF domain. The mixture of these chromophores in the GAF domain results in broad and diverse spectral features [54, 62]. These chromophores are arranged in an order from a longer wavelength absorbing system to a shorter wavelength absorber (BV > PFB > PCB > PVB). The longer wavelength of light is absorbed by the longer length chromophores. Sometimes, the PCB isomerizes to PVB at the GAF region. This sort of PVB linked GAF area in CBCR has been distinguished from the cyanobacterium *Acaryochloris marina* [54]. The chromophore binding species should possess a UV-to-blue absorber.

The absorption of the Cys-free form is highly affected by the linked species of chromophores. The chromophore PCB in AM1_1186g2 revealed a reversible photoconversion between a Cys-free red-absorber (Pr) and a Cys-dependent blue absorber Pb [63], while covalently linked PVB in TePixJg showed reversible photoconversion between a Cys-free Pg absorber to a Cys-linked Pb absorber [62, 64]. The Cys-linked Pb absorber in the CBCR GAF s is the same as the blue to green reversible, but the Cys-free teal-absorber (Pt) is often shifted to blue absorber in association with

the typical green absorber Pg. A twist in the D ring of the conserved Phe residues in the Pt absorber contributes to the blue-shift [65, 66]. The conversion of the Pt form into the typical red-shifted Pg form is mediated by the loss of these Phe residues [66]. Likewise in the XRG lineage, CBCR GAF s have red to green reversible photoconversion. Blue-shift of the Pg form to red absorbing Pr caused a small twist in the A and D ring [67]. This photocycle is mediated by the proton donor and the acceptor is Glu amino acid.

5.3 Dark reversion

The two distinctive light-harvesting types of the CBCR GAF domain are generally constant under the dark phase, thus these CBCR GAFs can detect the proportion of two wavelengths. Further, in some CBCR GAF showed unidirectional photoconversion and rapid dark reversion. This can identify the concentration of certain colors of light [68]. Some XRG CBCR GAF s are unidirectional photoconversion from Pr-to-Pg in dark conditions, and after 4–25 s it rapidly undergoes dark reversion from Pg-to-Pr [68]. The kinetics of these GAF s of the dark reversion is highly dependent on higher temperature [53, 69, 70]. Light and temperature were indulged in the regulation of these GAFs. These characteristics may be physiologically relevant to sense light intensity for efficient photosynthesis because the same light intensity with lower temperatures severely inhibited photosynthesis.

5.4 Engineering

Several CBCRs have been designed to change the color-tuning interaction and output activity. Inclusion of the second Cys residue and modifications of PCB-binding in the GAF s caused reversible photoconversion from red/green into blue/green. This is due to the isomerization of PCB to PVB by the incorporation of the second Cys residue which attaches to the chromophore in the reversible form [70, 71]. The twisted geometry of the D ring can also be removed [66]. The output activity of the native GAF was modified with other lineage s by adenylate cyclases [72–74] that respond to various light. Changing the length of the CBCR GAF linker region in CcaS and HisKA turns the light receptiveness of the green to red lineage [75].

6. Conclusions

Optogenetics, is a new branch of synthetic biology, is generally defined as the engineering of particular light-induced cellular reactions. This study was initiated with light-sensitive Phytochromes and bacteriochromes experimented in bacteria and mammalian neurons. Recently this research field is sensational due to the CBCRs from cyanobacteria are widely used as signaling components. These CBCRs in optogenetic systems performed the regulation of cellular responses spatially and temporally by precisely applying and removing light. A cyanobacterial photoreceptor-based optogenetic system was implemented to study the protein interaction and cell signaling in cyanobacteria, bacteria and mammalian cells. The application of CBCRs in optogenetic systems extends their usage in developing potential new therapeutics. Smaller size photosensory regions and autocatalytic activity of CBCRs are more advantageous than other photoreceptors.

Acknowledgements

The author S. Sivasankari thanks the DST-Government of India for financial support under the grant number DST/INSPIRE FELLOWSHIP/2014/IF140666.

Conflicts of interest

The author declares that there is no conflict of interest.

Abbreviation

CBCRs	Cyanobacteriochromes
CPH1	Cyanobacterial phytochrome 1
PCM	Photosensory core module
PAS	Per-ARNT-Sim
GAF	cGMP phosphodiesterase-adenylate cyclase-FhlA
PHY	Phytochrome-specific
Cph	Cyanobacterial Phytochromes
DGC	Diguanylate cyclase)
Phys	Phytochromes
UV	Ultraviolet
nm	Nanometer
Cys	Cysteine
PCB	Phycocyanobilin
PVB	Phycoviobilin
(PΦB)	Phytochromobilin
PFB	Phytochromobilin
Glu	Glutamine
Phe	Phenylalanine
BV	Biliverdin
MA	Methyl acceptor
ATP	Adenosine triphosphate
ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
CBS	Cystathionine beta-synthase
His	Histidine

DOI: http://dx.doi.org/10.5772/intechopen.97522

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