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REVIEWS

Bacterial Photosensory Proteins: Regulatory Functions and Optogenetic Applications

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Abstract—Three classes of light-sensory regulatory proteins, which have been identified in genomes of numerous phototrophic and nonphotosynthetic bacteria, are discussed: the UVA/blue light sensitive BLUF and LOV domain-containing proteins and red/far-red light-sensitive phytochromes. Light perception by these chromoproteins is provided by the flavin or bilin (in phytochromes) chromophores binding to their photosensory domains. Bacterial photoreceptors also contain a variety of effector domains with enzymatic DNA-binding and other functions, which compose modular light-switchable systems. In recent years, progress was achieved in uncovering the photoactivation mechanisms of such systems. Based on the chromophore phototransformation-induced changes in the domain structures, these mechanisms cause the biochemical signal cascades which can control the light-dependent physiological responses of the cells. The new information obtained is important not only for understanding the fundamental mechanisms of light perception and signal transduction by bacterial photosensory proteins but also as a basis for designing photo-switchable enzymes and light-inducible gene expression systems, which may be used in optogenetics, a new field in cell biology and biotechnology. The presents review is focused on the structural aspects of signal transduction in light-activated bacterial photoreceptors, on their regulatory functions, and on some recent advances in using LOV and BLUF photosensors in optogenetics for the regulation of biological processes.

Keywords: bacterial phytochromes, LOV- and BLUF-photoreceptors, signal transduction, regulation, optogenetics

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Light is an important adaptation stimulus, and living organisms adapt their metabolism to the environmental illumination conditions by perceiving light signals and responding to them physiologically. Detection of light signals occurs by means of chromoprotein photoreceptors, which transform them into biochemical signaling cascades to induce physiological cell responses. Photon sensors of photoreceptor proteins are chromophore molecules capable of undergoing phototransformation, which induces structural changes in the photosensor domain and subsequent signal transduction to effector domains of the photoreceptor or associated proteins and modulates their activity.

Plants with the best-developed light reception systems responsible for a variety of photoresponses possess several types of regulatory photoreceptors, whose spectral sensitivity enables them to cover all ranges of the optical spectrum. Their number includes phytochrome sensors of red (R)/far red (FR) light (600–750 nm) [1], cryptochrome [2] and phototropin [3], receptors of UVA/blue light (320–500 nm), as well as UVR8 protein sensor of UVB photons (290–320 nm) [4]. While phytochromes were discovered over 50 years ago, it was only in the recent decade that the blue and UV light sensors could be identified and characterized at the molecular level thanks to the development of novel techniques based on isolating and expressing the genes that encode these photoreceptor proteins.

At present, using genomic identification, homologs of plant photoreceptors have been found and studied in a wide range of species, including numerous prokaryotes (e.g., cyanobacteria, phototrophic, and nonphotosynthetic bacteria) [5–8] and fungi [9, 10]. In prokaryotes, three major types of regulatory photoreceptors have been identified: phytochromes, which contain a conserved photosensory module covalently binding the linear tetrapyrrole bilin chromophores [5]; phototropin-like receptor proteins with a highly conserved LOV domain (Light, Oxygen, Voltage) binding a flavin chromophore (FMN); and proteins with a BLUF domain (Blue Light sensing Using FAD). Photoactivated adenylyl cyclase (PAC), the first of the sensors with a BLUF domain, was described in *Euglena gracilis*) [11].

Analysis of a large number of prokaryotic genome sequences showed that a considerable portion of bacteria from different phylogenetic groups possess the

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genes encoding photoreceptor proteins of all the three types. A number of bacteria carry predominantly phytochrome and LOV-domain genes, while archaea and some bacterial species have only LOV-protein-encoding genes [12]. In prokaryotic photoreceptors, chromophore-containing photosensor modules are closely associated with various effector domains, including those whose functional activity can change as a consequence of light-induced chromophore transformations (e.g., histidine kinases, cyclases, DNA-binding domains, etc.) [12]. Importantly, bacterial sensors exhibit the same bilin and flavin chromophore reactions that were described in the corresponding plant photoreceptors. These observations support the hypothesis proposed as early as the 1970s suggesting that the ability of living beings to react to light is an ancient universal trait associated with the emergence and development of photoreceptors, which retain a close genetic relationship even among evolutionary remote groups of organisms [13].

In recent years, there has been significant progress in the understanding of the mechanisms underlying light perception and signal transduction by bacterial photoreceptors, including identification and investigation of the light-dependent biochemical and physiological processes they mediate. In this review, we analyze the fundamental aspects of the functioning of bacterial photoreceptors and also discuss some recent achievements in the application of LOV and BLUF photosensors in optogenetics, a new field of cell biology and biotechnology.

PHYTOCHROME R/FR LIGHT PHOTORECEPTORS

Phytochromes are bile protein photoreceptors initially described in higher plants, where they regulate photomorphogenesis and a number of other R/FR light-dependent biological processes [1]. It was long believed that phytochromes were exclusively plant photoreceptors. However, the discovery of phytochrome homologs in prokaryotes, including nonphotosynthetic bacteria, and in fungi, disproved this hypothesis and confirmed earlier data suggesting that phytochromes might be present in microorganisms [14].

The principle of phytochrome action is based on the photoconversion between two mutually convertible forms absorbing red and far red light (Pr and Pfr). Pr–Pfr conversion is associated with a reversible Z–E photoizomerization of the 15/16 double bond of bilin chromophores [15]. The exact nature of chromophores varies among different phytochrome subfamilies: phytochromes of plants and cyanobacteria contain phytochromobilin (PΘB) and phycocyanobilin (PCB), while phytochromes of anoxygenic phototrophic and nonphotosynthetic bacteria, as well as fungal ones, contain biliverdin IXα (BV). Bilin chromophores are located in the conserved photosensory module comprising PAS (PER, ARNT, SIM), GAF (cGMP phosphodiesterase, adenylate cyclase, FhlA), and PHY (phytochrome-specific) domains (Fig. 1a); they are immersed within the GAF domain and form a covalent bond with a conserved cysteine residue located either in the same domain (PΘB/PCB chromophores), or in the PAS domain (BV chromophores) [15].

Data from comprehensive spectroscopic studies and newly obtained crystal structures of the bacteriophytochrome photosensory modules provided detailed information on the conformational changes occurring in bilin chromophores during photoisomerization, as well as on the dynamical structural changes in chromophore–protein interactions during Pr–Pfr photoconversion [16–20]. Changes in the protein conformation are caused by the reorganization of certain conserved amino acid residues interacting with the chromophore as a result of photoizomerization of the latter [19]. These structural changes provide for transduction of a light-induced signal from the phytochrome sensory module to the C-terminal domain responsible for the signaling activity of photoreceptors [21]. In many bacterial phytochromes, the photosensory module is combined with different effector domains, most commonly with a histidine kinase (H-kinase) or a GGDEF/EAL domain (Fig. 1a); when activated, effector domains enable transduction of light signals to biochemical signaling cascades [6, 12].

The first phytochrome-like photoreceptors found in prokaryotes were RcaE from *Calothrix* sp. PCC 7601 and PlpA from *Synechocystis* sp. PCC 6803, which control chromatic adaptation in these cyanobacteria by means of a bilin chromophore bound to a conserved cysteine residue [22]. However, although these biliproteins possess confirmed photobiological activity, they do not exhibit the photochemical properties and the R/FR photoconversion typical of canonical phytochromes. The first prokaryotic phytochrome gene identified based on its homology to plant phytochrome genes was found in the *Synechocystis* genome. In contrast to PlpA, this cyanobacterial phytochrome, Cph1 (Fig. 1a), undergoes classical R/FR photoconversion in vitro, exhibits autocatalytic binding of a PCB chromophore (in the recombinant form), and contains an H-kinase motif; i.e., it is a light-regulated kinase biliprotein that can transfer a phosphate group onto a response regulator (RR) Rcp1 (Fig. 2). Two other canonical phytochromes containing H-kinase effector domains, CphA and CphB, were identified in *Calothrix* (Fig. 1a). They also undergo ATP- and light-dependent autophosphorylation with subsequent phosphate transfer onto the corresponding RRs: RcpA and RcpB (Fig. 2) [23].

Thus, in these three phytochromes, light signal transduction is enabled by means of a two-component signaling system comprising a photosensory module and an effector kinase domain coupled to a RR

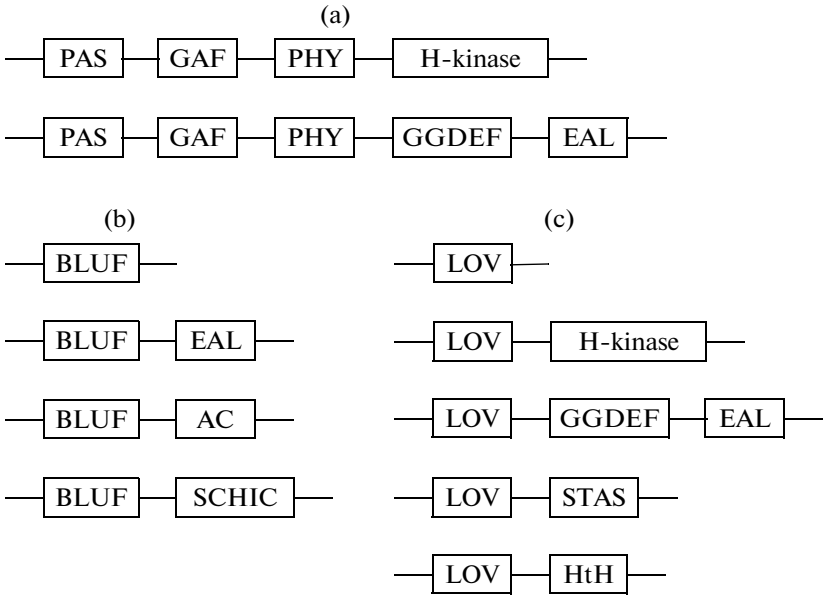


Fig. 1. Simplified domain structures of some functionally active bacterial photoreceptor proteins: phytochromes (a), BLUF photoreceptors (b), and LOV photorecetors (c). From top to bottom: (a) *Synechocystis* sp. (Cph2) and *Calothrix* sp. (CphA, CphB); *Synechocystis* sp. (Cph2) and *Rhodobacter sphaeroides* (BphG1); (b) *Synechocystis* sp. (PixD) and *Rhodopseudomonas palustris* (PapB); *Klebsiella pneumonia* (BlrP1) and *Escherichia coli* (YcgF); *Beggiatoa* sp. (bPAC/BlaC); *R. sphaeroides* (AppA); (c) *Pseudomonas putida* (PpSB1); *Caulobacter crescentus* (LovK), *Brucella abortus* (LOV-HK) and *P. syringae; Synechococcus elongatus; Bacillus subtilis* (YtvA); *Erythrobacter litoralis* (EL222). Conventional protein designations are given in parentheses. PAS–GAF–PHY-, BLUF-, and LOV- are photosensor domains linked to effector domains: H-kinase, histidine kinase domain; GGDEF/EAL, domains acting as diguanylate cyclase and phosphodiesterase in c-di-GMP synthesis and hydrolysis; AC, adenylate cyclase domain; SCHIC, Sensor Containing Heme Instead of Cobalamin; STAS, sulfate transporter/antisigma-factor antagonist; HtH, helix–turn–helix DNA-binding regulatory protein [6, 12, 33].

(Fig. 2). The kinase performs two functions: autophosphorylation of the conserved histidine residue and transphosphorylation activity involving subsequent phosphate transfer onto RR. Cyanobacteria possess several types of such regulatory proteins, including DNA- and protein-binding RRs that can potentially regulate the cell physiology [6].

Further bacterial phytochromes identified in *Synechocystis* (Cph2) and in an anoxygenic phototroph *Rhodobacter spaeroides* (BphG1) contain not H-kinase but GGDEF/EAL effector domains (Fig. 1a). These domains act as diguanylate cyclases/phospodiesterases in the transformation cycle involving cyclic dimeric guanosine monophosphate (c-di-GMP), a bacterial secondary messenger participating in complex physiological processes that underlie virulence, biofilm formation, etc. Interestingly, in vitro BphG1 exhibited R/FR reversible photochrome activity, while the construct containing only the GGDEF domain possessed light-dependent diguanylate cyclase activity [24]. BphG1 is the first bacterial phytochrome that was been shown to possess light-induced enzymatic activity not associated with the H-kinase phosphorylation cascade.

As apparent from the above data, R/FR photoreversible spectral characteristics and the processes of light-dependent regulation of enzymatic activity, especially H-kinase activity enabling the two-step light signal transduction via the auto- and transphosphorylation steps, have been studied in vitro for a number of bacterial phytochromes. However, currently available information concerning the involvement of canonical bacterial phytochromes in light-dependent physiological cell responses is scarce [6, 7, 25]. In particular, two R/FR-reversible effects were observed in *Candida guilliermondii* yeasts: photostimulation of cell growth and enhanced resistance to UVC radiation (254 nm) [14, 26]. The hypothesis implicating the phytochrome photoreceptor in the mediation of these effects is confirmed by the differential cell absorption spectrum (FR-minus-R light) with the maxima typical of plant phytochromes. Subsequent experiments showed that R light with a maximum at 680 nm induced photoreactivation (PR680) and photoprotection (PP680) of yeast cells subjected to UVB irradiation (290–320 nm) [27, 28]. These observations suggested that PR680 and PP680 phenomena are based on a common light-regulated mechanism of protection and reparation that is essentially different from the known mechanisms of enzymatic photoreactivation and photoprotection [29]. Due to the putative photoprotective role that phytochrome-like photoreceptors were found to play in yeasts, it seems interesting to investigate the photoprotective function of phytochromes identified in bacteria.

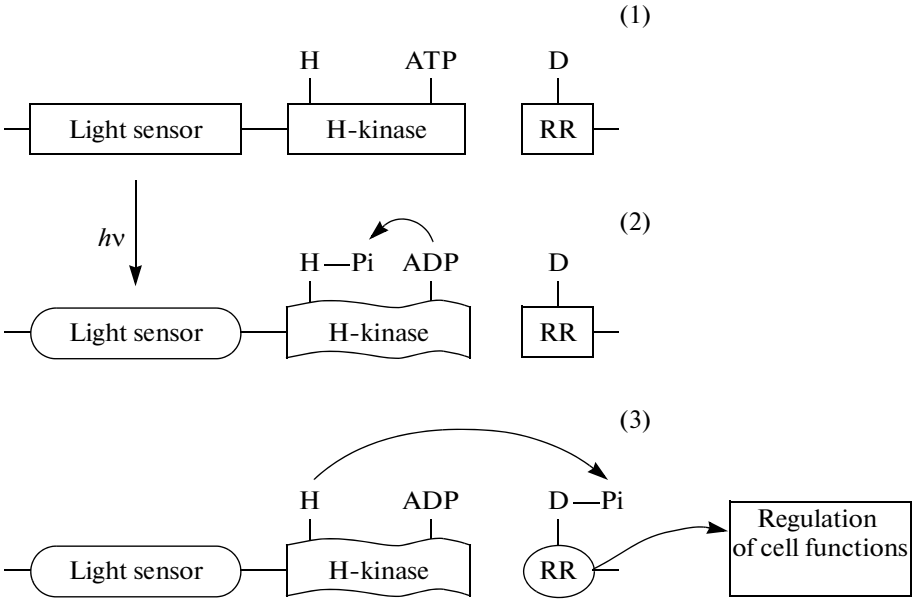
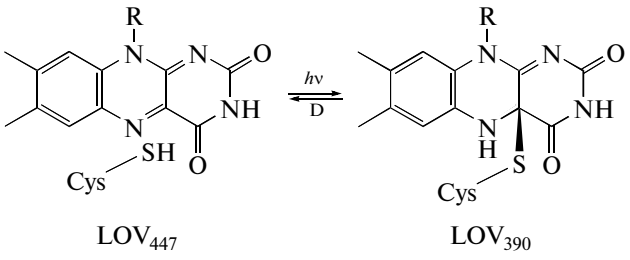


Fig. 2. Two-component system of light signal transduction. H-kinase domain of the photosensor in the inactive state binds ATP (1); photosensor activation by light ($h\nu$) induces domain structure changes triggering a phosphorylation cascade that transfers a phosphate group first onto the histidine residue (2) and then onto the aspartate (D) residue of the response regulator (RR), which can modulate cell functioning (3) [6, 12].Two-component system of light signal transduction. H-kinase domain of the photosensor in the inactive state binds ATP (1); photosensor activation by light ($h\nu$) induces domain structure changes triggering a phosphorylation cascade that transfers a phosphate group first onto the histidine residue (2) and then onto the aspartate (D) residue of the response regulator (RR), which can modulate cell functioning (3) [6, 12].

BLUF- AND LOV-TYPE UVA/BLUE LIGHT RECEPTORS

BLUF and LOV photosensory domains are short (100–110 amino acids) α/β-modules capable of binding flavin chromophores (FAD or FMN), which exhibit flexible photochemical activity. This property of flavins implies that they can undergo different light-induced transformations depending on the domain structure of the protein, first of all, on the conserved amino acid residue located near the chromophore. In LOV domains, the character of the photochemical reaction is determined by the conserved cysteine residue, which donates a proton to be transferred onto the photoexcited FMN moiety; this is accompanied by formation of a signaling FMN-cysteinyl adduct (Fig. 3). In BLUF domains, photoexcitation of FAD initiates proton-coupled electron transfer between the conserved tyrosine residue and the chromophore with subsequent reorganization of the network of surrounding hydrogen bonds (Fig. 4). The resulting structural changes in photosensory domains modulate the activity of effector domains or other proteins interacting with photoreceptors [11].



LOV_{447} LOV_{390}

Fig. 3. Light-induced ($h\nu$) formation of a dark-reversible (D) FMN–cysteinyl adduct in the LOV domain, as detected by the absorption maximum shift from 447 nm (LOV_{447}) to 390 nm (LOV_{390}) [42].

BLUF Photosensory Proteins

Many bacterial photoreceptors of this type possess only the conserved BLUF domain with two C-terminal α-helices; less frequently, they also comprise effector domains linked to BLUF, such as EAL or cyclase domains (Fig. 1b), whose activity can be modulated by the photoexcited BLUF domain [12]. Short BLUF photosensors transmit signals and regulate physiological responses by means of complex formation with signaling proteins, i.e., by light-dependent protein–protein interactions [30].

As was noted above, the FAD photocycle in a BLUF domain involves proton-coupled electron transfer from the tyrosine residue onto the photoexcited flavin converting it into a neutral radical. This induces reorientation of hydrogen bonds surrounding the flavin chromophore, resulting in the production of

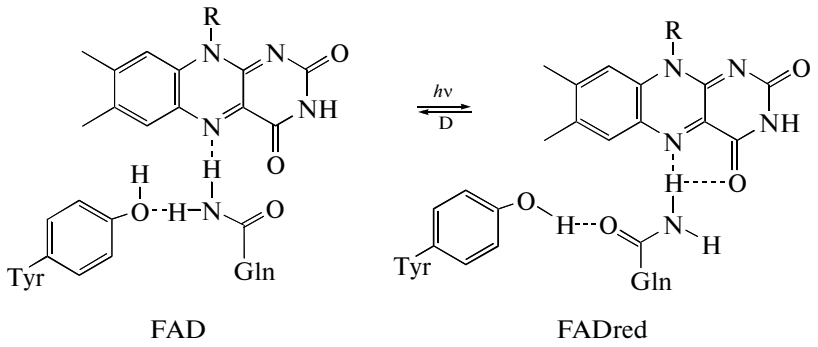


Fig. 4. Light-induced (*hν*) reorganization of the hydrogen bond network between FAD and tyrosine/glutamine residues results in the formation of a dark-reversible (D) FAD_{red} intermediate in the BLUF domain [31].

the dark-reversible FAD_{red} intermediate, with its absorption spectrum red-shifted by 10–15 nm [31]. The hydrogen bond responsible for the intermediate formation is donated by the conserved glutamine residue, which undergoes tautomerization during light-induced excitation of the BLUF domain (Fig. 4) [32]. Local changes in the hydrogen bond orientation cause conformational changes in the flavin-binding pocket, spreading across the β5-sheet and inducing structural changes in the C-terminal α-helices of the BLUF domain, which determines its modulating effect on the activity of the effector domains [33].

Some recent data suggest that photoexcited BLUF domains can regulate the catalytic activity of the enzymatic effector domains involved in the synthesis and degradation of secondary messenger molecules. The BLUF domain of BlrP1 (blue-light regulated phosphodiesterase), a photosensory protein from *Klebsiella pneumonia*, modulates the c-di-GMP-phosphodiesterase activity of the covalently linked EAL domain (Fig. 1b) [34]. Crystallographic analysis of BlrP1 showed that EAL domains form a dimer with a conserved contact produced by two dimerization helices and a shared helix formed by the short helices of either protomer. Importantly, photosensory BLUF domains are located close to this dimerization site, and light absorption by the BLUF domain of one subunit of an antiparallel homodimer stimulates phosphodiesterase activity of the EAL domain of the other subunit [34].

According to the model proposed in the first detailed study of the structure of the photoinduced BlrP1 state and the molecular basis of signal transduction, the BLUF sensor regulates the activity of the EAL domain by means of a two-way allosteric interaction between light-induced structural changes and the active center of EAL [35]. The crucial role belongs to the shared helix in the EAL dimerization contact site. Light signals from both BLUF domains are combined in the conserved EAL dimerization site and are transmitted to the active center of the enzymatic domain, inducing its activation.

In a novel photosensory protein recently described in *Beggiatoa* sp., the BLUF domain is linked to adenylyl cyclase, and light was found both to stimulate the enzyme activity and to increase the cellular cAMP level. When expressed in *Escherichia coli*, this bacterial photoactivated adenylyl cyclase, termed bPAC [36] (or BlaC [37]), exhibited low-level cyclase activity in the dark, which increased by two orders of magnitude in the light (Fig. 1b). Formation of a red-shifted flavin intermediate typical for BLUF photoreceptors was also detected. Interestingly, BlaC could be transformed into guanylate cyclase, as could be seen in the engineered nucleotidyl cyclase domain with replacement of substitute several amino acid residues presumably involved in substrate binding. A triple mutant designated BlgC exhibited photoactivated guanylate cyclase activity in vitro. Blue light strongly enhanced GMP synthesis in the BlgC-expressing *E. coli* strain [37].

Recent research provided some information on the physiological functions of BLUF photoreceptors. In different bacterial species, they control various light-dependent biological responses, such as phototaxis (Pix D from *Synechocystis*), biofilm formation (YcgF from *E. coli*, PapB from *Rhodopseudomonas palustris*), virulence (BlsA from *Acinetobacter baumannii*), and the production of components involved in photosynthesis (AppA from *R. sphaeroides*) [33]. The regulatory activity of these photoreceptors is based on light-dependent protein–protein interactions, including formation of complexes between photoreceptors and transcription factors. Recent studies provided new information concerning the molecular details of these light-regulated processes.

PixD, the short BLUF photosensor involved in phototaxis control in *Synechocystis* (Fig. 1b), interacts in the dark with the RR protein PixE, inducing the formation of a large oligomeric complex comprising ten PixD subunits and five PixE subunits [38]. Light-induced excitation of PixD causes conformational changes accompanied with decomposition of the complex into PixD dimers and PixE monomers [30].

Presumably, it is this light-induced process that triggers the signaling cascade controlling bacterial phototaxis, wherein PixD–PixE complexes serve to sense the intensity of light, rather than simply its presence [38].

The photophysiological function of another short BLUF protein, PapB from *R. palustris* (Fig. 1b), involves negative regulation of biofilm formation by means of PapB interaction with PapA, a c-di-GMP-specific phosphodiesterase whose activity increases in response to light-induced excitation of the photosensor. In contrast to PixD, which forms complexes with PixE only in the dark [38], PapB interacts with PapA in the light as well. This fact shows that these two photosensors interact with the corresponding signaling proteins using different mechanisms [33].

Experiments with a chimeric protein composed of the N-terminal core of the PixD BLUF domain and C-terminal α-helices of PapB showed that its interaction with PapA was light-independent, similarly to PapB–PapA interaction [39]. Based on this fact, it was assumed that the α-helical fragment of the BLUF domain acts as a mediator perceiving the light-induced signal detected by the flavin of the photosensory core and transmitting it to the interacting protein. The proposed model implies that the character of interactions between BLUF proteins and signaling cascade components—which, in turn, control various photobiological processes (e.g., phototaxis or biofilm formation)—is determined by different conformations of α-helices: they can be either perpendicular (PixD) or parallel (PapB) to the β-sheet of the core [33].

A BLUF protein from *E. coli*, YcgF, contains an EAL domain (Fig. 1b); however, in contrast to BlrP1, which has a similar domain structure (see above), YcgF does not bind c-di-GMP, and its EAL domain does not exhibit light-induced phosphodiesterase activity. It was shown that YcgF acts as an antagonist of the transcriptional regulator YcgE [40]. The latter acts as a repressor by binding to the promoters of the operon encoding the proteins responsible for biofilm matrix activation or otherwise involved in its formation via a two-component signaling system [40]. Light-induced excitation causes YcgF to form temporary homodimers, which results in dissociation of YcgE–YcgF complexes and releases the repressor from the operon; thus, YcgF performs a photosensory function in modulating biofilm formation in *E. coli* [40].

The above results show that a BLUF protein with a degenerate EAL domain can be induced by blue light to activate a nonenzymatic biological function by means of light-dependent protein–protein interaction. Winkler et al., who identified the key role of the EAL dimerizaton site in the regulation of its phosphodiesterase activity by the BLUF sensor of BlrP1, also supposed that, in the YcgF photoreceptor, the light signal could also be transmitted to the EAL dimerization site, modulating the affinity to the YcgE repressor potentially binding to the same region [35].

AppA, a photoreceptor from *R. sphaeroides* (Fig. 1b), acts as a light and redox regulator of the genes involved in photosythesis; it is composed of a BLUF domain and a redox-sensitive heme-containing SCHIC domain [33]. Light and oxygen are sensed by the AppA–PpsR regulator system, where PpsR is the repressor of photosynthesis-related genes containing a helix–turn–helix (HTH) DNA-binding motif, and AppA is an antirepressor capable of forming a noncovalent AppA–$PpsR_2$ complex via the SCHIC domain.

A recent study that analyzed the crystal structures of both proteins and their complex showed that light-induced activation of AppA changed the effector domain of PpsR within the complex. Moreover, it demonstrated the formation of a light-sensitive three-component complex AppA–PpsR–DNA, which can participate in signal transduction by means of allosteric structural changes. According to the proposed model, the photomodified App–$PpsR_2$ interacts with PpsR-binding sites on DNA and prevents the formation of PpsR–DNA–repressor complex, thus activating the expression of photosynthesis-related genes [41].

LOV Photosensory Proteins

The photosensory function of the LOV domain was first discovered when a plant phototropin containing two FMN-binding LOV domains was identified; of those two domains, LOV2, which is linked to a serine/threonine kinase effector domain via a Jα-helix, was central for the regulation of the photoreceptor activity [3]. FMN has an absorption maximum at 447 nm (LOV_{447}), and its photocycle involves dark-reversible formation of an FMN-cysteinyl adduct (LOV_{390}) (Fig. 3). The latter form is the signaling state of the photoreceptor associated with conformational changes in LOV2 induced by adduct formation; these changes trigger the destruction of the Jα-helix and subsequent upregulation of the kinase activity [3, 42].

Importantly, the same principle of photoactivation (except for some details in the structural mechanisms of signal transduction) is conserved among LOV proteins found in evolutionary remote phylogenetic groups, including fungi [10] and bacteria [11, 12, 25, 42–44]. All bacterial and fungal LOV photoreceptors contain a single LOV domain, and in many prokaryotes it is closely linked to various effector domains, which gives rise to module systems whose activity can be regulated by light.

In fungi, typical representatives of LOV photoreceptor systems [10] are two FAD-dependent LOV-domain proteins: White Collar (WC)-1 and VVD, which mediate light-dependent regulation of the circadian rhythm in *Neurospora crassa* [45]. The photo-

sensory protein WC-1 is a multidomain transcription factor that forms a functionally active heterodimeric WCC complex with a nonphotosensory WC-2 transcription factor. WC-1 photoactivation within this complex is initiated by formation of a cysteinyl-flavin adduct and leads to WCC homodimerization involving LOV domains of WC-1 [46]. Photoactivation of WCC induces the transcription of numerous light-inducible genes [47], including those that encode the FRQ central circadian oscillator and the VVD photosensor required for correct regulation of circadian rhythms [45, 48].

VVD is a small protein of 186 amino acids whose LOV domain carries only an N-cap packed on the β-sheet [47]. The N-cap is composed of an α-helix, a β-chain, and a short loop, which is attached to the same site of the β-sheet in the LOV core as the Jα-helix of LOV2 phototropin [49]. The key role in the propagation of conformational changes in the LOV/VVD induced by photoadduct formation belongs to the conserved glutamine and the 65–72 residues of the loop region; these changes result in VVD homodimerization and enable transformation of the light signal into altered protein interactions, which trigger the signaling cascade [50]. For instance, it was shown that a Ser substitution for Cys at position 71 in the loop inhibits VDD dimerization in vitro and compromises its function in vivo [48, 49, 51] due to the formation of a negative feedback from WCC in the control of circadian gene expression.

The current model of the antagonistic VVD effect on WCC implies their direct interaction and competitive VVD–VVD/LOV and WC-1/LOV heterodimerization [46, 48]. The formation of VVD : WC-1 heterodimers serves for the organism's adaptation to light, since it blocks the initial gene expression wave under continued light conditions, thus preventing overexpression of WCC-transcribed genes [46, 48, 51].

Bacterial LOV photoreceptors possessing various effector domains linked to the photosensor domain (Fig. 1c) were recently shown to exhibit light-dependent activity, and some of them were also found to perform photobiological functions [11, 43]. LOV-H-kinases identified in freshwater bacteria *Caulobacter crescentus* (LovK) [52], as well as in two pathogenic bacteria, *Brucella abortus* (LOV-HK) [52] and *Pseudomonas syringae* [53], exhibit the flavin chromophore photocycles typical of the LOV domains and accompanied by kinase autophosphorylation in vitro and phosphate transfer onto corresponding RRs. Thus, RRs associated both with LOV kinase and with phytochrome kinases (see Fig. 2) are two-component systems of light signal transduction; however, in contrast to phytochrome kinases, LOV kinases comprise the corresponding RRs as domains within the photoreceptor structure. Photoactivation of LovK and LOV-HK causes physiological responses in bacterial cells: *B. abortus* exhibited a 10-fold increase in the cell proliferation rate in macrophages, while *C. crescentus* responded by overexpression of *lovK* and the adjacent *lovR* resulting in a dramatic increase in cell adhesion [52]. Biochemical analysis showed that the midpoint reductive potential of the flavin cofactor in LovK ($E'_0 = -260$ mV) was close to the redox potential of cellular cytosol [54]. To enable a LOV domain to function as a photosensor, the flavin cofactor must be in the oxidized form; therefore, the redox state of the cell can affect the photoactivity of LOV by changing the redox state of flavin. For LovK, it was shown experimentally that FMN reduction impaired light-dependent regulation of H-kinase activity in vitro [54].

In cyanobacteria *Synechococcus elongates*, the LOV domain combined with GGDEF-EAL domains (Fig. 1c) mediates light-induced regulation of EAL phosphodiesterase activity in vitro [55]. Therefore, blue light-activated LOV domain can control the cellular c-di-GMP level, which is involved as a secondary messenger in the regulation of a range of physiological functions, such as cell motility or virulence. Taking into account that LOV-GGDEF-EAL photosensors constitute a considerable portion of bacterial LOV proteins, it is supposed that light-dependent regulation of the c-di-GMP concentration is a common functionally significant property of bacteria possessing such photosensors.

In *Bacillus subtilis*, the LOV-STAS protein YtvA (Fig. 1c) functions as a photoreceptor both in vitro and in vivo. In vitro, the STAS domain determines the blue light-modulated ability of YtvA to bind GTP [56], which acts as a second messenger in stress-induced responses in *B. subtilis*. Apart from that, photoactivated YtvA acts in vivo as a positive regulator of the general stress transcription factor σB [57]. Since mutations impairing GTP binding by the STAS domain or interdomain propagation of the light-induced signal also inhibit σB-dependent transcription activated by blue light in vivo, it was concluded that GTP binding is essential for YtvA functioning [57–59].

A study of structural changes occurring in the LOV domain of YtvA, which is connected to the effector STAS domain via a Jα-helix, showed that, in contrast to similar events in phototropins, they did not involve the destruction of the Jα-helix [58, 60]. This is explained by differences in the quaternary structure and Jα orientation, which has a coiled-coil configuration in the YtvA-LOV domain [57, 61]. Isolated YtvA-LOV domains are constitutive dimers with Jα-helices spreading away from the LOV core [60]. The key role in the propagation of light-induced structural transformations of YtvA-LOV belongs to the glutamine residue of the β-chain, whose side chain undergoes reorientation, as it is also the case in the LOV domains of phototropin and VVD [61]. At the same time, the spreading of conformational changes in YtvA-LOV to the STAS domain depends on two acidic residues (E105 and D109) [58]; the same two amino acids are

required for the light-dependent YtvA activity in vivo [57].

A recent study of the EL222 LOV-HTH protein from *Erythrobacter litoralis* (Fig. 1c) identified a structural regulatory mechanism similar to the regulatory mechanisms previously described for phototropins and VVD [62]. EL222 is a light-regulated protein that can bind DNA as a dimer. In the dark it exists in the monomer state, and its HTH domain is joined to the β-sheet of the LOV domain via a Jα-helix. Light-induced activation of LOV-EL222 destroys the binding contact between the LOV and HTH domains. This structural change ultimately results in the formation of a DNA-associated EL222 dimer via HTH–DNA binding that is possible only for this oligomeric state. Thus, photoactivation of the LOV domain can cause HTH detachment from the β-sheet and modulate the oligomeric state of EL222 in the presence of DNA.

The data obtained by studying the structural aspects of signal transduction in LOV photoreceptors suggest that the mechanism underlying the initial stage of light signal transduction is common for both bacterial and eukaryotic LOV proteins. It is based on conformational changes in the β-sheet of the LOV core caused by the formation of a flavin-cysteinyl adduct; the conserved glutamine residue that interacts directly with the photoactivated flavin chromophore is of crucial importance for the initiation of these changes [49, 63]. Glutamine transformation induces reorganization of the hydrogen bond network involving several peripheral amino acid residues that vary among different LOV proteins and act as specific signal transmitters. The structural mechanisms of subsequent signal transduction stages enabling LOV domains to regulate the activity of effector domains also vary [10, 43]. The differences among signal transduction mechanisms described in various LOV-domain proteins are determined by differences in their amino acid sequences and quaternary structures. The fact that LOV domains were found in a number of bacterial genomes makes it possible both to comprehensively study the molecular basis of LOV-induced signaling cascades and to investigate new regulatory functions of these ancient signaling photosensors.

Application of LOV and BLUF Photosensors in Optogenetics

Optogenetics is a new area of cell biology which combines optical and genetic approaches to noninvasive light-dependent control of cell processes using photosensory proteins. In recent years, optogenetics has become one of the most important biotechnologies, since genetically encoded photosensors can be functionally introduced in nearly any type of cell and regulate gene expression, enzyme activity, and other biological functions in response to photoactivation performed with a high spatial and temporal precision [64].

LOV and BLUF photosensors possess the key properties that make them ideally suitable for optogenetic applications, such as their small size and the use of photochemically flexible flavin chromophores, cofactors present in all cell types. Moreover, the fact that LOV and BLUF photosensors can form functional modular structures with various effector domains can be employed to design the constructs combining them with other proteins or enzymes, i.e., to engineer synthetic photoreceptor systems composed of photosensor domains and linked effector proteins whose activity is to be regulated by means of light-induced allosteric control [65–68].

In several studies, allosteric control of protein functions was performed using the phototropin LOV2 domain, which undergoes pronounced structural changes in response to photoactivation. LOV2-Jα-helix is attached to the target protein in such a manner that conformational changes in LOV2 induce conformational changes in the target. Using this mechanism, LOV2-Jα carries out light-dependent regulation of the enzymatic activity of dihydrofolate reductase [69], lipase A from *B. subtilis* [70], and Rac1 GTPase in animal cells expressing photoactivatable LOV2-Jα–Rac1 construct [71, 72]. Since Rac1 is a key protein in the regulation of cell motility, these data demonstrate that light can be used for high-precision spatial and temporal control of this important biological function.

LOV2-Jα also exhibited a photoregulatory effect on the DNA-binding activity of a hybrid protein containing a bacterial tryptophan repressor, TrpR [73]. Light-induced DNA binding is based on detachment of the Jα-helix from the LOV core, which is accompanied by a conformational change and by activation of the effector domain (TrpR). It should be noted that, due to the presence of the TrpR domain, the balance of attachment and detachment between the Jα-helix and the LOV core is shifted towards the detached state; that is, the LOV domain is functionally active even in the dark, and the hybrid protein exhibits a low level of light-dependent activation. However, using site-directed mutagenesis to stabilize the predominantly attached state of the Jα-helix in the dark, it was possible to augment 60–70-fold the regulatory effect of light on DNA binding by the hybrid protein, thus strongly increasing the system's sensitivity to light [74].

Along with LOV2-Jα, optogenetic research also concerns bacterial LOV photosensors. Based on the ability of many bacterial LOV-domain photoreceptors to bind H-kinase effector domains, Moglich et al. constructed a synthetic light-regulated H-kinase where the nonphotosensitive PAS-domain of the FixL oxygen sensor from *Bradyrhizobium japonicum* was substituted with the LOV domain of the YtvA photoreceptor from *B. subtilis* [75]. Further applications of this chimeric protein were related to plasmid engineering for the purposes of light-dependent induction

(pDawn) or repression (pDusk) of bacterial genes [76].

Genetic engineering techniques have also been applied to produce other light-regulated transcription systems [77]. A promising candidate for optogenetic applications is the construct composed of the VVD LOV photosensor from *N. crassa*, a DNA-binding domain, and an activation domain used for spatiotemporal control of gene expression in animal cells [78]. Considerable interest is attracted by the research of biochemical and functional properties of LOV domains identified recently in the genomes of various bacteria isolated from different habitats, including extreme ones [79]. Combinations of these photosensory modules with different effector domains can provide a broad variety of constructs with potential engineering and optogenetic applicability.

The pioneering work in the research of optogenetic applications of BLUF photosensors was performed with the cAMP cyclase-containing PAC photoreceptor from *E. gracilis* [80]. When expressed in animal neurons, this BLUF photoreceptor enables light-dependent cyclase activation accompanied by a rapid increase in the cAMP level, which controls gene expression and other biological processes via a phosphorylation cascade [80, 81].

Further optogenetic applications can be expected following the discovery of the bPAC/BlaC BLUF adenylyl cyclase from *Beggiatoa* sp. (*Gammaproteobacteria*) described above. Indeed, photoactivated bPAC is highly efficient in cAMP-regulated biochemical and physiological processes when integrated with various cell systems [36]. Moreover, using site-directed mutagenesis, BlaC can be converted into a photoactivatable cGMP-cyclase (BlgC), which broadens the area of its potential applicability [37]. It is known that adenylyl- and guanylyl cyclase products, cAMP and cGMP, are universal secondary messengers involved in the regulation of many processes. Therefore, the ability of bacterial PACs to control cAMP/cGMP synthesis in response to light, along with their small size and high efficiency of light activation, make BLUF-cyclase photoreceptors promising candidates for optogenetic applications.

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