Structure and Function of Plant Photoreceptors

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Abstract

Signaling photoreceptors use the information contained in the absorption of a photon to modulate biological activity in plants and a wide range of organisms. The fundamental—and as yet imperfectly answered—question is, how is this achieved at the molecular level? We adopt the perspective of biophysicists interested in light-dependent signal transduction in nature and the three-dimensional structures that underpin signaling. Six classes of photoreceptors are known: light-oxygen-voltage (LOV) sensors, xanthopsins, phytochromes, blue-light sensors using flavin adenine dinucleotide (BLUF), cryptochromes, and rhodopsins. All are water-soluble proteins except rhodopsins, which are integral membrane proteins; all are based on a modular architecture except cryptochromes and rhodopsins; and each displays a distinct, light-dependent chemical process based on the photochemistry of their nonprotein chromophore, such as isomerization about a double bond (xanthopsins, phytochromes, and rhodopsins), formation or rupture of a covalent bond (LOV sensors), or electron transfer (BLUF sensors and cryptochromes).

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INTRODUCTION

Light is a major developmental cue that generates an initial signal upon absorption of a photon of visible light, transmits this signal through elaborate molecular and metabolic pathways, and ultimately modifies the "behavior" of plants: It influences critical aspects of development, morphology, and metabolism. In this review, we adopt the perspective of biophysicists interested in processes of light-dependent signal transduction in nature and the three-dimensional structures that underpin them. We focus on the better-understood upstream events closest to the site of photon absorption and explore results at the molecular rather than the cellular or organismal level of structure. Important though they surely are, the influence of these higher levels of structure on the transduction of light into physiological function or behavior is not yet well understood.

Light that will modify behavior is directly absorbed by protein molecules known as signaling photoreceptors or more simply as photoreceptors. Because membranes are effectively transparent to light, most photoreceptors are cytoplasmic and water soluble. In other key molecules that absorb light, e.g., light-harvesting complexes or photosynthetic reaction centers, the energy derived from absorbing a photon generates an electrochemical potential gradient across a membrane that can be harnessed to drive energy-requiring chemical processes. Thus, in contrast to most signaling photoreceptors (but in common with chemoreceptors, whose small molecule ligands cannot traverse membranes), light-harvesting complexes and reaction centers are integral membrane proteins. Signaling photoreceptors and the more widely studied chemoreceptors thus have quite different cellular locations but may retain intriguing similarities in their signaling properties (99).

The absorption properties of photoreceptors match the spectrum of light falling on them, typically the solar spectrum at Earth's surface extending from the near UV (~350 nm) through the blue to the red/far red (~750 nm), filtered by, for example, a leaf canopy. Because the polypeptide backbone and amino acid side chains do not absorb in this visible range, all photoreceptors contain an organic, nonprotein component known as a chromophore that serves as the primary site of photon absorption and may be covalently or noncovalently bound to the protein. All chromophores are partly unsaturated, thus allowing electron delocalization across a conjugated π system. The larger the chromophore, the greater is the possible extent of electron delocalization and the longer is the wavelength at which it will absorb. Examples of plant chromophores include flavin adenine dinucleotide (FAD), which absorbs in the blue, and the

linear tetrapyrrole phytochromobilin (PΦB), which absorbs in the red to far red (**Figure 1**). The presence of chromophores enables various forms of UV/visible spectroscopies to be successfully applied. Photoreceptors are commonly classified by the chemical nature and photochemistry of their chromophore and at present, six distinct classes are known: light-oxygen-voltage (LOV) sensors, xanthopsins, phytochromes, sensors of blue-light utilizing FAD (BLUF), cryptochromes, and rhodopsins (**Figure 1**) (146).

The energy contained in a photon in the visible spectrum, 40–60 kcal mol^{-1}, is sufficiently large to easily drive chemical processes such as electron transfer, formation or rupture of a covalent chemical bond, or isomerization about a double bond, if suitably harnessed. Thus light can readily influence chemistry. Each of these processes is accompanied by changes in atomic position or extent of motion, both of which can change the affinity of one part of the photoreceptor for another part, or of the photoreceptor for another cellular constituent, e.g., a small molecule, protein, DNA, or membrane. A change in affinity is essentially thermodynamic in nature; thus a signal possesses both thermodynamic and structural aspects (99). Each chromophore exhibits a specific photochemistry and photophysics, and particular photoreceptors thus utilize different chemical processes. Photoreceptors have evolved to maximize the quantum yield for generating a signal and, correspondingly, to minimize the quantum yields for nonproductive, rapid, competing processes of vibrational/thermal and fluorescence de-excitation. In practice this means that the initial changes in the excited state of the chromophore must be efficient, specific, and very fast, e.g., intersystem crossing, electron transfer, excited state proton transfer, or facile isomerization about one or more double bonds.

Two features are very characteristic of both photoreceptors and chemoreceptors: They are modular in their architecture, and each module is associated with a different aspect of receptor function (110). A photoreceptor typically comprises several discrete protein units known as modules or domains, each of which forms a covalently connected, compactly folded portion of the sequence. Domains are linked together by elements of secondary structure such as α helices or by extended loops. One or more modules may bind the chromophore that absorbs light and serves as a sensor or input domain; another may promote dimerization or association with another protein, small molecule, or membrane; yet another may exhibit light-dependent catalytic activity or DNA binding and serve as an effector or output domain, which interacts with one or more components further downstream in the signaling pathway. The sensor domain is usually (but not exclusively) located N-terminal to the effector domain. Although less emphasis is typically placed on the linker segments and extensions at the N and C termini, their structures are often also light dependent and play important roles in signal transduction (99). The modular structure of representative photoreceptors from each of the six known classes is illustrated in **Figure 2**. Some photoreceptors such as phototropins, neochrome, and *Rhodospirillum centenum* Ppr (**Figure 2**) contain two or more chromophores, which introduces the possibility of interaction between signals. For example, signals may originate in absorption of both blue and red light, or in absorption of blue light and a redox or other chemical signal. A representative tertiary structure of each class of sensor domain is shown in **Figure 3**.

Comparative genomics suggests that the number of different types of domains has been conserved during evolution; this number is roughly the same in the worm, fly, plants, and humans. It appears that individual domains have been substantially conserved in both structure and function, but their combination into multidomain proteins differs in plants and other evolutionarily distant organisms. Thus, human proteins contain almost twice the number of multidomain combinations in a single polypeptide chain than do proteins from lower organisms (79). Combinatorial mixing of domains during evolution apparently has conferred greater phenotypic complexity in signaling and regulation (85). Thus, a single class

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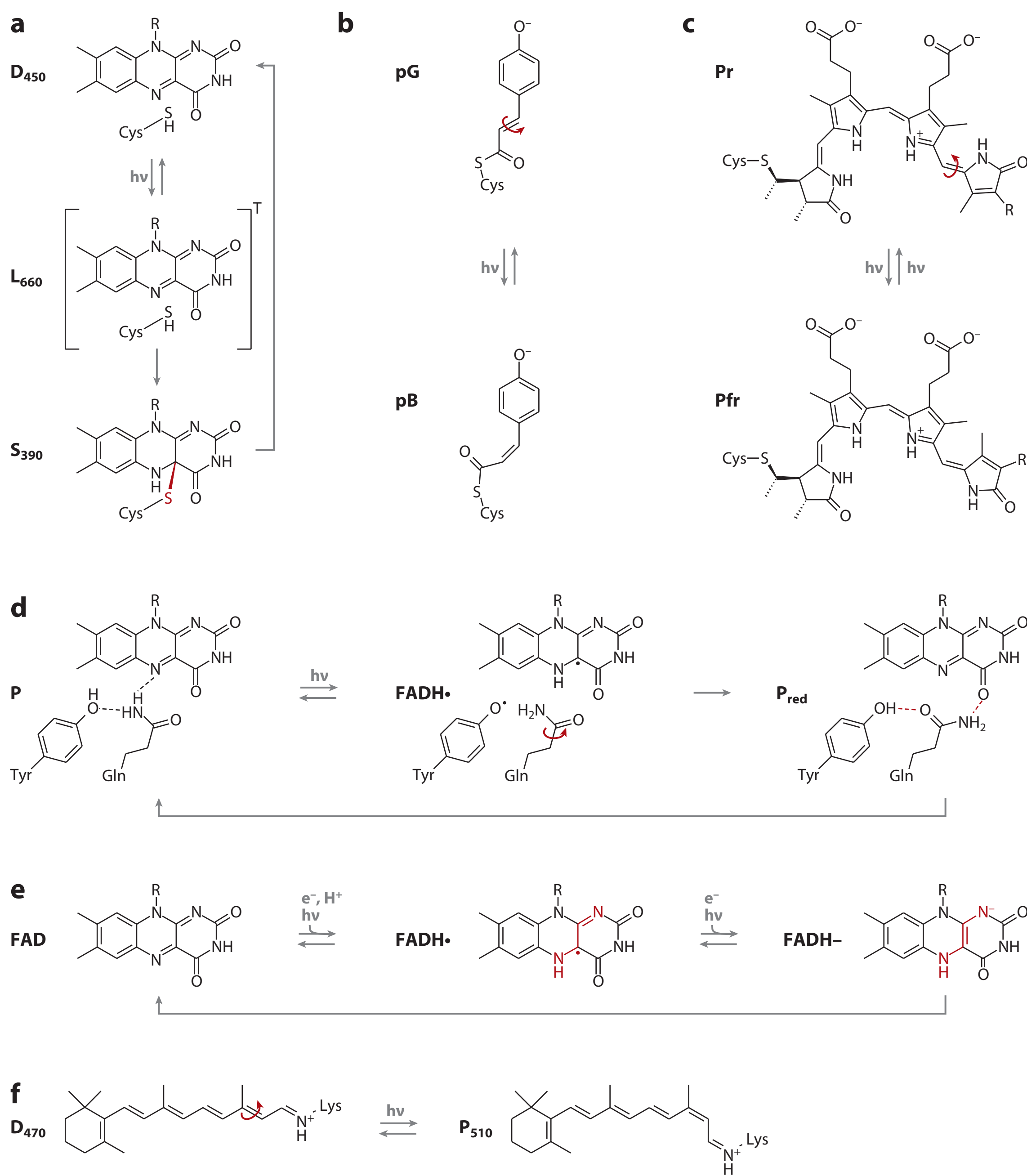

Figure 1

Chromophores and simplified photochemistry of the six photoreceptor classes. (*a*) Light-oxygen-voltage (LOV). (*b*) Xanthopsin. (*c*) Phytochrome. (*d*) Blue-light sensors using FAD (BLUF). (*e*) Cryptochrome. (*f*) Rhodopsin.

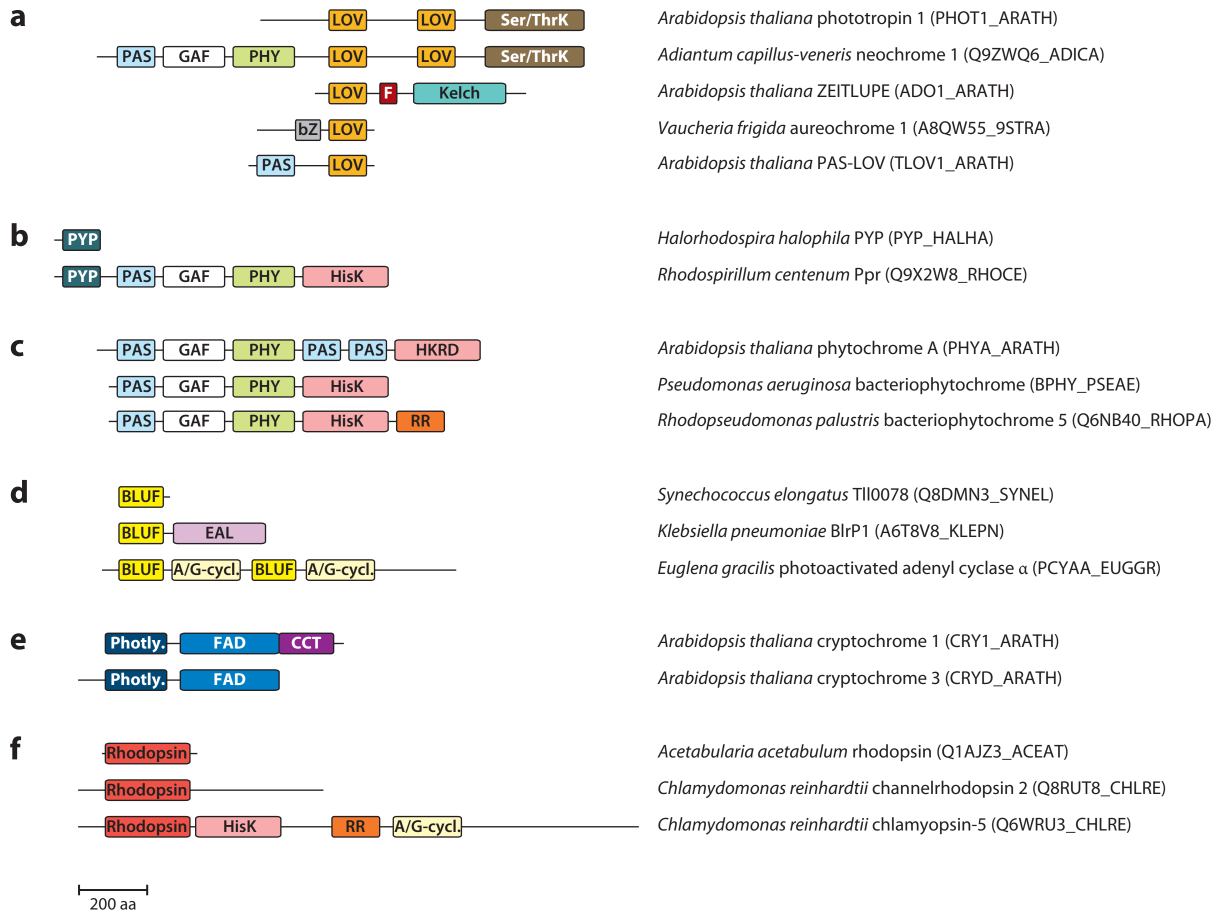

Figure 2

Domain composition of representative photoreceptors of the six classes according to the Pfam database (41). (*a*) Light-oxygen-voltage (LOV). (*b*) Xanthopsin. (*c*) Phytochrome. (*d*) Blue-light sensors using FAD (BLUF). (*e*) Cryptochrome. (*f*) Rhodopsin. Proteins are drawn approximately to scale and labeled with their UniProt identifiers (145). Domain abbreviations are Ser/ThrK (serine/threonine kinase), GAF (GAF domain), PHY (phytochrome), F (F box), Kelch (Kelch repeat), bZ (basic zipper), HisK (histidine kinase), HKRD (histidine kinase-related domain), RR (response regulator), EAL (diguanylate phosphodiesterase), A/G-cycl. (adenylate/guanylate cyclase), Photly. (photolyase α/β domain), FAD (photolyase α domain), CCT (cryptochrome C-terminal domain).

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of photosensor domain such as the LOV blue-light sensors is found covalently attached to a very wide range of effector domains that differ markedly in their tertiary structure and biological activity (28). Conversely, a single class of effector domains such as phosphodiesterases is found covalently attached to more than one class of sensor domains, e.g., to LOV or BLUF domains. The effectiveness of combinatorial mixing of domains during evolution implies that it can be used as a powerful principle for the design of novel sensor molecules. This has indeed proved to be the case (81, 98, 133), as we discuss below.

The modular nature of photoreceptors has enabled a "divide and conquer" experimental strategy in which individual domains within a longer, complex photoreceptor are identified, isolated, and characterized separately in both structural and functional aspects. The compact nature of each domain implies that it folds more or less independently of all other domains

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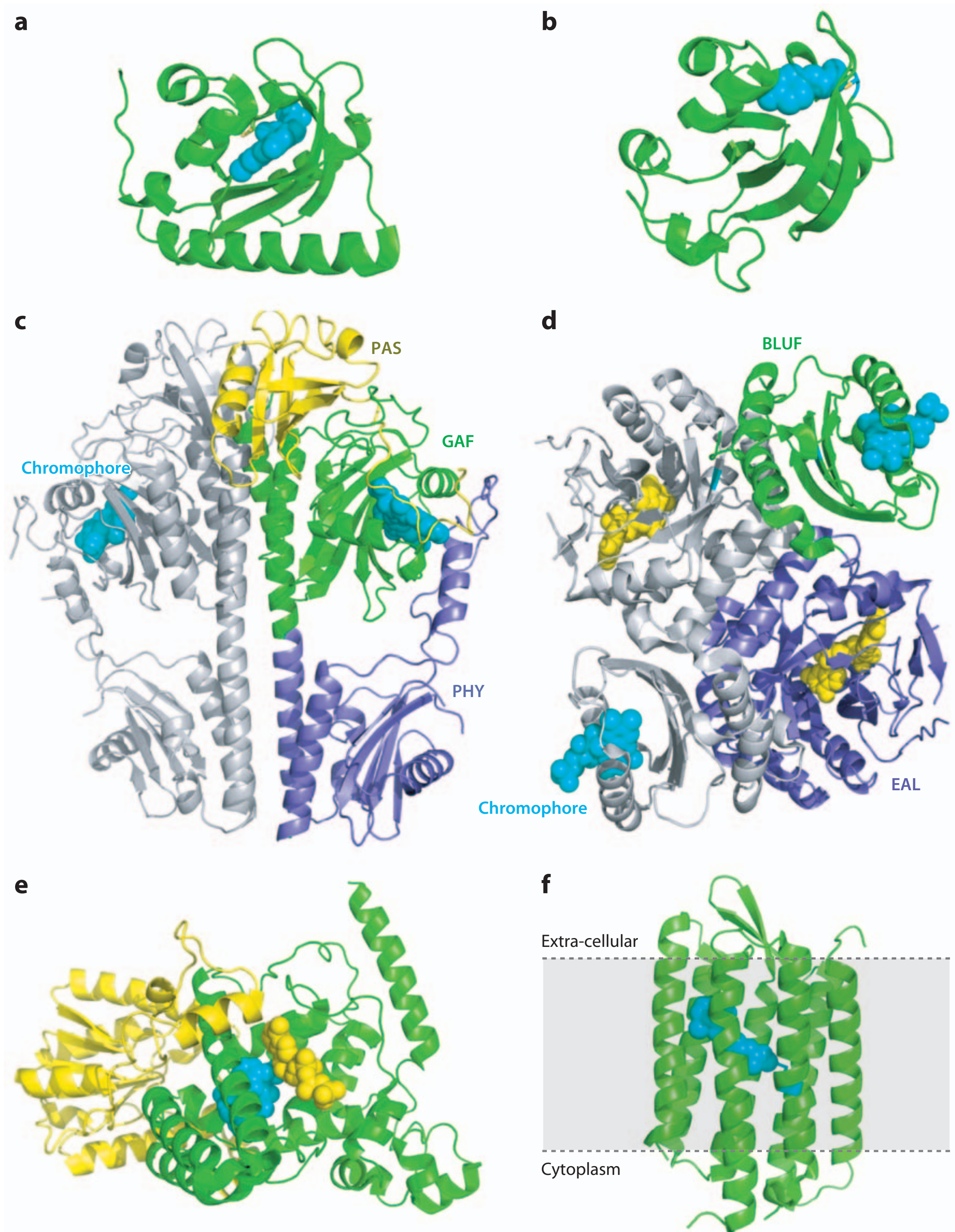

present in the intact, full-length photoreceptor and, hence, that when isolated, it is likely to retain critical aspects of both its structure and more importantly, its function. For example, plant phototropins contain two LOV input or sensor domains, a serine/threonine kinase output or effector domain whose kinase enzymatic activity is light dependent, several linkers between these domains, and small extensions at the N and C termini (**Figure 2*a***) (21, 23, 24). Each LOV domain has been isolated from intact phototropin and effectively studied. Nevertheless, an intact photoreceptor is more than the sum of its parts. The interfaces and interactions between domains are lost in separated domains, the linkers between domains are disrupted or absent, and the critical functional property of long-range signal transmission is absent. Further, photoreceptors in vivo may associate noncovalently with other proteins and cellular constituents, and their overall function may be importantly influenced by these associations, as for example the interaction of phototropin and 14–3-3 proteins (73). Isolation extracts its price.

The modular nature also enables results on photoreceptors from other organisms to be related with some confidence to those on plants, because distant organisms share common domains and identical (or closely related) chromophores and photochemistry (**Figures 1** and **2**). Although there is little direct information available today at the atomic level specifically on plant photoreceptors, a review of photoreceptors across all kingdoms does enable extrapolations toward plant photoreceptors. We organize this review by class of photoreceptor and concentrate on those of most relevance to plants.

LIGHT-OXYGEN-VOLTAGE SENSORS

Light-oxygen-voltage (LOV) domains utilize flavin nucleotide cofactors to detect blue light. First discovered as tandem sensor domains in the plant photoreceptor phototropin (23), LOV domains have since been found in several plant, fungal, and bacterial proteins (28, 89). In plants, to date, three families of bona fide photoreceptors utilizing LOV domains have been identified (**Figure 2*a***). First, phototropins 1 and 2 mediate a variety of relatively fast, light-induced responses in plants including phototropism, chloroplast and leaf movements, and stomatal opening (21, 23). Second, proteins of the ZEITLUPE family control slower light responses such as entrainment of the circadian clock and onset of flowering (32, 105). Third, aureochromes occur in photosynthetic stramenopiles and regulate aspects of morphogenesis in response to light (138). The *Arabidopsis thaliana* PAS-LOV protein is a further, putative plant photoreceptor for which blue-light-dependent protein interactions were reported but neither correct flavin incorporation nor the characteristic LOV photoreaction has yet been demonstrated (107).

LOV Photochemistry

LOV domains are distinguished from other flavin-based blue-light photoreceptors such as BLUF domains by the characteristic LOV photochemistry (77, 96, 122) (**Figure 1*a***). After absorption of a photon in the blue spectral region around 450 nm by the dark-adapted D_{450} state, the flavin nucleotide cofactor undergoes efficient intersystem crossing in picoseconds to yield a triplet L_{660} state (136). Within

Figure 3

Three-dimensional structures of representatives of the six photoreceptor classes. Chromophores are shown as space-filling models in cyan, chromophore-binding domains in green, and additional protein domains in yellow and blue. Gray color denotes the second subunit within homodimeric structures. (*a*) LOV: *A. sativa* phototropin 1 LOV2 domain (PDB entry 2V0U) (52). (*b*) Xanthopsin: photoactive yellow protein from *H. halophila* (2PHY) (12). (*c*) Phytochrome: *P. aeruginosa* bacteriophytochrome (3C2W) (157). (*d*) BLUF: *K. pneumoniae* BlrP1 (3GFZ) (10). (*e*) Cryptochrome: cryptochrome 1 from *A. thaliana* (1U3D) (17). (*f*) Rhodopsin: *Halobacterium salinarum* bacteriorhodopsin (1C3W) (91).

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microseconds a covalent, thioether bond between atom C(4a) of the flavin ring and a conserved, spatially proximal cysteine residue is formed, the S_{390} state (117). This reaction probably proceeds through an intermediate radical pair (96, 122). The overall quantum yield for the photoreaction leading to formation of the S_{390} state is high, ~0.3 in phototropin LOV2 domains (71). Usually, the photoreaction is fully reversible and the signaling state thermally reverts to the ground, dark state. Despite closely similar sequence and structure, individual LOV domains differ markedly in the kinetics and quantum yield of their photocycle (24, 69). For example, the time constants for dark recovery in phototropin LOV domains are about 10–100 seconds (69), but much longer (or even irreversible) in the isolated LOV domain of *A. thaliana* FKF1 (63) and in a LOV histidine kinase from *Brucella melitensis* (137). However, photochemical properties of LOV domains depend on the protein context, providing a specific example of a difference in properties between isolated domains and full-length proteins (24). The basis for these differences may be linked to protein flexibility, or to solvent accessibility, and the specific environment of the flavin chromophore (3), where mutations can strongly affect photocycle kinetics (22).

LOV Domain Structure

LOV domains constitute a subclass of the Per-ARNT-Sim (PAS) family whose members serve as versatile sensor and interaction domains in diverse signaling proteins (99, 139). The first structure of a LOV domain and indeed of any plant photoreceptor, that of the LOV2 domain of *Adiantum capillus-veneris* neochrome 1 (26), displayed the canonical PAS fold. As illustrated for the LOV2 domain of *Avena sativa* phototropin 1, the PAS core domain comprises a five-stranded antiparallel β sheet, whose strands are in the topological order Bβ-Aβ-Iβ-Hβ-Gβ (that is, 2-1-5-4-3), and several α helices (Cα, Dα, Eα, Fα) are packed on either side of the sheet (**Figures 3*a*** and **4*a***). A flavin nucleotide cofactor, flavin mononucleotide (FMN) in plant LOV proteins, is bound in a cleft formed by the central β sheet and helices Eα and Fα. Residues involved in FMN coordination and the photoreaction are largely conserved and line the flavin-binding pocket. LOV domains frequently display

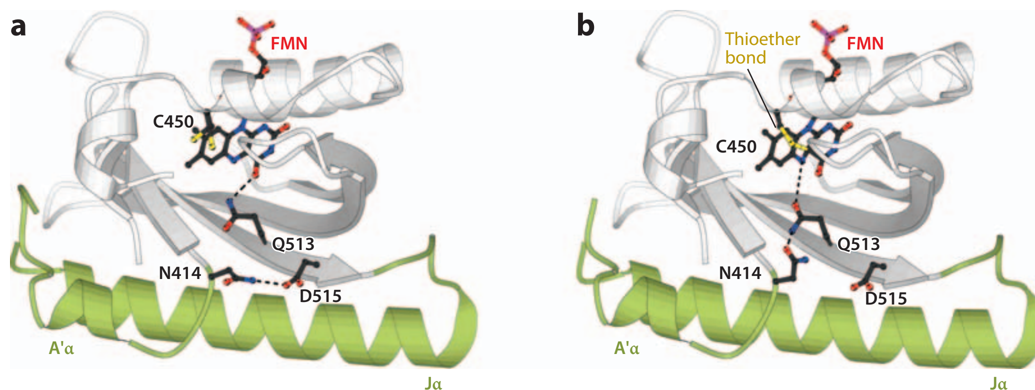

Figure 4

Light-induced structural changes in the *A. sativa* phototropin 1 LOV2 domain (52). The LOV core domain is shown in white and N- and C-terminal α-helical extensions, A'α and Jα, in green. For clarity, helix Cα is not displayed. (*a*) In the dark the active-site cysteine 450 adopts two conformations. Hydrogen bonds are formed between Q513 and atom O4 of the FMN ring, and from N414 to D515. (*b*) Upon blue-light absorption, a thioether bond (*yellow*) forms and induces a slight tilt in the FMN ring. Q513 presumably flips its side chain to form new hydrogen bonds to the FMN ring and N414. Conformational changes could thus be propagated to the terminal helices and could cause unfolding of the Jα helix (54). Note that alternate conformations of residues N414 and Q513 are not shown (52).

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structured N- and C-terminal extensions flanking their core, which predominantly adopt an α-helical conformation and may be crucial for signal transduction (see below and Reference 99).

Crystallographic studies identify blue-light-induced structural changes around the flavin cofactor that are similar in all LOV domains studied to date (27, 38, 52) (**Figure 4*a***). Formation of the covalent thioether bond causes a tilt of the isoalloxazine ring of the FMN by about 6° and slight movements of the coordinating residues. The side chain of a nearby, conserved glutamine residue is proposed to rotate and thereby changes its hydrogen-bonding pattern (27, 38, 163). For the *A. sativa* phototropin 1 LOV2 domain, further structural changes occur within the β sheet and the C-terminal Jα helix that extends from the core (52, 54) (**Figure 4*b***). Indeed, nuclear magnetic resonance (NMR) data suggest that this Jα helix unfolds (54). Infrared spectroscopic results suggest that this reaction proceeds through distinct intermediate structures (66) that remain to be fully characterized.

Light-induced structural changes are generally small in extent and (with the clear exception of the Jα helix) largely confined to the immediate vicinity of the flavin, which may be due to several reasons. Light-induced conformational changes may be qualitatively affected or attenuated in magnitude by the crystal context or by isolation of the photoreceptor domain, or the changes may be largely dynamic in nature, not associated with substantial atomic translation.

LOV Signal Transduction

We provide here a brief overview of plant LOV photoreceptors with a focus on structural and mechanistic aspects; an in-depth treatment is provided in recent reviews (21, 32).

Phototropins. Phototropins comprise two LOV domains, LOV1 and LOV2, and a serine/threonine kinase domain (23) (**Figure 2*a***). Phototropins undergo autophosphorylation at several serine and threonine residues but no other physiological phosphorylation target has been identified (21). Recent data indicate that autophosphorylation of a particular serine residue in the kinase activation loop is essential for all phototropin-dependent responses; phosphorylation at other sites may be required for specific phototropin-dependent responses (64). Repression of phototropin kinase activity in the dark is relieved upon blue-light absorption by the LOV2 domain (97). In contrast, the photochemically identical LOV1 domain is not essential for light regulation of kinase activity; rather, it attenuates the effect of LOV2 and may contribute to dimerization (97). Structural (54) and functional (53) data implicate unfolding of the Jα helix in the light regulation of phototropin kinase activity. However, it is unclear whether the Jα helix and its unfolding are strictly necessary, because the LOV2 domain can exert its regulatory effect on the kinase domain *in trans* and in the absence of the Jα helix (97). Further, recent data from infrared spectroscopy suggest that the role of the Jα helix differs among phototropin LOV domains; in the *Adiantum* neochrome 1 LOV2 domain, this helix does not unfold upon blue-light absorption (78). Helical extensions similar to the Jα helix are also found at the N and C termini of several phototropin LOV domains and may be involved in signal transduction, possibly also undergoing light-induced conformational changes (52, 103). Although the molecular details remain elusive, light-dependent interactions both among individual phototropin domains (104) and between phototropin and other proteins, such as 14–3-3 proteins (73) and presumably the unidentified phosphorylation targets of phototropins, play key roles in signaling (99).

ZEITLUPE family. The *A. thaliana* ZEITLUPE, FKF1, and LKP2 proteins contain a LOV domain followed by an F-box domain and several Kelch repeats (**Figure 2*a***) (130). These proteins mediate ubiquitin-dependent protein degradation in a light-controlled manner (92), ultimately leading to photoperiodic expression (121)

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and/or accumulation (72) of key proteins involved in regulating the circadian clock and flowering onset. No structural information is available yet on ZEITLUPE proteins, and their detailed mechanism of action is only beginning to be understood (32). However, as for phototropins, light-regulated protein interactions are crucial for signal transduction of the ZEITLUPE-family proteins (72, 121).

Aureochromes. Stramenopile aureochromes are transcription factors that comprise an N-terminal basic-zipper DNA-binding domain and a C-terminal LOV domain (**Figure 2*a***), thus resembling the modular composition of bacterial one-component systems (144). Blue-light absorption increases the affinity of the basic-zipper domain for DNA (138). Although the molecular details remain to be elucidated, aureochromes regulate cell branching and differentiation (138). Because basic-zipper domains act as homo- and heterodimers (41), it is likely that light-dependent protein interactions and changes in quaternary structure and dynamics will be revealed as components of signal transduction.

XANTHOPSINS

Photoactive yellow protein (PYP) is a small, cytoplasmic photoreceptor of 126 amino acids that, upon absorption of blue light, undergoes a fully reversible photocycle spanning around 1 second that contains several spectroscopically and structurally distinct intermediates (146). PYP thus represents an isolated sensor domain (**Figure 2*b***), the paradigm of the xanthopsin class of photoreceptors. Despite considerable effort, no other protein with which PYP might interact in a noncovalent, light-dependent fashion has been identified nor has its physiological role been identified in any organism. Thus, interest in PYP is more evident among biophysicists than physiologists. No homolog of PYP has been identified in plants. Nevertheless, PYP serves as the structural paradigm for the PAS domain family (99), of which LOV domains (see above) form a prominent subfamily. Through application of cryocrystallography, time-resolved crystallography, NMR, and ultrafast spectroscopic techniques, PYP has also provided the most detailed results on the photocycle of any photosensor domain at the atomic level.

PYP contains a 4-hydroxycinnamic acid chromophore, covalently attached by a thioester bond to a cysteine residue and completely buried in the interior of the protein (**Figures 1*b*** and **3*b***). The primary photochemical event is *trans* to *cis* isomerization about the sole double bond in the tail of its chromophore. Reversion to the *trans* state occurs purely thermally as the photocycle concludes. In the dark, ground state, the phenolate anion at the head of the chromophore is stabilized by unusually short hydrogen bonds (7) to nearby glutamic acid and tyrosine side chains. Thus isomerization must occur in a molecularly constrained environment, in which one end of the chromophore is pinned by the covalent bond and the other by these hydrogen bonds. Complete isomerization occurs within a few nanoseconds and introduces severe strain into the chromophore and the surrounding protein. Relaxation of this strain initiates a long series of changes in tertiary structure: rupture of these hydrogen bonds; ejection of the chromophore toward the solvent; partial unfolding of the Cα helix, which contains these glutamic acid and tyrosine residues; and, ultimately, unfolding of a pair of short, N-terminal α helices packed on the distal side of the β sheet, which forms the structural core of all PAS and LOV domains including PYP (99, 139). Time-resolved crystallographic studies identify each of the intermediates as relaxation occurs over the timescale from nanoseconds to seconds [Ihee et al. (62) and references therein]. These intermediates differ in tertiary structure. Each therefore represents a distinct structural signal, which could differ in affinity for another protein to which the signal could be transmitted. More recent studies (H. Ihee et al., personal communication) have extended the time resolution toward 100 picoseconds to

explore isomerization itself and characterize earlier stages of the photocycle.

Although ultimately unsatisfying from a physiological standpoint, these studies on PYP point the way to an understanding at the atomic level of the generation of a structural signal in an isolated photosensor domain. But small is not simple; even PYP turns out to be deceptively complicated when studied in detail.

PHYTOCHROMES

Phytochromes are red/far-red photoreceptors and, together with cryptochromes and phototropins, constitute one of the three major regulators of photomorphogenesis in plants (20). Although the first plant phytochrome was discovered about 50 years ago, progress in understanding these photoreceptors has been greatly stimulated by the recent discovery of homologous microbial phytochrome systems, denoted bacteriophytochromes (30, 61, 149). Phytochromes and bacteriophytochromes utilize a linear tetrapyrrole, bilin chromophore to sense red/far-red light (**Figure 1*c***). Substituents on the pyrrole rings and their mode of covalent attachment to the protein differ between phytochromes and bacteriophytochromes (113). In both, absorption of a photon causes the red-absorbing (Pr) spectroscopic state to photoconvert to the far-red-absorbing (Pfr) spectroscopic state; absorption of a second photon causes reversion to the Pr state. In some (bacterio-)phytochromes the Pr state forms the dark, ground state and in others, the Pfr state. Two N-terminal domains (PAS and GAF) together form a chromophore binding module (CBM) that displays only limited photoconversion from the dark state. However, addition of the phytochrome-specific (PHY) domain yields the three-domain, PAS-GAF-PHY, photosensory core module (PCM) that retains the full photoconversion properties of full-length proteins (113). The C-terminal effector domain in bacteriophytochromes is a histidine kinase (HK) domain and forms part of a two-component signaling system (**Figure 2*c***). In all five classes of plant phytochromes (denoted phyA–E in *A. thaliana*), the C terminus contains a histidine-kinase-related domain (HKRD) in place of an authentic HK domain and two additional PAS domains are inserted between the PCM and HKRD (**Figure 2*c***). When difference spectra between the Pr and Pfr states are probed by UV-vis absorption, resonance Raman, or Fourier transform infrared (FTIR) spectroscopy (6, 43, 147), they exhibit striking similarities between plant and microbial phytochromes, which suggests that they share a common photoconversion mechanism. The extent to which findings on microbial phytochromes are relevant to plant phytochromes is not established, but a working hypothesis is that they are closely related (113).

The phytochrome field has been the subject of excellent reviews (112, 113). Here, we focus on recent advances in structural studies and current views on the molecular basis of photoconversion and the signal transduction mechanism.

Phytochrome Structure

Bacteriophytochromes (BphPs) and cyanobacterial phytochromes (Cphs) share a similar domain structure and high sequence homology with plant phytochromes (Phys), particularly in the N-terminal CBM that binds the bilin chromophore and absorbs red light (113). The crystal structure of the CBM from *Deinococcus radiodurans* bacteriophytochrome (DrBphP) was the first for domains of any phytochrome (150). Two recent crystal structures of *Synechocystis sp.* Cph1 and *Pseudomonas aeruginosa* PaBphP in their dark state, Pr and Pfr respectively, advance our understanding of the Pr↔Pfr photoconversion (37, 157). Both structures include the entire PCM and retain the photoconversion properties of the corresponding full-length proteins.

The PAS, GAF, and PHY domains share a common core fold defined by a central, antiparallel β sheet with the strands in the order of 2–1-5–4-3 and a connecting element between strands 2 and 3 containing an α helix. Although the overall spatial arrangement of these three domains is linear in a beads-on-a-string fashion, they are closely integrated via the N-terminal

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extension of the PAS domain, a highly unusual knot (150), and an arm extending from the PHY core domain (37, 157). All three domains converge near the chromophore binding site. In addition, the GAF and PHY domains are linked by a long, continuous helix spanning both domains that forms part of a helical bundle (37, 150, 157).

In the crystals, PaBphP-PCM forms a head-to-head, parallel dimer with an extensive helical bundle at the dimer interface, but in contrast Cph1-PCM packs as an antiparallel dimer. A parallel dimer is also observed in other crystal structures of bacteriophytochromes (150, 159) and may well prove to be more biologically relevant for phytochromes in general (157).

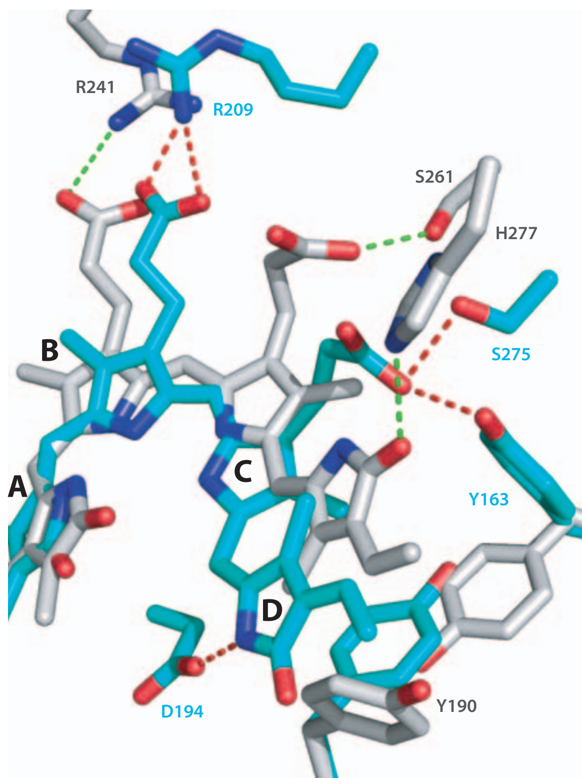

Figure 5

Flip-and-rotate model for Pr↔Pfr photoconversion in bacteriophytochromes as shown for *P. aeruginosa* BphP (158). Pyrrole rings of biliverdin are labeled A–D. Residues interacting with the D ring are shown in cyan for the Pfr state and in gray for the Pr state.

Phytochrome Photochemistry

All four pyrrole nitrogens in the bilin chromophore are protonated in both the Pr and Pfr states (76, 115, 132) (**Figure 1*c***). Femtosecond- to picosecond-absorption spectroscopy revealed the presence of two electronic states before fully isomerized photoproducts are formed. These photoproducts occur in both the Pr→Pfr and the Pfr→Pr directions and are denoted Lumi-R and Lumi-F, respectively (29). Transient proton release and uptake in the chromophore may accompany the formation and decay of the later (micro- to milliseconds) intermediates denoted Meta-R and Meta-F (14). However, the structural bases of these reaction intermediates and the identity of transient proton donor/acceptors in the reactions are not yet established (151).

It is generally accepted that the primary photochemical event in the reversible Pr↔Pfr photoconversion in Phys and BphPs is Z/E isomerization around the C15 = C16 double bond between rings C and D in the bilin chromophore (**Figures 1*c*** and **5**) (113). Indeed, crystal structures directly confirm that the chromophores of Cph1-PCM, DrBphP-CBM, and RpBphP3-CBM adopt distinct configurations in the Pr and Pfr states (37, 150, 157). We have recently proposed a "flip-and-rotate" model to summarize conformational differences between the Pr and Pfr states in the chromophore and its surrounding protein matrix (158). Upon absorbing a photon, rapid isomerization occurs and ring D flips about the C15 = C16 double bond. Eventually, the entire chromophore slightly rotates relative to the protein matrix around an axis roughly centered at ring A and approximately perpendicular to rings B and C. Consequently, ring D and the propionate substituents on rings B and C interact with different sets of protein side chains in the Pr and Pfr states (**Figure 5**). In addition, a pair of aromatic side chains near ring D undergoes concerted rotamer changes coupled to flipping of ring D (157, 158).

Whether this flip-and-rotate model applies to photoconversion reactions in both forward and reverse directions and how it progresses

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through intermediate structures are open questions. The model requires more pronounced conformational changes around ring D (distant from the rotation axis) and the propionate side chain of ring C, consistent with observations in Cph1 and phyA (13, 114). However, other evidence suggests that structural changes are more dramatic around ring A in Cph1-PCM (148). Ring D remains nearly unchanged between the Pr and Pfr structures of the GAF domain of a "PAS-less" phytochrome (25, 143) (A. Ulijasz, personal communication).

Phytochrome Signal Transduction

How do signals originating in the chromophore and its binding pocket propagate to the spatially remote effector domain? Any answer must be indirect, because no crystal structure is yet available for full-length phytochromes of any kind. In our model for dimeric, full-length PaBphP, the C-terminal helix of the PHY domain is directly fused to the N-terminal helix of the HK domain, thus further extending the helical bundles at the dimer interface (157). The relative positioning between the phosphoacceptor histidine and the kinase active site in the HK domain supports the proposed *in trans* autophosphorylation. A similar interdomain linkage and disposition of the sensor and effector domains may be present in plant phytochromes.

The extended arm of the PHY domain found in both the PaBphP and Cph1 structures seems to be conserved in all phytochromes. Its close proximity to the chromophore makes the PHY domain an effective and direct mediator of transduction of the signal toward the HK domain. Conformational changes in the chromophore binding pocket could be transmitted to the HK domain either via direct, interdomain interactions or via alterations in the stability of the helical bundle that comprises much of the dimer interface. Long interdomain helices are found in a wide range of signaling proteins (4), where they may play important roles in signal transduction and in spatially aligning multiple domains.

BLUF SENSORS

BLUF domains comprise a family of photosensor domains that use FAD to detect blue light (49). BLUF domains predominantly occur in prokaryotes (41) and were first identified in AppA from *Rhodobacter sphaeroides* where they regulate expression of photosynthesis genes (48, 49, 93). BLUF domains occur either as isolated domains or covalently linked to effector domains mostly involved in cyclic nucleotide metabolism, e.g., adenylate/guanylate cyclases and phosphodiesterases (41, 49) (**Figure 2*d***). BLUF domains are also found in eukaryotes such as euglenozoa (65) and fungi (41, 49), but to date no BLUF proteins have been identified in plants.

In contrast to the flavin-containing LOV domains, upon absorption of blue light by a BLUF domain, the FAD cofactor undergoes minimal conformational changes and the signaling state is rapidly formed on the subnanosecond timescale (46, 94) (**Figure 1*d***). Upon light absorption by the ground state P, an electron is transferred from a conserved tyrosine residue to the flavin ring and gives rise to a short-lived radical pair (46). The side chain of a nearby, conserved glutamine residue is assumed to rotate (8), followed by back transfer of an electron to the tyrosine (46) to form the signaling state, P_{red}. P_{red} shows a slightly red-shifted absorption spectrum and differs from the ground state in the hydrogen bonds that the flavin ring forms. In both the ground and signaling states, FAD maintains its fully oxidized state. P_{red} is formed with a quantum yield of ~0.25–0.4 and thermally reverts to the ground state within seconds (46). Details of the BLUF photocycle are still under debate; an alternative mechanism based on tautomerization of the side chain of the conserved glutamine residue has been advanced (33).

BLUF domains are oligomeric, are usually dimeric, and adopt a ferredoxin-like core fold comprising a five-stranded mixed parallel/antiparallel β sheet with a strand order of 4-1-3-2-5 and two α helices running parallel to the strands (8, 68, 74) (**Figure 3*d***). The FAD

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cofactor is embedded in a cleft between the two α helices. A C-terminal, α-helical cap of ∼40–50 residues packs against the outer face of the β sheet in orientations that differ from domain to domain (67, 74, 153). As discussed above, blue-light-induced rotation of the side chain of a conserved glutamine residue alters its hydrogen bonding pattern. Movement of a conserved methionine residue in strand β_5 out of the FAD binding pocket leaves a void that is filled by the side chain of another residue (tryptophan in AppA) (8, 68). In addition, light also induces conformational changes within the β sheet and the C-terminal cap (51, 153).

Very recently, a groundbreaking study reported the first crystal structure of a full-length photoreceptor, that of *Klebsiella pneumoniae* BlrP1, which comprises BLUF and EAL phosphodiesterase domains (10). Briefly, within an antiparallel dimer the BLUF domain of one molecule interacts with the EAL domain of the other molecule through its C-terminal cap. Light-induced conformational changes could thus be propagated to the effector domain, leading to changes in quaternary structure and enzymatic activity (10). Interestingly, changes in quaternary structure and dynamics are common components of signal transduction in BLUF (160), LOV (see above), and PAS proteins (99).

CRYPTOCHROMES

Cryptochromes are widely distributed blue-light photoreceptors mediating various responses in plants and animals (1, 18, 47, 86, 87, 100, 118). Examples in plants are *A. thaliana* cryptochrome 1 and 2 (AtCry1 and AtCry2) that entrain the circadian clock and trigger developmental processes such as de-etiolation and flower induction (84, 125, 126). Cryptochromes are flavoproteins whose photosensory domains are closely related to DNA photolyases, but typically lack their DNA repair activity (87). *A. thaliana* cryptochrome 3 (AtCry3) belongs to a different class of cryptochromes found in *Drosophila*, *Arabidopsis*, *Synechocystis*, and *Homo* (cry-DASH) (18). Cry-DASH proteins bind both double-stranded and single-stranded DNA and can also repair single-stranded DNA containing cyclobutane-pyrimidine-dimer (CPD) lesions, but their function in signal transduction, if any, is not yet known (60, 111).

Compared to photolyases, cryptochromes often possess additional terminal regions of various lengths that may be involved in signaling (see below) (75). AtCry1 and AtCry2 are composed of an N-terminal photolyase homology region (PHR) and a C-terminal extension (CCT); AtCry3 contains a shorter N-terminal extension of the PHR domain (**Figure 2*e***). Although largely divergent in sequence, these N-terminal or C-terminal regions carry three short sequence motifs known as the DAS motif, DQXVP, followed by acidic and serine-rich sequences.

The structural biology of DNA photolyases and cryptochromes has been extensively discussed in several reviews (36, 86, 87, 100). We focus here on the characteristics of cryptochromes as photoreceptors directly relevant to mechanisms of light perception and signaling.

Cryptochrome Structure

The photosensory PHR domains in AtCry1 and AtCry3 share the same architecture with DNA photolyases, composed of an N-terminal α/β domain and a C-terminal α-helical domain (17, 60, 75, 111) (**Figure 3*e***). The α/β domain adopts a dinucleotide-binding fold consisting of a five-stranded parallel β sheet surrounded by five helices. The helical domain consists of 16 helices and noncovalently binds the FAD chromophore in a U-shaped conformation. The linker region between the α/β and helical domains is extended and largely unstructured, with conformations that vary among photolyase and cryptochrome structures. In the crystal structure of AtCry3 (and as in photolyases), a second light-harvesting chromophore, 5,10-methenyltetrahydrofolate (MTHF), is located at the interface between these domains (111).

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In contrast to DNA photolyases and DASH cryptochromes such as AtCry3, the surface of AtCry1-PHR is predominantly negatively charged, which accounts for its lack of DNA binding activity (17, 100). However, the nucleotides ATP (adenosine triphosphate) or AMP-PNP [adenosine 5′-(β,γ-imido)triphosphate] bind to AtCry1-PHR in close proximity to the FAD chromophore, in a manner similar to the binding of the substrate cyclobutane pyrimidine dimers to photolyase (17).

The putative signaling CCT domains of cryptochromes are not yet characterized structurally, nor is any sequence-based homology model available. Both secondary structure prediction and circular dichroism (CD) and NMR spectroscopy suggest that the isolated AtCry1-CCT domain mostly adopts a flexible, extended structure with short stretches of helices (109). However, in the context of the full-length protein, it adopts a more stable conformation through interaction with its cognate PHR domain. Such direct interdomain contacts occur in the crystal structure of AtCry3, in which a 40-residue N-terminal extension containing the DAS motif in an extended structure wraps around the helical domain of PHR (111).

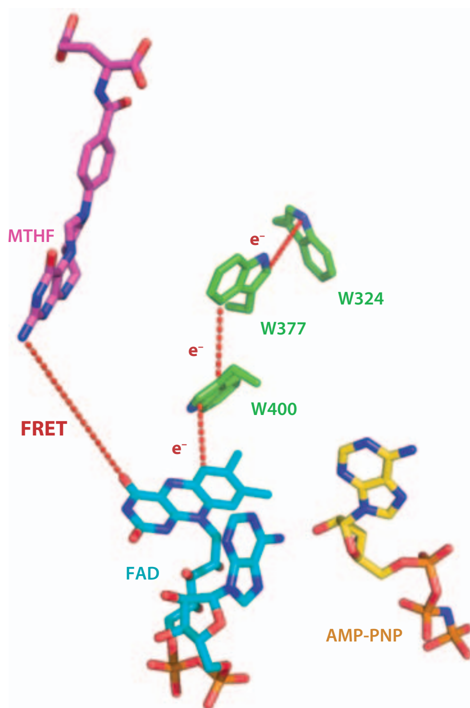

Figure 6

Spatial configuration of key components involved in the photoreaction of cryptochromes based on the structural superimposition of *A. thaliana* cryptochromes 1 and 3 (PDB entries 1U3D, 2VTB) (17, 111). Putative paths of electron and resonance energy transfer are marked in red dashed lines. Residue numbering refers to cryptochrome 1.

Cryptochrome Photochemistry

Absorption of light by DNA photolyases causes photoreduction of the oxidized ground state of FAD to a fully reduced, catalytic $FADH^-$, the light-activated state, which further enables electron transfer to DNA to split and repair the UV lesions. In contrast, plant and animal cryptochromes exhibit more complicated photochemistry. Absorption of blue light by the oxidized ground state of FAD in AtCry1 and AtCry2 induces formation of a radical intermediate state (semiquinone), FADH•, that accumulates in the activated signaling state. Absorption of green light by FADH• causes further reduction to $FADH^-$, which abrogates signaling. $FADH^-$ reoxidizes to the fully oxidized form during dark reversion (9, 16).

In the vicinity of the FAD chromophore, a chain of three tryptophan residues and antenna pigments such as MTHF or 8-hydroxy-5-deazariboflavin (8-HDF) are conserved among DNA photolyases and cryptochromes (**Figure 6**). This Trp triad is required for FAD reduction in AtCry1 (161) and participates in intramolecular electron transfer to the FAD chromophore upon photoactivation. Light energy absorbed by an antenna pigment is transferred to FAD by fluorescence resonance energy transfer to ensure efficient photoreduction. Although not observed in the structure (17), biochemical evidence shows that MTHF also binds to AtCry1, where it functions as an antenna (58).

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Cryptochrome Signal Transduction

The biochemical nature of the output signals remains elusive for plant cryptochromes. The cryptochrome C-terminal extensions CCT probably form the effector domain as evidenced by a constitutive photomorphogenetic phenotype resulting from overexpression of the isolated CCT domains of either AtCry1 or AtCry2 (156) and an inactive cry1 allele that carries mutations in the C-terminal region (2). Signaling is likely mediated by direct interactions between the CCT domain and downstream effector proteins such as the ubiquitin ligase COP1 (152, 155). Proteolysis experiments on full-length AtCry1 in the dark and after illumination suggest that the CCT domain undergoes a light-induced order-to-disorder transition particularly in the serine-rich region of the DAS motif (109).

Light-dependent autophosphorylation, common among photoreceptors, has also been examined as a potential output signal in AtCry1 and animal cryptochromes. AtCry1 binds ATP and exhibits FAD-dependent kinase activity in vitro (15). The crystal structure of AtCry1-PHR reveals binding of the nonhydrolyzable analog AMP-PNP near the FAD chromophore. Indeed, the autokinase activity of AtCry1 is not affected by light in vitro, and AtCry2 displays no detectable kinase activity in the light or dark (108). Blue-light-dependent phosphorylation of AtCry1 and AtCry2 in vivo may arise from other light-dependent kinases for which the disordered CCT domain is a substrate.

Like other plant photoreceptors, AtCry1 and AtCry2 form homodimers in vivo and in vitro (119). Dimerization is mediated by the PHR domain and appears to be essential for signaling in AtCry1 and AtCry2 (116). AtCry3 and photolyases, however, appear to be mostly monomeric in solution (75).

RHODOPSINS

In contrast to the five other water-soluble photoreceptor classes, rhodopsins are integral membrane proteins. Light detection is achieved via a retinal chromophore that is covalently bound to a lysine residue as a protonated Schiff base. Long recognized as the photoreceptor in animal vision, related rhodopsins were subsequently identified as photoreceptors in microorganisms (11, 106) and algae (44). Known plant rhodopsins belong to the microbial (type-1) rhodopsins (55, 131), which are exemplified by bacteriorhodopsin (106). Channelrhodopsins mediate phototaxis in flagellate algae (55, 128), and were first identified in *Chlamydomonas reinhardtii* as light-gated cation channels (101, 129, 135) (**Figure 2*f***). *Chlamydomonas* also expresses other rhodopsins for which no clear functional role has yet been ascertained (55). The first identified chlamyopsins 1 and 2 are prevalent in the *Chlamydomonas* eyespot but their physiological role is unclear (45, 55, 70). In chlamyopsins 5–7, known as the enzymerhodopsins, rhodopsin sensor domains are covalently connected to histidine kinase, response regulator, or adenylate/guanylate cyclase effector domains (70). Other algal rhodopsins (142) function as light-driven H^+-pumps akin to bacteriorhodopsin although their physiological role in photosynthetic organisms remains mysterious (55).

Rhodopsin Photochemistry

As in bacteriorhodopsin, the retinal chromophore of algal rhodopsins adopts the *all-trans* conformation in its dark-adapted state D_{470} (56, 80). Upon blue-light absorption around 470 nm, the retinal moiety isomerizes to the 13-*cis* form (56, 80) (**Figure 1*f***). Long hampered by limited protein availability, detailed spectroscopic studies of the photocycle of *Volvox carteri* channelrhodopsin became feasible recently (35). Briefly, the initial photoexcited state P_{500} interconverts to the P_{510} state within 200 μs through an intermediate with a deprotonated Schiff base. Based on electrophysiological experiments, P_{510} is the major signaling, ion-conducting state (35). P_{510} thermally relaxes to the ground state through a spectroscopically silent intermediate denoted N with

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time constants of 20 and 100 ms, respectively. The quantum yield for retinal isomerization in rhodopsins is typically high, e.g., 0.64 in bacteriorhodopsin (140).

Rhodopsin Structure

In the absence of high-resolution structures, plant rhodopsins are expected to structurally resemble homologous microbial rhodopsins (57). As shown for bacteriorhodopsin in **Figure 3*f*** (91), microbial rhodopsins belong to the large family of seven-helix transmembrane proteins (41). Seven α helices connected by short loops traverse the plasma membrane and assemble into a barrel-like bundle. The retinal chromophore is embedded in the middle of this bundle through residues that are largely conserved in algal rhodopsins (101). Studies by crystallography and cryoelectron microscopy on bacteriorhodopsin revealed in great detail its structure and light-induced conformational changes throughout the photocycle (57, 90, 91). However, these findings may not fully apply to algal rhodopsins, and corresponding homology models are almost certainly deficient in their molecular details (55). Reliable answers will be provided by three-dimensional structures of plant rhodopsins, the prospects for which appear improved by recent advances in the recombinant production of channelrhodopsins (35).

Rhodopsin Signal Transduction

We focus on key aspects of rhodopsin function and refer to Reference 55 for a current and authoritative treatment. Phototaxis in *Chlamydomonas* and related algae involves photocurrents driven by cation influx across the plasma membrane (88). Such photocurrents depend on the action of channelrhodopsins (44, 129) and appear within microseconds after light excitation (55). Heterologous expression of channelrhodopsin in oocytes established their function as light-gated ion channels (101, 102). Although in algae the photocurrent is mainly carried by Ca^{2+} and H^{+} (59, 88), channelrhodopsins are also permeable for other mono- and divalent cations (102). These properties are exploited in so-called optogenetic applications in neuroscience (31), where channelrhodopsin-2 (102) is largely used because it achieves higher photocurrents than channelrhodopsin-1 (101).

The gating mechanism of channelrhodopsins is little understood but certain aspects might be shared with bacteriorhodopsin. Specifically, channelrhodopsin-2 also displays H^{+} pumping activity and could thus be considered a "leaky proton pump" (39). In native algae, part of the channelrhodopsin photoreceptor function might be mediated by regulation of as yet unidentified downstream proteins (128, 129) similar to microbial sensory rhodopsins (120). Such a function might reside in the long C-terminal half of channelrhodopsins, which is not required for light-gated channel activity (101). Related scenarios may also apply to the less studied enzymerhodopsins (55, 70). The observation that transmembrane rhodopsin photoreceptors can be linked to the same types of effector domains (e.g., histidine kinases) as soluble photoreceptors is intriguing. Certain principles and mechanisms of signal transduction might be shared among quite disparate photoreceptor classes.

COMMON STRUCTURAL AND SIGNALING PRINCIPLES

All photoreceptors face the same principal task: the information provided by absorption of a photon must be efficiently converted into productive "jiggling and wiggling of atoms" (40) to elicit the appropriate physiological response. Our analysis of the six photoreceptor classes reveals certain recurring principles of structure and signal transduction mechanisms.

Photoreceptors of the LOV, PYP, phytochrome, and BLUF classes are based on the same modular domain architecture in which a photosensor domain is covalently linked, usually to the N terminus but occasionally to the C terminus of an effector domain. Strikingly, different sensor domains can regulate the same type of effector domain and in some proteins

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several photosensors occur in combination, e.g., in neochromes. These classes bury their chromophores, inaccessible to solvent, within a core consisting of a central β sheet and several α helices. The structural similarity among them and resemblance to certain chemosensor domains may reflect a common evolutionary origin (5, 99). The modular structure appears to have evolved by fusion of individual domains characterized by distinct functions, e.g., signal sensing, catalysis or propensity to oligomerize, and which retain those functions, albeit in modified form, in the multidomain photoreceptors we see today. These photoreceptors usually form dimers or higher-order oligomers, which introduces the possibility of quaternary structural changes. Linkers or extensions at the N or C termini of the sensor domains are mainly α-helical, often located at a dimer (or oligomer) interface and involved in signal transduction. Their conformations are light dependent and may take the form of order-disorder transitions, which in turn may modulate quaternary structure. Thus, changes in quaternary structure and dynamics often form part of the signal transduction mechanism (99).

However, cryptochromes and rhodopsins differ architecturally from the other photoreceptors, lack a clear modular structure, and incorporate their chromophores into a compact unit that also contains an enzyme active site or ion channel. This architecture almost certainly reflects their evolution from light-absorbing proteins that utilize photons primarily as a source of energy rather than for their information content. However, the recent discovery of enzymerhodopsins implies that to some extent signaling strategies are shared between the integral membrane rhodopsins and several of the water-soluble, modular photoreceptor classes.

PHOTORECEPTOR BIOTECHNOLOGY

The identification of photoreceptors and elucidation—even in its earliest stages—of their photoreactions, structures, and signaling mechanisms have recently enabled applications in biotechnology and protein engineering. As natural photoreceptors frequently employ near-ubiquitous metabolites such as flavins and bilins as cofactors and spontaneously incorporate them to form a functional photosensor, photoreceptors can readily be deployed in vivo in heterologous systems. For example, a plant-derived photoreceptor may be functionally expressed in *Xenopus* (101) or *Mus* (50).

Because most current biotechnological approaches are based on plant photoreceptors, we summarize these approaches briefly and loosely group them into four categories (**Figure 7**).

First, various efforts are directed at altering properties of photoreceptors such as their absorption, action and emission spectra, quantum yield, or photocycle kinetics (**Figure 7*a***). Examples include spectral tuning in rhodopsins (162), incorporation of alternate chromophores into LOV domains (95), and mutant photosensors with altered photocycle kinetics (22).

Second, photoreceptor domains were engineered to act as fluorophores (**Figure 7*b***). As noted in the Introduction, natural photoreceptors are evolutionarily optimized to efficiently undergo productive photochemistry upon light absorption and conversely unproductive de-excitation pathways such as internal conversion or fluorescence are minimized. If productive photochemistry is blocked, e.g., in mutant proteins, other processes of de-excitation such as fluorescence can become dominant. For example, removal of their active-site cysteine renders LOV domains fluorescent (19, 34). Similarly, several bacteriophytochromes have been made fluorescent by mutating key residues in such a way as to block efficient Pr↔Pfr photoconversion (42, 127). High-resolution crystal structures of photoreceptors guide the rational design of variants with impaired photochemistry and increased fluorescence. Fluorescent photoreceptor variants represent interesting alternatives to the widely used jellyfish fluorescent proteins for which GFP is the paradigm (141). The orthogonal chromophores and

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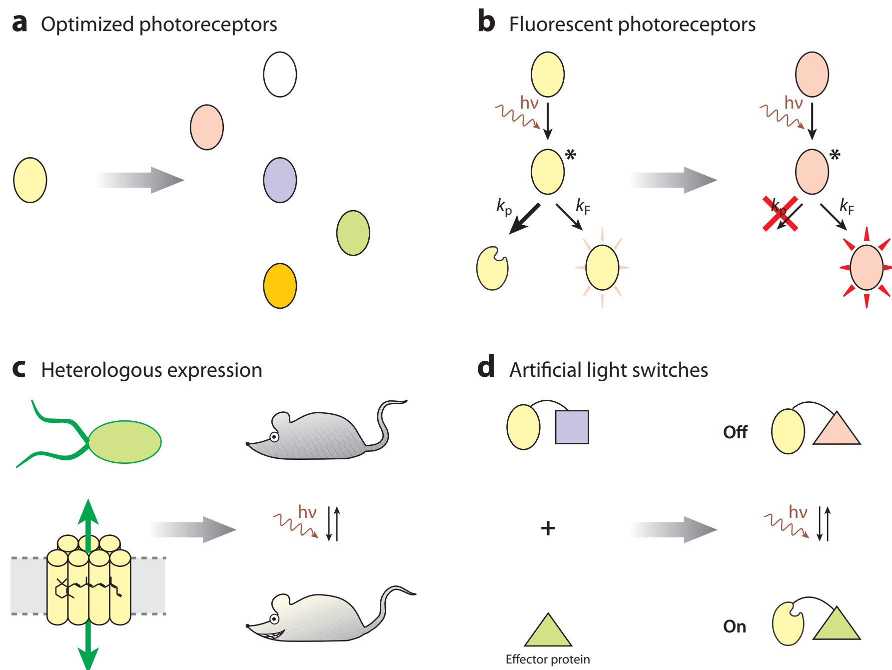

Figure 7

Application of plant photoreceptors in biotechnology and protein engineering in four general areas. (*a*) Alteration and improvement in properties of photoreceptors such as action and fluorescence spectra, quantum yield, and lifetime of the signaling state. (*b*) Upon light absorption, a photoreceptor efficiently undergoes productive photochemistry (k_P) (*left*). Competing de-excitation processes such as radiative decay (fluorescence, k_F) are minimized but may become dominant in mutant proteins with impaired photochemistry (*right*). (*c*) Natural photoreceptors, e.g., channelrhodopsin, were isolated from plants (*Chlamydomonas*) and expressed in other organisms (*Mus*) to control their behavior by light. (*d*) Design of modular photoreceptors by fusion of effector proteins (*green triangle*) with photosensor domains.

photochemistry in photoreceptors differ greatly from GFP and its variants in important properties such as fluorescence emission spectrum (127).

Third, (plant) photoreceptors were heterologously expressed to control behavior of cells and even of entire organisms (**Figure 7*c***). Based on their ability to elicit action potentials in neurons, channelrhodopsins (101) have found widespread use (e.g., Reference 50), thus spawning the vibrant field of optogenetics (31). Photoactivated adenyl cyclase (65) was employed to increase cellular levels of the second messenger cAMP (3′,5′-cyclic adenosine monophosphate) upon light absorption (123). These approaches are particularly powerful because the underlying photoreceptors need not be added exogenously but can be genetically encoded and endogenously expressed in the desired cellular location. This facilitates noninvasive and reversible control over physiological processes with excellent spatial and temporal resolution. Most versatile are those photoreceptors that regulate elementary processes of importance in diverse cell types and organisms (cf. channelrhodopsins).

Fourth, artificial photoreceptors were designed (**Figure 7*d***) via approaches that exploit the modularity of photoreceptors to bestow light sensitivity on effector proteins by covalently linking them to suitable photosensor domains. For example, LOV domains

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were fused (carefully) to regulate, in a blue-light-dependent manner, the activities of a DNA-binding protein (133), dihydrofolate reductase (81), histidine kinases (98), and most recently and spectacularly, the small GTPase Rac1 (154). A bacteriophytochrome was used to bestow control by red light on histidine kinase activity (83). Further, the light-dependent association of plant phytochromes with their interacting factors (PIF) was harnessed to regulate actin metabolism (82). A key challenge to designers is to efficiently couple photosensor and effector domains, thus translating a significant portion of the free energy available from photon absorption into a change of biological activity (99). The nature of the linker between sensor and effector domain is therefore crucial in determining the extent of light regulation (83, 98, 133). Knowledge of natural photoreceptors, their structures, and signaling mechanisms informs the design of artificial photoreceptors (99); and *vice versa*, the properties of artificial photoreceptors yield insights relevant for natural systems (98).

FUTURE CHALLENGES AND OUTLOOK

The identity, structure, and photochemistry of many photoreceptors have only been elucidated recently. Advances in DNA technology have greatly facilitated the identification of new members of known photoreceptor families (e.g., References 101, 138). Although perhaps less likely, completely new photoreceptor families based on novel photochemistry may remain to be discovered, as may combinations of photosensors with other metabolic sensors. For example, flavin cofactors serve as redox sensors in many proteins (124). It has long been known that light can lead to photoreduction of the flavin ring, though in most cases photoreduction processes appear to be of no physiological relevance. However, a recent report suggests that the *Oryza sativa* protein HAL3 acts as a combined redox and light sensor in vivo (134).

A major challenge is to extrapolate from data at the molecular level to the physiology of living plants. In the following, we highlight some of the difficulties and how they might be addressed. Recently, microbial proteins have served as powerful model systems to study photoreceptor structure, photochemistry, and signaling mechanisms. Although the primary photochemical events are expected to be closely similar in plants, that is very unlikely to be the case for downstream events. At the physiological level, photoreceptors such as phototropins and phytochromes occur as several subtypes with partly overlapping, partly differing functions. Photoreceptor function may vary in different cell compartments (e.g., cytosol versus nucleus) or tissues (e.g., root versus leaf), and may further depend upon time of day and developmental stage.

As we note in the Introduction and in the section on LOV photoreceptors, sensor and effector domains are frequently studied in isolation and their properties can differ from that of the full-length protein. It is not fully known for any plant photoreceptor how multiple domains are arranged in space and how they interact with each other. To give one example, there is no detailed information on the atomic structure of any plant phytochrome. We therefore strongly advocate structural studies on full-length proteins (10), preferably at atomic but also at lower resolution.

Experimental results would be invaluable—and far superior to any model—but one has to be realistic. Such studies place high demands on sample quality (purity) and quantity. They are complicated by the structural heterogeneity of signaling proteins: Structural changes in response to relatively small inputs of free energy are inherent in all signaling systems, and flexibility forms an essential aspect of their function (99). A further challenge derives from the fact that in vivo photoreceptors do not act in isolation but in concert with other sensors and cellular components, many of which are as yet unidentified. Nevertheless, one should not be deterred.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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