The Evolution of Flavin-Binding Photoreceptors: An Ancient Chromophore Serving Trendy Blue-Light Sensors

Aba Losi[1] and Wolfgang Gärtner[2]

[1]Department of Physics, University of Parma (**http://www.unipr.it**), 43100 Parma, Italy; email: aba.losi@fis.unipr.it

[2]Max Planck Institute for Bioinorganic Chemistry, 45470 Mülheim, Germany; email: gaertner@mpi-muelheim.mpg.de

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Abstract

Photoreceptor flavoproteins of the LOV, BLUF, and cryptochrome families are ubiquitous among the three domains of life and are configured as UVA/blue-light systems not only in plants—their original arena—but also in prokaryotes and microscopic algae. Here, we review these proteins' structure and function, their biological roles, and their evolution and impact in the living world, and underline their growing application in biotechnologies. We present novel developments such as the interplay of light and redox stimuli, emerging enzymatic and biological functions, lessons on evolution from picoalgae, metagenomics analysis, and optogenetics applications.

Contents

1. INTRODUCTION: FL-BLUES, FLAVIN-BINDING UVA/BLUE-LIGHT SENSORS

Fl-Blues: flavin-binding UVA/BL sensors

LOV domain: light-oxygen-voltage domain

HB: hydrogen bond

BLUF domain: BL sensing using flavin domain

Cry: cryptochrome

The past decade has seen a blooming in the study of flavin-binding UVA/blue-light (BL) sensors (Fl-Blues) belonging to the LOV (light-oxygen-voltage), BLUF (blue-light sensing using flavin), and cryptochrome (Cry) protein families (65). Fl-Blues have a long history in science and a much longer one in nature (Sections 1 and 4). Their roles, well documented in plants, are mirrored now by a growing understanding of their involvement in light-to-signal conversion in microorganisms (Section 3). Photoexcitation of the flavin chromophores induces chemical reactions that are well known from their role as enzyme cofactors (10), such as covalent bond formation (LOV), hydrogen-bond (HB) rearrangements (BLUF), and redox reactions (Cry). The primary photochemical events (Section 2) lead sequentially to (*a*) conformational changes of the chromophore and any covalently linked protein element (proximal changes), (*b*) intermediate structural changes involving the surrounding microenvironment, and (*c*) distal conformational changes reaching the photosensory domain surface, often affecting the networks of protein-protein interactions that eventually lead to the biological response (127).

The widespread presence of Fl-Blues in general, and their presence in metagenomes in particular, offers a unique opportunity to explore the evolution of light sensing within the three domains of life (Section 4). Finally, given the ubiquitous availability of flavins, these photoreceptors are drawing increasing attention as tools to control cellular processes with light (optogenetics) and as fluorescent reporters, paralleling the well-known applications with channelrhodopsins and green fluorescent protein (GFP) (Section 5).

1.1. Fl-Blues Enter the Third Millennium

In 2006, following the outburst of structural and functional data for Fl-Blues within a few years of their molecular identification, Briggs (14) wrote a fascinating historical review. For almost 200 years, scientists had reported on light-color effects on plant growth and morphogenesis, but it was only during the past 20 years that the mysterious Cry proteins and the long-sought phototropins (phots)—responsible for phototropism—were identified. It soon became clear that plant BL photoreceptors bind riboflavin derivatives, immediately initiating a new discussion as the dogma of photoisomerization was broken (63, 75).

Cry proteins preferentially bind flavin adenine dinucleotide (FAD), whereas phots favor flavin mononucleotide (FMN) within two tandem-arranged protein domains, LOV1 and LOV2. The subsequent characterization of a photoactivated adenylyl cyclase (PAC) in *Euglena gracilis*, binding FAD within a novel type of protein domain called BLUF (34, 49), confirmed that the BL world was entering a new research era. In the same exciting year (2002), a LOV protein and a BLUF protein were each described in bacteria: The former, YtvA from *Bacillus subtilis* (*Bs*), was discovered via a combined in silico and molecular biology approach (67); the latter, AppA, was isolated while searching for the light and redox sensor involved in regulation of photosynthesis genes in *Rhodobacter sphaeroides* (*Rs*) (13). Since then, the prokaryotic LOV and BLUF proteins have grown steadily in number and have become a case story in their own right (42, 65, 92).

phot: phototropin

FAD: flavin adenine dinucleotide

FMN: flavin mononucleotide

PAC: photoactivated adenylyl cyclase

HK: histidine kinase

PL: photolyase

1.2. Structural Criteria: LOV, BLUF, or Cry?

Before discussing the light activation pathways of these photoreceptors, we should address structural criteria: what makes a LOV, BLUF, or Cry protein? LOV domains, a subgroup of the PAS (Per Arnt Sim) superfamily, and BLUF domains are minimal and compact photosensing modules (~100–110 amino acids) with α/β folds. The secondary structure elements are named, respectively, $A\beta B\beta C\alpha D\alpha E\alpha F\alpha G\beta H\beta I\beta$ and $\beta_1\alpha_1\beta_2\beta_3\alpha_2\beta_3$ (63). For both classes the keyword is modularity: LOV and BLUF domains are linked to diverse effector domains that in most cases define the functionality of the protein (**Figure 1**), e.g., kinases, phosphodiesterases, or DNA-binding proteins. However, many LOV and BLUF units are simply flanked by short variable regions and are known as short sensors. Phots seem to be unique among LOV proteins, as they possess two LOV domains in tandem, LOV1 and LOV2, linked to a serine/threonine kinase domain.

Additional sensing or regulating domains are present in many cases, especially in the variegate prokaryotic LOV proteins. The majority of prokaryotic LOV proteins are histidine kinases (LOV-HKs), whereas almost 80% of BLUF bacterial proteins are of the short type (65). The sole criterion for photofunctionality of LOV and BLUF domains is the ability to form a flavin-cysteine adduct and a transient species with a red-shifted absorption spectrum, respectively (**Figure 2**).

Cry proteins, conversely, are two-chromophore proteins, similar in structure to the photolyase (PL) DNA-repairing enzymes, with which they form the large Cry/PL family (21, 101). PLs catalyze the light-dependent repair of UV-induced cyclobutane pyrimidine

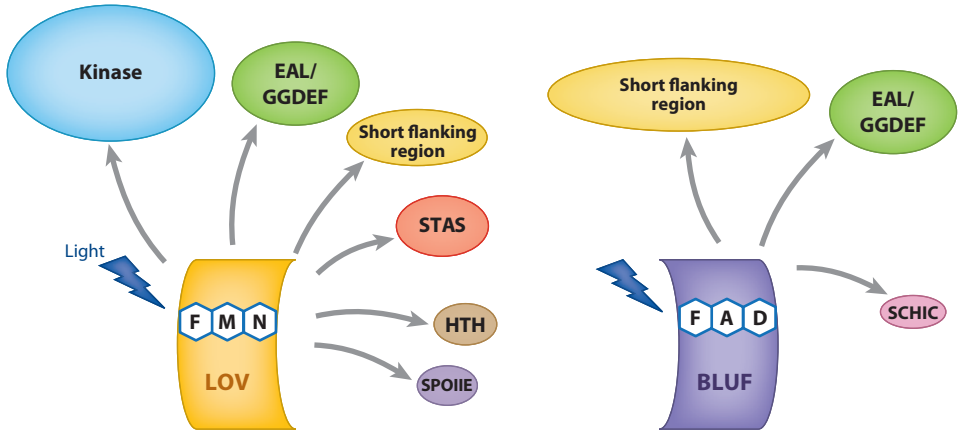

Figure 1

A simplified sketch of the structural and functional modular concept for prokaryotic LOV and BLUF proteins. The photosensory moiety is associated with diverse effector domains or solely with short flanking regions. Other sensing/regulatory domains are often present on the same protein. UVA/blue-light (*lightning bolts*) activation of LOV and BLUF domains results in regulation of the effectors ultimately responsible for signaling via networks of protein-protein interactions. The relative abundance of each protein type is proportional to the area of the corresponding inset. Classification of domains is as in the InterPro database. FMN, flavin mononucleotide; kinase, ATP-binding histidine kinase; EAL (named after a conserved sequence), cyclic diguanylate phosphodiesterase; GGDEF (named after a conserved sequence), synthesizer of cyclic diguanylate; STAS, sulphate transporter/antisigma-factor antagonist; HTH, helix-turn-helix regulatory protein, DNA binding; SPOIIE, sporulation stage II, protein E C-terminal/protein phosphatase 2C-related; FAD, flavin adenine dinucleotide; SCHIC, sensor containing heme instead of cobalamin.

dimers (CPDs) or (6-4) pyrimidine-pyrimidone photoproducts (119). Cry proteins are generally defined as PL-like proteins that have lost or strongly reduced DNA repair activity and instead have gained signaling roles (21). The Cry/PL family consists of 55–70-kDa proteins that contain two noncovalently bound prosthetic groups: a photoredox-active FAD and an antenna chromophore of pterin or flavin type (8-hydroxy-5-deazaflavin, FMN, FAD) (21). Both cofactors are held within the so-called PL domain, referred to as Cry_{PL}, formed by an α/β subdomain and an all-α subdomain, joined by a variable linker. Cry proteins from plants and animals bear an additional C-terminal tail [cryptochrome C-terminus (CCT)], involved in signaling (Section 3). The group previously named Cry-DASH (*Drosophila*, *Arabidopsis*, *Synechocystis*, and *Homo* cryptochromes) comprises proteins that can carry out single-strand DNA photorepair (ssDNA-PL) (104), but their signaling role is not clearly defined (40).

2. LIGHT ACTIVATION OF LOV, BLUF, AND CRY PROTEINS

The photochemical properties of flavins have been recently reviewed (60). Flavins can switch between different oxidation states, referred to as *ox* (fully oxidized), *sq* (semiquinone, one-electron reduced form), and *hq* (hydroquinone, two-electron reduced form). At physiological pH in aqueous solution, *sq* and *hq* can be further subjected to ionic equilibria, with pK_a values of approximately 8 and 6, respectively (60).

In the UVB-visible range, the *ox* absorption spectrum comprises three major $\pi\pi^*$ bands, centered at approximately 446, 370, and 265 nm (63, 100). The *sq* species shows a strongly red-shifted maximum at approximately 650 nm. The *hq* species shows an unstructured spectrum with a maximum in the UVB range (62). A fundamental parameter to understand the photochemistry of flavins is the change of their redox potential upon photoexcitation (from approximately −0.3 V to approximately +1.9 V),

CPD: cyclobutane pyrimidine dimer

CCT: cryptochrome C-terminus

Cry-DASH: *Drosophila*, *Arabidopsis*, *Synechocystis*, and *Homo* cryptochromes

***ox*:** oxidized

***sq*:** semiquinone

***hq*:** hydroquinone

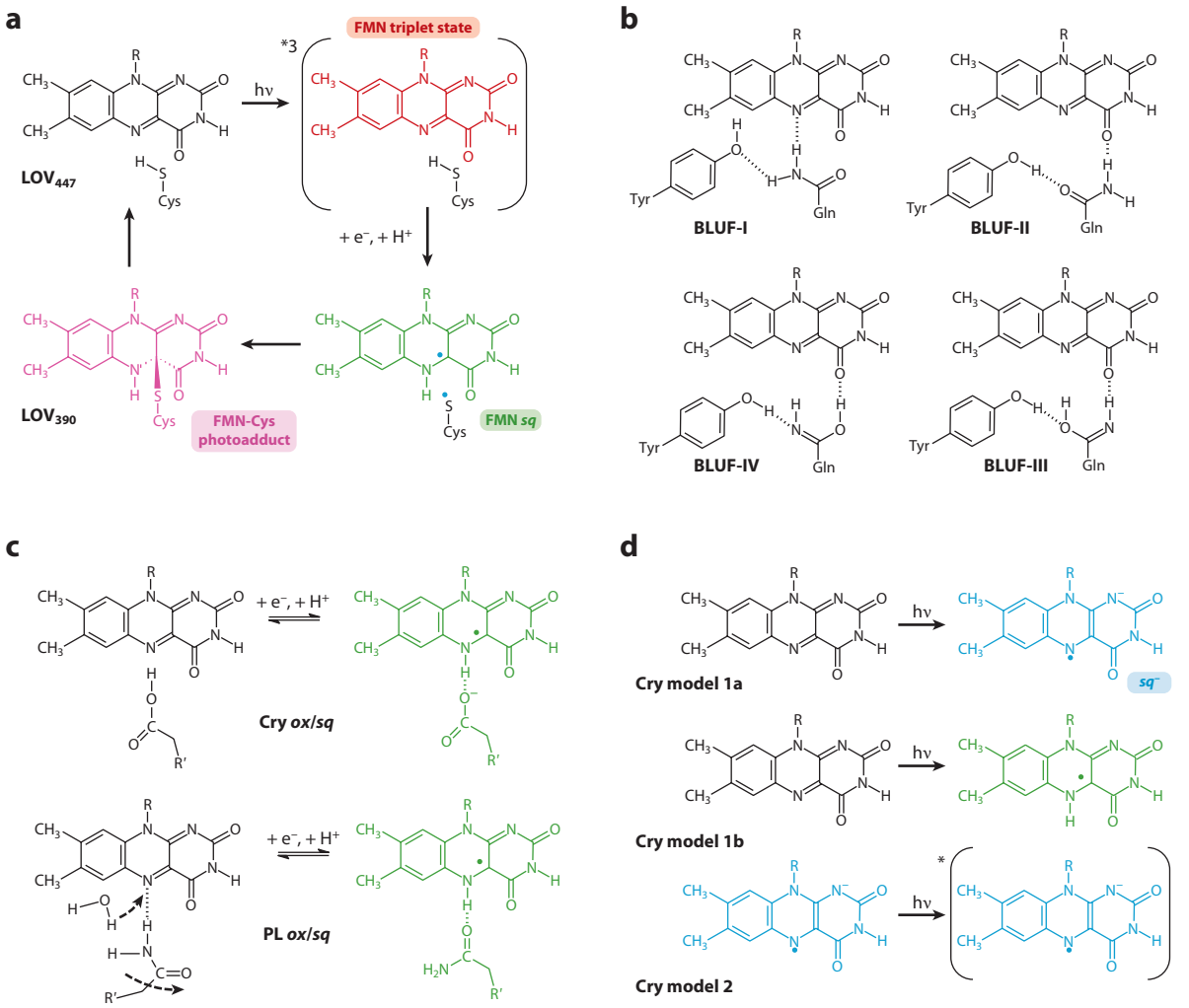

Figure 2

(*a*) Recently proposed mechanism for the photocycle of LOV domains: the flavin mononucleotide (FMN) triplet state is shown in red, the FMN *sq* (semiquinone) is shown in green, and the FMN-cysteine (FMN-Cys) photoadduct is shown in magenta. Subscript numbers denote absorption maxima. (*b*) Four possible hydrogen-bond network models of the tyrosine/glutamine (Tyr/Gln) region for BLUF domains. The light-activated, red-shifted state ($BLUF_{RED}$) configuration I has recently been ruled out. (*c*) Dark-state *ox* (oxidized)/*sq* equilibria for plant cryptochrome (Cry) proteins and photolyases (PLs) and proposed proton-coupled electron transfer reactions. In PLs, the site close to N(5) is occupied by an asparagine residue, which confers a higher reaction barrier for oxidation with respect to the corresponding aspartate in plant Cry proteins because it requires proton transfer from bound/mobile water and rotation of the asparagine lateral chain (*arrows*). Although in both cases oxidation is thermodynamically favored, PLs tend thus to be stabilized in vitro in the *sq* form. In contrast, plant Cry proteins tend to be purified with an *ox* form of flavin adenine dinucleotide. (*d*) Proposed light-triggered reactions in some Cry proteins. Model 1a, with the formation of sq^- (*blue*), has been derived for insect Cry proteins; model 1b has been derived for plant Cry proteins. An alternative model has recently been put forward for insect Cry proteins (model 2), where protein conformational changes are triggered by the excitation of sq^-.

indicating that flavins in the excited state are much stronger oxidants than they are in the ground state. The second important property is their high triplet quantum yield (approximately 0.5–0.7) and high fluorescence quantum yield (0.25–0.3), with the exception of FAD in solution (60, 63).

A "dangerous" aspect of flavins is the photosensitizing effect potentially carried out by flavin triplet states (given their high redox potential of approximately +1.7 V) and the efficient energy transfer to molecular oxygen. Low-efficiency or diffusion-limited reactions, however, can take advantage of the high energy content of the long-lived triplet state (68).

2.1. LOV and BLUF Photocycles: Covalent Bonds, Hydrogen-Bond Switches, and Radicals

The photocycle of LOV domains starts from the dark-state LOV_{447} (the suffix indicates the absorption maximum) in which FMN is in the noncovalently bound *ox* state. The photoprocess involves the formation of a covalent bond between FMN-C(4a) and a conserved cysteine (lit state or LOV_{390}) via the short microsecond decay of the FMN triplet state, red-shifted with respect to *ox* (LOV_{710}) (110). LOV_{390} decays thermally to the parent state with breakage of the covalent bond on a timescale of seconds to hours at room temperature (63). The driving force for this recovery reaction results from the high energy content of LOV_{390}, approximately 110–140 kJ mol^{-1}, indicative of a strained protein conformation (66, 68). Formation of LOV_{390} involves the establishment of new C(4a)-S and N-H(5) bonds, most probably via the fast decay of an $FMNH^{\cdot}$-$H_2CS^{\cdot}$ radical pair (8).

The extended HB network stabilizing the chromophore can also modulate the quantum yield, kinetics, and thermodynamics of the photocycle (95). The thermal recovery to the dark-adapted state can be strongly influenced by mutations, even quite distant from FMN, a feature important for understanding details of the reaction mechanism (65, 106, 118).

In BLUF domains a transient and reversible red-shifted absorption spectrum, corresponding to the signaling state $BLUF_{RED}$, is dictated by an HB switch reaction involving N(5), O(4), and two conserved tyrosine and glutamine residues (**Figure 2*b***) (51, 63, 71). The recovery lifetime in the dark ranges from a few seconds to several minutes. $BLUF_{RED}$ formation seems to involve light-driven electron and proton transfer from the conserved tyrosine residue to FAD, followed by HB rearrangement and radical-pair recombination (11, 31). An alternative mechanism implies glutamine rotation during the lifetime of the biradical state of the system, followed by biradical recombination leading to the enol tautomeric form of glutamine (56) (**Figure 2*b***).

Even though some molecular details of LOV and BLUF photoactivation remain unclear, both photoreceptors have an *ox* flavin in their ground state. This has recently opened a new topic: changes in the flavin state with changes in the intracellular redox potential. Could this be a mechanism to tune light inputs and responses to the redox state of the cell? Integration of light and redox sensing has been shown to be possible for a LOV protein from *Caulobacter crescentus* (93) and for *Rs*AppA (2).

2.2. The Many Photoredox Facets of Cryptochromes

The photochemistry of the Cry/PL family has been recently reviewed (21, 62). In PL the light-harvesting antenna transfers excitation to $FADH^-$ (FAD in the hq^- form), which in turn transfers an electron to the pyrimidine dimer during DNA repair. After repair, the electron is transferred back to the flavin chromophore. The hq^- active form can also be restored by intraprotein photoreduction via a triad of conserved tryptophans. A current working idea proposes that the dark-adapted state of Cry instead contains *ox* FAD as a chromophore and that the primary photochemistry involves

photoreduction via the triad of aromatic amino acids, leading to *sq* or sq^- representing the signaling state (21, 83) (**Figure 2*d***). A correlation of in vivo effects with the photoreducing triad has up to now been reported solely for *Arabidopsis thaliana* (*At*) Cry1 (32). Furthermore, *Rs*CryB is photochemically and biologically active but does not bear the aromatic amino acids triad (41).

The question is obvious: Does the *ox* form in Cry reflect the in vivo situation? In *At*Cry1, Asp396 in the vicinity of N(5) (asparagine in PLs and Cry-DASH) has been proposed to downshift the redox potential of the bound FAD, thus thermodynamically favoring the oxidized form of the flavin in the dark state (4, 21). A mere thermodynamic view of this problem has been challenged by recent data obtained for *Anacystis nidulans* PLs that favor a kinetic mechanism instead (23). The authors proposed that the aspartic acid proximal to N(5) in plant Cry is protonated in the ground state and can act as a proton shuttle to reduce the kinetic barrier for FAD oxidation (**Figure 2*c***) with respect to PLs that carry an asparagine residue in this position. As a consequence, purified PL tends to be stabilized in vitro in the *sq* form, whereas plant Cry tends to be purified with a bound *ox* FAD.

Photoreduction of FAD via the aromatic triad might not be the reaction responsible for Cry activation, and the mechanism derived from in vitro experiments could reflect a double photon absorption, first from *ox* and then from sq^-. Photoexcitation of sq^- is in fact responsible for the light-induced conformational change of insect Cry (86). A similar hypothesis has been put forward in a recent review (62) (**Figure 2*d***).

Photoredox reactions of Cry appear to be important for addressing another puzzling question: their role as light-regulated magnetoreceptors (78, 82). The most recent hypothesis on the possible underlying mechanism involves an oxygen radical, with formation of $[FADH^{\cdot} \ldots HO_2^{\cdot}]$. The spin-correlated $FADH^{\cdot}$-superoxide radical would be responsible for magnetic sensing during its short lifetime (78).

2.3. Propagating Signals: Coupling Photons to Signal Transduction

Photochemical events trigger intraprotein signal propagation via conformational changes that travel from the chromophore cavity to molecular surfaces, where they affect interdomain or protein-protein interactions (127). Information on these pathways is still sparse, with a few notable exceptions (6, 81, 122). The high modularity of these proteins poses a major question: do LOV and BLUF domains from different origins communicate with effector partners through the same or partially overlapping surfaces? In both cases, variations of a general β-sheet/helical cap mechanism for activation have been suggested (121).

The β-scaffold of the LOV core is formed by five antiparallel β-strands (63). This extended β-sheet is a true chromophore/environment interface: on one side it hosts residues directly interacting with the isoalloxazine ring of FMN, and on the other side it communicates with helical regions flanking the LOV core (N-terminal cap and/or Jα-linker) (35, 91, 128) or communicates directly with effector domains (81). A conserved, switching glutamine on the terminal strand Iβ (Q123 from YtvA numbering) that forms an HB with FMN well illustrates this idea: Q123 in YtvA not only modulates the kinetics and yield of the photocycle, but also dictates the extent of light-driven conformational changes and the photoresponses in vivo (3, 95). A similar role for this switching glutamine has been described for other LOV domains (65).

LOV2 of phot1 has offered the first identification of a signal transmission model involving the β-scaffold: self-phosphorylation of phot1 is enhanced by the light-induced undocking of the so-called Jα-linker helix that connects LOV2 to the kinase domain (36). In the dark, the Jα-linker is mostly docked to the β-scaffold of LOV2; light activation shifts the equilibrium of the Jα-linker from mostly docked (inactive kinase) to mostly undocked (active kinase) (**Figure 3*a***).

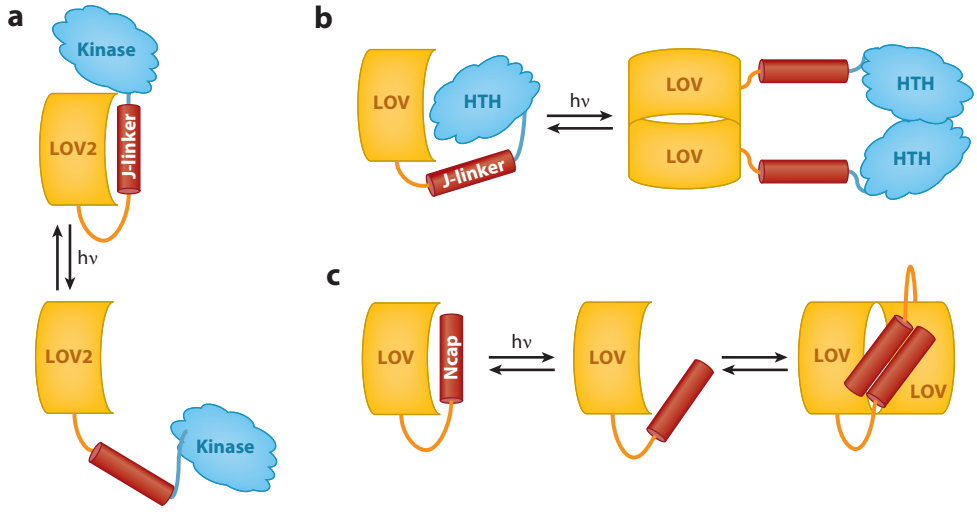

Figure 3

Signal propagation concepts in LOV proteins. (*a*) The extended antiparallel β-scaffold and regions flanking the photosensing LOV core play major roles in light-to-signal transmission. In *Arabidopsis thaliana* photolyase 1 (phot1), light relieves inhibition of the kinase activity by promoting undocking of the J-linker helix, mostly clamped at the β-scaffold of LOV2 in the dark. (*b*) In *Erythrobacter litoralis* (*El*) LOV-HTH the dark dimerization of the helix-turn-helix (HTH) domain is blocked by LOV-HTH interaction via the LOV domain β-scaffold. Light weakens the contact, thus promoting dimerization of *El*LOV-HTH and binding to target DNA. (*c*) The helical N-terminal cap (Ncap) is docked at the β-scaffold in the *Neurospora crassa* short-LOV protein VVD (Vivid). Light promotes dimerization, necessary for signaling, by partial undocking of the Ncap.

The first crystal structure of a full-length LOV protein from *Erythrobacter litoralis* (*El*) (81), however, depicts a different scenario. *El*LOV-HTH bears a helix-turn-helix (HTH) DNA-binding motif, but can bind to its target only after being photoexcited and undergoing dimerization of HTH. In the dark state, the helix involved in HTH dimerization is sequestered into interactions with the β-sheet surface of the LOV domain. Furthermore, in the dark state, the LOV domain interacts with HTH precisely where DNA should bind, constituting a further inhibition factor (81) (**Figure 3*b***).

In *Bs*YtvA, two acidic residues localized on strands Hβ, E105, and D109, conserved within YtvA-like proteins, are involved in signal transmission (3, 114) through a mechanism that is still ill-defined if taking into account published structures of the dimeric LOV domain (73) and a model-building of the whole protein (52). A torque mechanism has been proposed involving the Jα-linker arranged as a coiled coil in the dimeric structure (74), although E105 is not part of the dimeric LOV-LOV interface.

The β-scaffold surface also participates in LOV-LOV dimerization, but the relevance of this phenomenon in full-length proteins is unclear (65). As an example, *At*phot1 undergoes light-dependent dimerization in vivo, coinciding with a mechanism of light-driven autophosphorylation in *trans* (53). LOV1 is nevertheless not necessary for dimerization, although the isolated LOV1 is dimeric (99). In contrast with crystal structures, dimerization of LOV proteins/domains in solution may be a highly dynamic phenomenon, as shown for the short-LOV fungal protein VVD (Vivid). VVD is monomeric in the dark and tends to undergo a light-driven dimerization, but the lit structure appears to be a rapid monomer-dimer equilibrium (61, 126).

HTH: helix-turn-helix (DNA-binding protein motif)

In BLUF domains the β-scaffold is formed by five strands, only partially arranged antiparallel, named $\beta_1\beta_2\beta_3\beta_4\beta_5$. Studies with the *Escherichia coli* (*Ec*) YcgF protein and with *Synechocystis* (*Sy*) PixD suggest that the helical region comprising helices α_3 and α_4, C-terminal to β_5, is important in signal propagation (37, 38). Molecular dynamics simulations for BlrP1 from *Klebsiella pneumoniae* (*Kp*) reveal significant light-induced conformational changes in the $\alpha_3\alpha_4$ loop (55, 121). The extensive work on *Kp*BlrP1-BLUF suggests that after BL illumination, the C-terminal helical cap undergoes a reorientation process that might be associated with the conformational changes of $\beta_4\beta_5$ and $\alpha_3\alpha_4$ loops and strand β_5 (121). *Kp*BlrP1 bears a BLUF associated with an EAL domain (named after a conserved sequence) with phosphodiesterase activity [hydrolysis of cyclic diguanylate (c-di-GMP)]. *Kp*BlrP1-BLUF is a stable monomer in solution, whereas full-length *Kp*BlrP1 is dimeric (6, 121). This enzymatic activity (upon light absorption) is apparently regulated via allosteric communication between the two proteins in the dimer (6). A similar phenomenon was observed for *Ec*YcgF, a BLUF-EAL protein similar to *Kp*BlrP1, as YcgF exists in a fast and temperature-dependent monomer-dimer equilibrium, and light excitation results in transient dimerization of the monomeric species (80) (**Figure 4*a***).

c-di-GMP: cyclic diguanylate

Perhaps the most intriguing functional aspect for BLUF oligomerization has been elucidated for the short-BLUF protein *Sy*PixD, which forms a 10-subunit complex comprising two stacked pentameric rings (123). A recent analysis of the interactions of *Sy*PixD with its cognate response regulator PixE revealed that the latter drives aggregation of *Sy*PixD

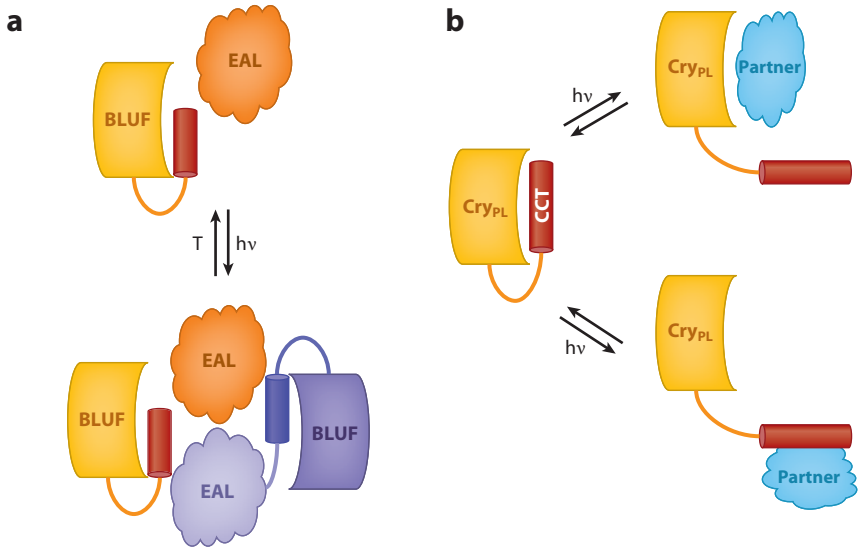

Figure 4

Signal propagation concepts in (*a*) BLUF and (*b*) cryptochrome (Cry) proteins. (*a*) In the *Escherichia coli* BLUF protein YcgF, the monomer-dimer equilibrium of the dark state is dependent on temperature and light. Light shifts the equilibrium toward the dimeric form. In the *Klebsiella* YcgF-like protein BlrP1, a light-regulated cyclic diguanylate phosphodiesterase, the enzymatically active form is the dimer. (*b*) In Cry, the photolyase (PL)–like domain (Cry$_{PL}$) is followed by a C-terminal tail [cryptochrome C-terminus (CCT)]. CCT can act as a direct repressor (*above*), inhibiting contact with partner proteins in the dark, e.g., in *Drosophila* Cry; alternatively, once released by light activation, CCT can act as a recognizing element for partner proteins (*below*). Abbreviation: EAL (named after a conserved sequence), cyclic diguanylate phosphodiesterase.

dimers into a *Sy*PixD$_{10}$-PixE$_5$ complex under dark conditions. Photoactivation destabilizes the complex into monomers of PixE and dimers of *Sy*PixD, most probably via conformational changes occurring at strand β_5 and at the β_4-β_5 loop (70, 113, 124, 125).

2.4. Signaling Issues in Cryptochromes

Cry proteins still remain cryptic even in their signaling mechanism. In plant and animal Cry, the CCT sequence flanking the PL-like domain is certainly involved in signaling, but it can act either as a repressor or as a recognizing element for partner proteins (**Figure 4*b***) (127). A detailed analysis of CCT in plant and human Cry proteins showed that this segment is intrinsically disordered in solution and interacts with Cry$_{PL}$ in the dark (87). In insect Cry, the removal of CCT renders the protein constitutively active (17), indicating that CCT inhibits an interaction interface for partner proteins. In plant Cry, CCT can interact with partner proteins—chiefly COP1 (constitutive photomorphogenesis protein 1)—upon light activation, promoting COP1-mediated degradation of a transcription factor. Dimerization is needed for this response (97). Bacterial Cry-DASH (ssDNA-PL) is void of regions flanking the PL-like domain; *At*Cry-DASH bears an N-terminal sequence, but its role in signaling is doubtful given that it is not conserved in all plant Cry-DASH (21). *Rs*CryB, in contrast, does not have N- or C-terminal extensions; therefore, signal propagation pathways that do not rely on CCT must exist (41).

Cry proteins neither repair DNA nor possess a kinase domain, but they can bind ATP and undergo self-phosphorylation (85). In plant Cry this process is light regulated and may depend on photoreduction via the conserved tryptophan triad (12). ATP binding stabilizes the *sq* form after photoactivation and affects protein conformation (16). This issue has recently been discussed (21, 127), but one important aspect should be kept in mind: According to a proposal by Liu et al. (62), electron transfer from $FAD^{\cdot -}$ to ATP may promote the self-phosphorylation reaction and conformational change of the photoreceptor.

3. PHOTOBIOLOGICAL RESPONSES MEDIATED BY LOV, BLUF, AND CRY PROTEINS

The biological roles of LOV, BLUF, and Cry proteins and the underlying signal-transduction cascades have been recently reviewed (21, 24, 65, 127). In plants, phot and Cry are originally related to phototropism and to the inhibition of hypocotyl elongation during photomorphogenesis, respectively (14). Other processes relying on phot light activation are stomatal opening, leaf expansion, chloroplast accumulation at low light intensity, chloroplast avoidance movement at high light intensities, and inhibition of hypocotyl growth during photomorphogenesis. In the green alga *Chlamydomonas reinhardtii*, phot regulates the algal sexual life cycle and modulates the expression of photosynthesis genes (44, 47). The main molecular partners of phot during signal transduction are NPH3 (NON-PHOTOTROPIC HYPOCOTYL-3), RPT2 (ROOT PHOTOTROPISM-2), and PKS1 (phytochrome kinase substrate 1) (24, 65).

In plants and animals, Cry proteins act as BL sensors regulating processes ranging from circadian entrainment to plant growth and development, and they trigger responses such as de-etiolation, anthocyanin accumulation, and flower induction. The main partner of Cry during light-controlled development is COP1, a ubiquitin ligase that suppresses photomorphogenic cellular development (21, 75, 127).

Recently, it was shown that light-activated *Drosophila melanogaster* (*Dm*) Cry can evoke changes in the resting potential of neurons within 100 ms (28). *Dm*Cry is otherwise a regulator of circadian rhythms, resetting the clock by light-activated targeting of TIM (TIMELESS) for degradation (88). *Dm*Cry-TIM interaction is nevertheless not necessary for Cry depolarization of neurons, a process most likely modulated by potassium channels. This

new phototransduction mechanism can also be transferred to neurons that are not normally photoreceptive (28).

Cry proteins are also the best candidates for light-dependent magnetic field sensors (photomagnetoreceptors), e.g., in insects, migratory birds, and fish (120). The underlying molecular mechanism is still under debate, but recent data indicate that the UV/violet cones could host these photomagnetoreceptors in birds (82).

3.1. Kinases and Circadian Regulators: From LOV Proteins to Cryptochromes and Back

Phots are serine/threonine kinases that undergo light-regulated self-phosphorylation, a key event in signaling (48). In the prokaryotic world they are mirrored by a large number of LOV-HKs of the bacterial two-component signal-transduction systems (65). A similar LOV-HK system modulates cell adhesion in *C. crescentus* (94).

Are LOV-HKs indeed the bacterial counterpart of plant phot? LOV proteins identified in the picoalga *Ostreococcus tauri* (*Ot*) and in other Chlorophytae species seem to contradict this idea (25). Unusually, this organism bears both a plant-like phot protein and a bacterial-type LOV-HK, whose LOV domains seem to have a different evolutionary history than those from other Chlorophytae species (25). Although the role of *Ot*phot has not been established (118), it is clear that *Ot*LOV-HK is involved in circadian rhythms (25). In higher plants, LOV proteins related to circadian rhythms are built entirely differently: in the ZTL/LKP2/FKF1 family, a single LOV domain is linked to an F-box and six Kelch repeats (75). These proteins mediate ubiquitin-dependent protein degradation, ultimately leading to photoperiodic expression/accumulation of proteins involved in circadian clock regulation and flowering onset (7, 29, 65). Among fungi, the best-known LOV-based circadian system is that from *Neurospora crassa* (*Nc*). *Nc*WC-1 (white-collar-1), a zinc-finger-LOV transcription factor, belongs to the white-collar complex that acts together with VVD during photoresponses (102).

Circadian regulation and photoperiodism bring us back to Cry proteins: in plants, Cry1 and Cry2 also act as circadian inputs in shortening the period length in the light. Molecular partners for these responses are partially in common with the ZTL/LKP2/FKF1 LOV proteins (e.g., *CONSTANS*) (21). Circadian rhythms are also the field for animal Cry—type I being responsive to light (e.g., in *Drosophila*), and type II acting independent of light (e.g., in humans) (88).

The picoalga *O. tauri* again offers a striking example of an unusual Fl-Blues: *Ot*CPF1 is a (6-4) PL but also influences circadian rhythms (40). In other words, this protein is a PL with an additional light-sensing role, a feature inconceivable under the old criterion that Cry are light sensors without DNA repair capability (19). Other bifunctional Cry/PL proteins have been described recently: in *Aspergillus nidulans*, CryA regulates sexual development and has a CPD PL activity (9); in the marine diatom *Phaeodactylum tricornutum*, CPF1 has a (6-4) PL activity and at the same time regulates (BL-dependent) transcription of genes involved in photosynthesis (22); and in the cyanobacterium *Synechocystis*, Cry-DASH has a putative role in gene regulation and phototaxis (76) and a weak PL activity (15).

If circadian rhythms and photoperiodism have brought LOV proteins close to Cry in their biological roles, then from the molecular point of view we recall here the light-regulated self-phosphorylation of Cry (see Section 2.2) (21), reminiscent of the same process in phot and hybrid LOV-HK/response regulator proteins (18).

3.2. Lights On and Gene Transcription

The photoresponses described in Section 3.1 ultimately affect gene expression, often as part of a chain of events. Some LOV proteins instead are equipped with effector domains able to directly interact with target genes, such

as the fungal zinc finger containing WC-1 (5), the algal basic leucine zipper (bZIP), AUREOCHROMEs (AUREOs) (112), and the bacterial *El*LOV-HTH transcription factor (81). The LOV-bearing AUREO from the stramenopile *Vaucheria frigida* binds to target DNA and regulates gene expression in a BL-dependent way. The physiological response regulated by this AUREO1 is cell branching (50, 112). BL absorption increases the affinity of the bZIP domain for DNA, probably by affecting homo- and heterodimerization patterns (64), similarly as described above for *El*LOV-HTH (Section 2.3). A few other bacterial proteins have been found with HTH domains (65), but their proposed functions are not proven. They are, nevertheless, among the most interesting proteins for biotechnological applications (Section 5), given the intriguing possibility of directly modulating gene expression by light.

An indirect method of induction of gene expression is carried out by YtvA in *B. subtilis* and in the pathogen *Listeria monocytogenes* via upregulation of the alternative stress sigma factor B (σ^B) of the RNA polymerase holoenzyme (30, 84). The σ^B factor is one of the key components in the general stress response of this group of microorganisms and controls the transcription of more than 200 genes (1). It is not clear whether light input and the LOV protein are of any importance during *Listeria* infection (84); however, a direct and dramatic effect (in increasing virulence) has been convincingly demonstrated for a LOV-kinase in *Brucella abortus* in a cell-culture assay (111).

A different way to photoregulate gene transcription was found for *Ec*YcgF, built of a BLUF domain and a degenerate EAL domain unable to hydrolyze c-di-GMP. YcgF directly binds to the repressor YcgE upon BL irradiation, thus inducing expression of proteins involved in biofilm formation. Furthermore, the YcgF-YcgE system integrates BL and other stress signals, being induced at low temperature and under starvation conditions (115). The integration of light and temperature signals via YcgF is modulated by the temperature dependency of gene expression (115), and it also depends on a monomer-dimer equilibrium (80).

The activity of YcgF is reminiscent of *Rs*AppA. AppA binds the repressor protein PpsR constitutively at low oxygen tension, whereas under fully aerobic conditions PpsR is released from AppA and binds to the promoter of certain photosynthesis genes, repressing their transcription. These responses are light independent, but at intermediate oxygen concentration, BL determines whether AppA releases PpsR (72). Thus, AppA integrates both redox and light signaling. Redox sensing relies chiefly on the oxygen-binding ability of the SCHIC (sensor containing heme instead of cobalamin) domain (77), and probably involves a C-terminal cysteine-rich sequence (57). Recently, the bound flavin has also been proposed to contribute to the redox–light sensing interplay (2). The activity of these systems in *R. sphaeroides* is aimed at maximizing photosynthesis under favorable conditions and reducing it when the risk of photooxidative damages is high.

3.3. Fl-Blues Regulation of Biofilms and Cyclic Nucleotides

In several cases, BLUF-dependent responses in bacteria appear to be related to the turnover of cyclic nucleotides, important second messengers in prokaryotes (33). Besides a few examples of LOV and BLUF domains linked to adenosine monophosphate (AMP) and guanosine monophosphate (GMP) cyclases (65), a considerable number of these proteins are predicted to be involved in the turnover of c-di-GMP, a second messenger regulating the formation of biofilms, motility, and virulence, among others (96). The turnover of c-di-GMP is accomplished by the GGDEF (cyclase; as with EAL, named after a conserved sequence) and EAL (phosphodiesterase) domains. In *Kp*BlrP1 from *K. pneumoniae* (ortholog to *Ec*YcgF), the EAL domain indeed shows light-activated phosphodiesterase activity (6). A related system was found in *Rhodopseudomonas palustris*, where PapB, a short BLUF protein, interacts with

the c-di-GMP phosphodiesterase PapA in a light-regulated way, influencing biofilm formation (54).

BlsA from the opportunistic pathogen *Acinetobacter baumannii* strain ATCC 17978 is also a short BLUF protein (79). Several responses appear to be modulated by BL through BlsA: motility and biofilm formation are negatively regulated, whereas virulence is positively regulated. Furthermore, temperature has a cross-effect with BL, probably owing to differential expression of the protein at different temperatures (79) or to a temperature-dependent monomer-dimer equilibrium (80).

In eukaryotes, the only known examples of BLUF proteins are the PACs of euglenoids (*Eg*). *Eg*PAC consists of two subunits, PAC-α and PAC-β, each comprising approximately 1,000 amino acids. Each protein (α and β) carries two BLUF + adenylate cyclase motifs arranged in tandem (49). They function in a PAC-α/PAC-β heterodimeric complex as BL-regulated adenylate cyclases, responsible for step-up photophobic responses and both positive and negative phototaxis. A similar but much smaller protein from the bacterium *Beggiatoa* sp. is also a light-regulated adenylate cyclase, but its function for the bacterium is unknown (98, 107).

4. EVOLUTION AND ECOLOGICAL SIGNIFICANCE OF FL-BLUES

The interplay between detrimental and beneficial effects of light and their impact in evolution is most evident in the UVA/BL range. Potential damage to nucleic acids and photosensitizing effects, mediated by endogenous absorbers, are counteracted by stimulation of growth and photosynthesis (43). In addition, BL penetrates the deepest of all spectral qualities into a water column and constitutes a ubiquitous information source. BL is thus an ambivalent environmental factor: On the one hand, it represents an attracting light quality allowing activation of the photosynthetic apparatus and is essential for the function of light-activated DNA-repairing enzymes (101); on the other hand, ubiquitous porphyrins and flavins, the same cofactors that optimize respiration and photosynthesis, are powerful photosensitizers, mainly by forming toxic oxygen species (65). The interplay between oxygen and the high-energy UVA/BL spectral range is certainly a selective factor in evolution and has induced development of highly specialized light-sensing systems. The large number of sequences that are now available from genome projects is of great help in elucidating these patterns of evolution. Environmental genomics or metagenomics are likely to bring rapid change by discovering novel proteins, as recently was demonstrated with LOV, BLUF, and Cry/PL proteins (89, 105). Similar approaches have recently added—unexpectedly—members of the phytochrome family to the UV/BL-sensing photoreceptors (see sidebar, New Kids on the Block: Members of the Red/Far-Red-Sensing Phytochromes with UV/Blue-Light-Sensing Functions) (46).

NEW KIDS ON THE BLOCK: MEMBERS OF THE RED/FAR-RED-SENSING PHYTOCHROMES WITH UV/BLUE-LIGHT-SENSING FUNCTIONS

The ambivalence of the BL region calls for a delicate balance between its pros and cons. Accordingly, many photoreceptor types have been developed to sense this spectral quality. Animal rhodopsins are the most striking paradigm (note the yellow spots in our eyes that—in the region of highest visual performance—filter away most of the BL!). Rhodopsins, however, with few exceptions, are not present in the bacterial world. Besides the Fl-Blues discussed in this article, a group of photoreceptors have added sensitivity to this spectral region that had not been expected to serve this purpose. The phytochromes—photoreversible red- and far-red-sensing receptors in plants—perform their function via a double-bond photoisomerization of their covalently bound bilin chromophore. Recent genome digging and molecular biology studies have revealed their presence in a large number of bacteria with a remarkable spectral and photochemical variation. These studies have identified not only reversibly red-to-green switching chromoproteins, but also representatives that transiently interact with a cysteine residue, generating a bilirubin-type chromophore with absorbances in the near-UV/BL region.

4.1. Cryptochromes and Photolyases: Evolution of a Big Family

The Cry/PL family is particularly suitable for a sequential and phylogenetic analysis owing to its structural arrangement: the photosensing Cry_{PL} unit has a stand-alone function and is coupled to a CCT tail in plant and animal Cry or, more rarely, an N-terminal cap (21). A recent comprehensive work has screened 882 sequences from Archaea, Eubacteria, and Eukarya (69) and identified six major groups: (*a*) CPD class II PLs, present in Archaea, Eubacteria, Eukarya, and viruses; (*b*) the large heterogeneous group of CPD class I PLs in Archaea, Eubacteria, and Eukarya; (*c*) (6-4) PLs and animal Cry proteins in Eukarya and, atypically, in the cyanobacterium *Gloeobacter violaceus*; (*d*) ssDNA-PL/Cry-DASH in Archaea, Eubacteria, and Eukarya; (*e*) plant Cry proteins and a group of CPD bacterial PLs, first described in *C. crescentus* as CPD class III PLs, that cluster close to CPD class I PLs, including two fish proteins likely acquired via horizontal gene transfer; and (*f*) a novel prokaryote-specific PL group, clustering next to (6-4) PLs and animal Cry proteins. The first four PL subfamilies were most likely already present in the common ancestor of this protein in the three domains of life, but were repeatedly lost. At least two of them, plant and animal Cry, acquired photosensing functions. The sixth group is novel but could be evolutionarily important given its wide taxonomic range (suggesting an ancient origin) and its clustering close to (6-4) PLs. The authors of this study suggested that (6-4) PLs may have evolved even before the eukaryotes (69).

Further insight into Cry/PL evolution may be derived from the study of *O. tauri* PL-like proteins *Ot*CPF1 and *Ot*CPF2, similar to (6-4) PL/animal Cry and Cry-DASH, respectively. *O. tauri* does not carry genes for plant Cry. The two PL proteins certainly have a double activity as DNA repair enzymes and as light sensors (40) but function differently than plant Cry. Interestingly, the authors suggested that bifunctional Cry/PL could help enhance growth at low light intensities and could act as classical PLs at high intensities (40). The number of such bifunctional Cry/PL proteins is likely to increase, and the work with *O. tauri* substantiates previous reports on Cry-DASH (15) and on fungal and diatom Cry/PL (9, 22).

4.2. The Evolution of LOV Domains

O. tauri and other Chlorophytae species also offer a glimpse of the evolution of LOV proteins (25). The contemporary presence of plant-like phots and bacterial-like LOV-HKs involved in circadian regulation has offered the opportunity to investigate the origin and evolution of LOV proteins in the "green lineage." A phylogenetic analysis suggests that phots and LOV-HKs have a different evolutionary history (25). Chlorophytae/plant phot-LOV domains cluster close to AUREO-LOV but not to the LOV domains from Chlorophytae LOV-HK (25). Up to this point, LOV-HKs of the two-component system were known solely from prokaryotes (65, 116).

An extensive phylogenetic analysis on prokaryotic and eukaryotic LOV domains had already suggested an independent evolution of the LOV domains of the ZTL/ADO/FKF1 family of circadian regulators with respect to other eukaryotic LOV domains, i.e., originating from two distinct endosymbiotic events (59). The prokaryotic proteins cluster as a monophyletic group with cyanobacterial LOV domains, whereas the eukaryotic domains are related to alphaproteobacteria clades. LOV-based circadian regulators with distinct origin—e.g., ZTL/ADO/FKF1 and fungal WC-1—seem to have undergone convergent evolution to a similar function (59). LOV domains thus appear to be of bacterial origin, and their gene must have undergone frequent horizontal gene transfer, massive gene duplication, and gene loss in prokaryotes.

LOV domains, together with red-light-sensing phytochrome-like proteins, are mainly represented in bacterial groups where photosynthesis is also active, chiefly in cyanobacteria and alphaproteobacteria (65). In

addition, LOV proteins are the sole Fl-Blues present in Archaea, although their role has not been determined. Archaeal LOV domains, restricted to a few mesophilic Euryarchaeota species, are well separated from the bacterial proteins in a phylogenetic tree. It is nevertheless not possible to establish whether they represent the evolutionarily oldest sequences or whether Archaea acquired LOV genes by horizontal gene transfer (59).

4.3. Going Out: Metagenomics of Fl-Blues

Genome screening from environmental samples—the arena of metagenomics—reveals the occurrence of Fl-Blues in practically every environment. More than 10,000 Cry/PL orthologs from four metagenomes (surface seawater from the Sargasso Sea, farm soil, acidic mine runoff, and deep-sea whale fall) have been identified, the large majority of which are localized in surface water or on top of microbial mats, consistent with the higher UV radiation in such locations (105). This study has also uncovered two novel Cry/PL families with still unknown functions.

BLUF proteins have thus far been found solely in bacteria and protists, and in most cases these proteins consist of a stand-alone BLUF domain with flanking regions (65). The same observation holds for metagenomes, for which, in this case, gene neighborhood analysis is probably the best tool to characterize novel functionalities. Of the 73 identified metagenomic BLUF domain proteins, 36 were analyzed in such a way, confirming BLUF function in phototaxis, nucleotide metabolism, and the repression of anoxygenic photosynthesis, but also uncovering novel functions: luciferase synthesis, nitrate metabolism, and quorum sensing (105).

Mining in metagenome databases also yielded for the LOV domains a relatively small number (approximately 580) of open reading frames with signatures strictly conserved in this BL-photoreceptor domain. Defining a positive hit for a LOV domain has turned out to be relatively difficult, as LOV domains share a great sequential and structural similarity with the ubiquitous PAS domains. In addition, an individual inspection of each marked sequence had to identify those amino acids that are essential for the photochemistry and stabilization of the chromophore in the binding pocket. Yet this survey revealed that nearly three-quarters of the LOV domain sequences can be considered novel, taking a conservative value of 80% sequence similarity with LOV domains from bacterial genomes (90). A recent DNA-microarray-based environmental screening yielded a multidomain protein in a soil sample that carried a LOV domain followed by an HK and a fused response regulator; the phylogenetically nearest neighbor protein exhibiting practically the same domain arrangement was found in the genome of *Methylibium petroleiphilum* strain PM1 (89). Going one step further with this metagenomic gene, this study presented a functional proof by heterologously expressing the LOV domain in a fully photochemically functional form.

5. BIOTECHNOLOGICAL APPLICATIONS AND NEW TRENDS

The term optogenetics has been coined for the use of light-gated proteins originally designed by nature as tools to photomodulate cell activities (39). Prompted by the success of the light-sensitive, retinal-protein channelrhodopsin that provides a nontoxic, inheritable mechanism for the selective manipulation of cell membrane potential, optogenetics applications are now also becoming available with Fl-Blues of the LOV and BLUF families (58, 74). Involving LOV and BLUF proteins adds a fascinating additional aspect to this novel research field. These proteins are usually built from domains (modules)—i.e., the light-sensing part and the signal-generating/transducing part. Considering the already wide variety of signaling domains in Fl-Blues (preferentially for the LOV proteins), one sees immediately the exciting potential: Not only changes in the cell potential as for channelrhodopsin but also

a number of other regulatory functions—even those that do not exist in nature (or have not yet been found)—can be imagined to be modulated by light. The PAC from *E. gracilis* (carrying a BLUF domain and an AMP cyclase) has initiated optogenetic research with Fl-Blues (103). Its function, light-regulated cyclic AMP (cAMP) formation, leads to activation of neurons upon functional expression of PAC. The rapid increase in cAMP, which in turn regulates gene expression in eukaryotic cells via a phosphorylation cascade, is also a promising aspect. A bacterial BLUF adenylyl cyclase from the gammaproteobacterium *Beggiatoa* sp. PS, bPAC (98), appears to be even more efficient and versatile than the *Euglena* PAC once integrated into a host cell system (107). bPAC can also be converted into a cyclic GMP (cGMP) cyclase by mutations, extending its range of applications (98) (**Figure 5*a***). The system is turned off within seconds by thermal recovery to the dark-adapted state and by phosphodiesterases present in the host cell (**Figure 5*a***): Tuning the photocycle precisely,

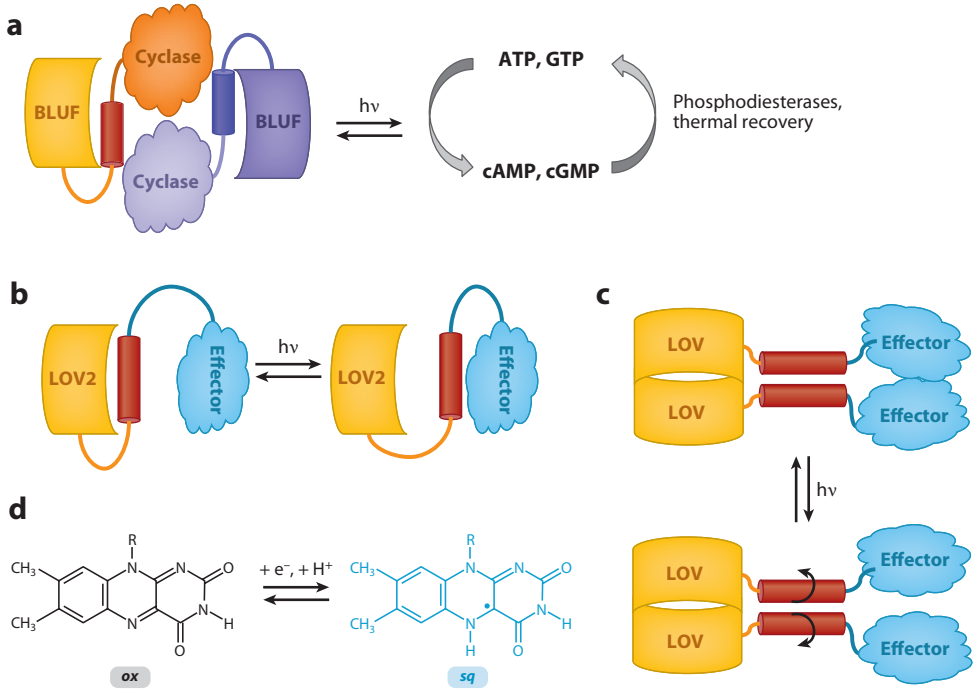

Figure 5

Key issues in LOV- and BLUF-based optogenetics. (*a*) Photoreceptors bearing an enzymatic domain able to regulate the concentration of metabolites. Crucial are specificity, duration of the photocycle, and enzymatic inactivation (here phosphodiesterase). BLUF cyclases can be expressed in nonnatural hosts and produce cyclic nucleotides in a light-regulated way. Specificity toward cyclic guanosine monophosphate (cGMP) could be acquired via specific mutations in a bacterial BLUF cyclase. Abbreviation: cAMP, cyclic adenosine monophosphate. (*b*) Undocking of the Jα-linker in LOV2 constructs activates a linked effector. Engineering aims to minimize constitutive activation in the dark and optimize light regulation via mutations that affect the thermodynamic barrier between the docked and undocked (inactive-active) states. (*c*) The torque mechanism requires dimerization, with the Jα-linker adopting an α-helical coiled-coil conformation. Light activation induces a torque effect on the coiled linker, thus switching the effector activity on or off. (*d*) Depending on the redox potential of the cell, the chromophore could be in a photochemically competent *ox* (oxidized, *black*) or noncompetent *sq* (semiquinone, *blue*) state.

by means of site-directed mutagenesis, to the enzymatic characteristics of the host cell is mandatory for future applications of this and other photoswitchable proteins (65, 74).

The latest exciting news came out for Cry proteins. The novel phototransduction mechanism operating in neurons coupling *Dm*Cry to a millisecond membrane depolarization (Section 3) is transferable to inherently light-insensitive neurons (28). Tests with olfactory projection neurons proved that *Dm*Cry can autonomously confer light responsiveness to nonclock neurons, highlighting a mechanism that couples light activation to rapid changes in membrane potential, independent of clock proteins (28).

5.1. Exploiting Modularity and Engineering Novel Light Tools

The engineering approach takes advantage of the natural modularity of LOV and BLUF systems for the construction of hybrid proteins. A direct light-regulated DNA binding was accomplished by joining a phot-LOV2 domain to the *E. coli* tryptophan repressor protein (TrpR), making use of the light-induced undocking of the Jα-helix from the LOV core (108). Yet in the LOV2-TrpR hybrid protein, the degree of light activation was modest. This is linked to the fact that the docked-undocked equilibrium of the Jα-helix is shifted in LOV2-TrpR toward the undocked state, rendering the protein mostly functionally active even in the dark (109) (**Figure 5*b***). Using site-directed mutagenesis, the regulatory effect of light on DNA binding by LOV2-TrpR could be hugely improved (109). Similar exciting applications for light-induced DNA binding can be foreseen for derivatives of AUREO or *El*LOV-HTH (27, 81, 112).

A recent construct emphasizes the engineering approach: The Rho-like GTPases Rac and Cdc42 have been placed under the control of a LOV domain; these proteins are activated upon irradiation in living cells to produce protrusions, filopodia, etc. Such constructs can also serve as spatiotemporal probes for signaling pathways in living cells (122).

Employing the building principle of many LOV domains—being fused to HK in bacterial proteins—the LOV domain of *Bs*YtvA was fused to the HK from the oxygen-sensing protein FixL. The light-promoted dampening of kinase activity in this construct seems to rely on a torque effect (**Figure 5*c***) (73, 74) and requires dimerization.

5.2. Fluorescent Reporters and the Redox Question

The relatively high fluorescence quantum yield of LOV domains allows applications such as fluorescence-based cellular studies. As fluorescence is lost upon formation of the photoadduct, this last process can be annihilated by mutating the reactive cysteine into alanine or serine, yielding a permanently fluorescent molecule (26). In fact, compared with GFPs, where chromophore formation is oxygen dependent, LOV domains are advantageous for applications in anaerobic or microaerobic environments as well as for investigating viral infections of plants (20, 26).

An intriguing aspect is represented by the above-discussed redox state of the flavin chromophore (Section 2.1). The redox potential of a living cell is close to the midpoint potentials measured for LOV- and BLUF-bound flavins, i.e., −250/300 mV (2, 93), and such Fl-Blues are photoactivated only when the chromophore is fully oxidized (**Figure 5*d***). This might be a drawback for optogenetic applications, but could be helpful for fluorescence studies similar to applications already performed with targeted GFP variants for the real-time estimation of redox potentials within specific cell compartments (117).

6. CONCLUDING REMARKS AND OUTLOOK

Powerfully emerging from a long-lasting quest and an ancient biosynthetic pathway, Fl-Blues are rapidly conquering the field of

UVA/BL photobiology in all three domains of life. Physiology, chronobiology, microbiology, evolutionary biology, biophysics, and biotechnology are the major research areas involved. Although few case stories have been described, Fl-Blues also seem to constitute light-perceptive systems in prokaryotes, a sort of primitive visual tool integrated with other signal-transduction systems for screening and responding to environmental conditions. Genomics and proteomics of picoeukaryotes and other microorganisms, together with environmental genomics, provide important clues on the phylogeny and evolution of Fl-Blues. Parallel to basic research, biotechnological applications are likely to grow in number in the near future, given the possibility of exploiting Fl-Blues in a large variety of cellular systems.

SUMMARY POINTS

1. The discussion on the details of photochemical reactions in Fl-Blues continues. The hottest topic is the nature of the dark-adapted state in BLUF and Cry proteins.
2. The interplay of light and redox input on the flavin chromophore, modulated by the microenvironment, is one of the most interesting aspects that has recently emerged.
3. Researchers are now in a position to modulate the duration of photocycles without impairing function, which had been one of the major issues for biotechnological applications.
4. Various signal-transduction mechanisms for Fl-Blues are now being elucidated, offering a picture where surprising analogies as well as surprising variations on themes are emerging.
5. The occurrence of bifunctional Cry/PL proteins has broken a paradigm and is important for understanding the evolution of this variegate protein family.
6. The biological effects of Fl-Blues are well understood in plants and in some insects, but much less so in animals and prokaryotes. Microbiologists are now increasingly interested in this topic, and we may expect a substantial progress in the field.
7. Genomics, metagenomics, and phylogenetic analyses are enlarging our understanding of the spread and evolution of Fl-Blues. The case story of picoalgae is emblematic.
8. Optogenetics and other biotechnological applications with Fl-Blues are a reality and in expansion. The latest news about Cry-mediated activation of neurons opens novel exciting possibilities for applications.

DISCLOSURE STATEMENT

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