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Photoreceptor Proteins, "Star Actors of Modern Times": A Review of the Functional Dynamics in the Structure of Representative Members of Six Different Photoreceptor Families

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ABSTRACT
Six well-characterized photoreceptor families function in Nature to mediate light-induced signal transduction: the rhodopsins, phytochromes, xanthopsins, cryptochromes, phototropins, and BLUF proteins. The first three catalyze *E/Z* isomerization of retinal, phytochromobilin, and *p*-coumaric acid, respectively, while the last three all have a different flavin-based photochemistry. For many of these photoreceptor proteins, (many of) the details of the conversion of the light-induced change in configuration of their chromophore into a signaling state and eventually a biological response have been resolved. Some members of the rhodopsins, the xanthopsins, and the phototropins are so well characterized that they function as model systems to study (receptor) protein dynamics and (un)folding.

1. Introduction

Light-sensing proteins, i.e., biological photoreceptors, are optimally suited to study the role of dynamical alterations in protein structure in relation to their function. First, such proteins can be triggered with (laser) flash illumination, and therefore excellent time-resolution is achievable in studies of the dynamical alterations in their structure. Second, because they are signal-transduction proteins, one may anticipate large conformational transitions to be involved in their signaling state formation (and its subsequent decay), which is indeed borne out by the experiments (e.g., ref 1). Third, the (changing) color of these proteins often is an excellent indicator as to which time scale is relevant to resolve structural transitions. Significant and unsurpassed insight along these lines has been obtained for a number of different photoreceptor proteins. Hence, they can, indeed, be considered as "star actors" in the pursuit to understand, in general terms, the atomic details of the dynamics of functional conformational transitions [i.e., (partial) un/folding] in these proteins required for their functioning.

The many different photoreceptor proteins that have been described in the literature can be classified into a limited number of families. The most rational approach is to base this classification on the chemical structure of the light-absorbing chromophores involved, but in addition, arguments derived from protein sequence alignment have to be used to discriminate the many photoreceptor proteins that bind a flavin derivative. Accordingly, the most important families are the rhodopsins,[2,3] the phytochromes,[4] the xanthopsins,[5] the cryptochromes,[6] the phototropins,[7] and the BLUF proteins[8] (see also Table 1). The primary photochemistry of activation of these photoreceptor proteins changes the configuration of their chromophore. For the first three families in Table 1, this change in configuration is *E/Z* isomerization, but recently also other types of photochemistry been uncovered (like transient cysteinyl-adduct formation in the LOV domains of phototropins[9]). This change in configuration then initiates formation of a signaling state of sufficient stability to communicate the process of photon absorption to a downstream signal transduction partner.

Possibly, Table 1 does not (yet) cover the full richness of Nature. Although the most important photoreceptor proteins responding to visible and (infra)red light have been uncovered, several systems responding to UV irradiation remain to be characterized. It is one of the remaining challenges in photobiology to establish whether handling of high-energy radiation requires involvement of dedicated chromophores, like carotenoids, chlorophyll precursors, and/or vitamin B_{12}.[10]

The photoreceptor proteins feed their signals into distinct photobiological response pathways. Although, for most of these, significant detail is known, for all of them gaps in our understanding still exist. Here, we will discuss the current status of understanding of each major photoreceptor family and its downstream signal transduction chain separately, through discussion of their best-understood representatives, thereby extending earlier reviews of this field [2,11,12]

2. Rhodopsins

The photoreceptor family that is most senior and has been characterized in most detail is the rhodopsins. Nevertheless, even this family still continues to expand. Besides the visual rhodopsins from Eukarya and Archaea, and the ion-translocating prokaryotic rhodopsins from the Ar-

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Table 1. Well-Characterized Classes of Chromophores and Photosensor Families[a]

CHROMOPHORES classes	example	key structural element	PHOTOSENSOR FAMILY	PHOTOCHEMISTRY
tetrapyrroles	phytochromobilin		Phytochromes	*trans* ↔ *cis*
polyenes	retinal		Rhodopsins	*trans* ↔ *cis*
	coumaric acid		Xanthopsins	*trans* ↔ *cis*
'aromatics'	flavin		Cryptochromes	electron transfer?
			Phototropin	cysteinyl adduct formation
			BLUF proteins	proton transfer?

[a] The curved arrow identifies the vinyl bond subject to photoisomerization. In retinal, both the 11,12- and the 13,14-vinyl bonds can undergo isomerization, like in mammalian and in bacterial sensory rhodopsins, respectively.

chaea, new members of this family have recently been discovered in *Chlamydomonas*[13,14] and *Neurospora*,[15] in proteobacteria (i.e., proteorhodopsin),[16] in cyanobacteria,[17] and in the retina of vertebrates (i.e., melanorhodopsin).[18]

Visual rhodopsins from the higher Eukarya and from the Archaea channel their information into the well-characterized G-protein and Htr/Che networks, which ultimately leads to neural signaling and behavioral swimming responses, respectively. Also the *Chlamydomonas* sensory rhodopsin(s) (note the confusing nomenclature[13,14]) are phototaxis receptors, although the associated signal transduction pathway has not been characterized yet.[13] Proteorhodopsin, upon discovery, was introduced as a proton pump.[16] More recently, options for a sensory function of this proteobacterial rhodopsin are also considered.[19]

The structure of bacteriorhodopsin, with its motif of seven transmembrane α-helices, was elucidated as the first molecular structure of a membrane protein.[20] Its structure has meanwhile been refined to better than 2 Å. This structure has gained an enormous impact because of the fact that it is a major target in pharmacology. The recent elucidation of the crystal structure (now at 2.6 Å resolution) of bovine visual rhodopsin may be expected to boost further studies significantly.

Transient optical (UV/vis and vibrational) spectroscopy has resolved the sequence of states involved in signal generation in many rhodopsins, but particularly in bacteriorhodopsin. In this light-driven proton pump, the Franck–Condon state is referred to as I, with all subsequent transient (hot) ground-state intermediates named alphabetically from J to O. Their kinetics has been resolved in great detail. The initial intermediates are red-shifted as compared to the dark state, and subsequent intramolecular proton transfer leads to the formation of blue-shifted intermediates, a pattern found in many photoreceptor proteins. Although controversy has existed as to the primary photochemistry that activates bacteriorhodopsin, recent evidence indicates[21] that the change of configuration of the retinal molecule from all-trans to 13-cis really is the primary event, taking place with subpicosecond kinetics. Subsequently, strain within the isomerized retinal relaxes (typically in the nanoseconds to microseconds time scale) and next that in the surrounding opsin protein. The photocycle of bacteriorhodopsin is completed in about 10 ms, but for many of the signaling rhodopsins this recovery step is 10- to 100-fold slower. Through the use of retinal analogues, long-living blue-shifted intermediates have been identified as signaling states for the visual and the Archaeal rhodopsins. The relevant intermediate in bacteriorhodopsin shows significant outward movement of helix F.[22]

Unfortunately, rhodopsin crystals lose diffraction upon illumination. With bacteriorhodopsin crystals, however, at cryogenic temperature different intermediate states can be trapped that do diffract to high resolution, although it is not possible to trap exclusively one single intermediate at a time. Nevertheless, the structures of several low-temperature intermediates have now been solved.[23,24] The resolution achieved for the dark structure (i.e., 1.4 Å[25]) allows determination of key bond angles of the retinal

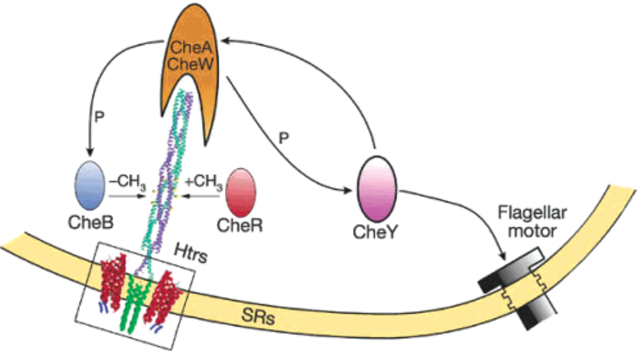

FIGURE 1. Schematic representation of signal transduction mediated by sensory rhodopsin II. The small box in the membrane highlights the interaction between the photosensor protein SRII and the transducer HtrII. P represents the phosphoryl-transfer reactions, common to the (*E. coli*) bacterial chemotaxis system. Reprinted with permission from *Nature* (http://www.nature.com), ref 29. Copyright 2002 Nature Publishing Group.

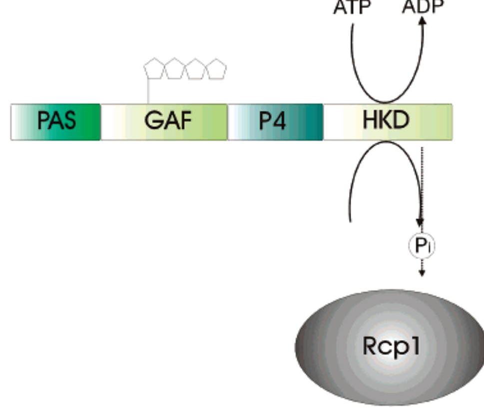

FIGURE 2. Schematic picture of the domain structure of the bacterial phytochrome Cph1 and its response regulator Rcp1. Highlighted are the chromophore linkage to the GAF domain and the phosphoryl transfer from Cph1 to Rcp1.

chromophore, free from model-based assumptions. From these studies, it is clear that retinal in bacteriorhodopsin initially changes from the all-trans to the 13-cis,15-anti configuration, with several additional bonds significantly constrained in the K intermediate, the first that can be trapped. In subsequent photocycle intermediates, the retinal changes to a relaxed 13-cis,15-anti configuration, the hydrogen-bonding network near the Schiff base becomes distorted, and key residues involved in proton translocation change their p*K*. This ultimately leads to transmembrane proton transport, although the change in chromophore configuration brought about by photoisomerization may also be a steric trigger of subsequent conformational transitions. In bacteriorhodopsin, the C_{20} methyl group of retinal and the indole ring of W182 may form the two parts of this "trigger structure".[26] The structure of these intermediates can be linked with their dynamic properties, as detected in optical spectroscopy at room temperature, thus providing a detailed representation of the dynamics in bacteriorhodopsin structure. Many aspects of the initial transitions in bacteriorhodopsin [e.g., (vibrational) spectra, trajectory of isomerization, conformational transitions, etc.] can now be reproduced accurately with quantum and classical dynamics calculations.[27]

Visual rhodopsin is activated by a light-triggered change of its 11-cis retinal chromophore to an initially strained all-trans configuration, which relaxes through β-ionone ring relocation. Subsequently, the salt bridge between the Schiff base and E-113 is disrupted, and the cytoplasmic loop containing E-134 and R-135 increases its affinity for G-protein binding (see, e.g., ref 28).

For sensory rhodopsin II, the most detailed information regarding the entire photobiological signal transduction chain is available (see Figure 1). This includes its spectral tuning and electronic energy levels, structure, signaling state formation, receptor/transducer interaction and signal transfer,[29] and its modulation of flagellar rotation in *Halobacterium salinarium* and in *Escherichia coli*. This even beats the insight into the human visual transduction system. Most importantly, the resolved structure of the SRII/HtrII receptor/transducer complex forms a bridge in our understanding of the initial and downstream signaling events. It is proposed that retinal isomerization—through kinking of helix F—induces a screwlike movement in helix 2 of the cognate transducer.[29] This may constitute a very general mechanism in photo- and chemoreception.

3. Phytochromes

The phytochrome photoreceptor family was discovered as the receptors responsible for red/far-red light reversible plant responses. They exist as dimers in the eukaryotic cytoplasm and translocate to the nucleus upon far-red light activation.[30] A linear tetrapyrrole is their light-sensitive chromophore, bound via a thioether linkage. Red light triggers a cis-to-trans change in the configuration of the extended "all-cis" chromophore, converting it into the far-red light-absorbing Pfr form. Subsequently, it slowly reverts back in the dark (on a time scale of hours) or almost instantaneously upon absorption of far-red light. During these transitions, structural changes take place in the protein, on the micro- and millisecond time scale, as well as proton uptake and release reactions. Their C-terminal domain consists of a combination of PAS (related) domains (PRD) and histidine kinase (related) domains[31] and interacts with signaling partners like PIF3, but may also be involved in stabilization of the (Pfr) form of the protein.[32] Light absorption activates intrinsic kinase activity, but it is not clear whether this is for signal transfer or for adaptation.

In the sequencing project of *Synechocystis* PCC6803, a phytochrome-like gene was discovered,[33] which has led to the identification of several additional bacterial phytochromes, both in phototrophic and in chemotrophic bacteria (e.g., in *Pseudomonas aeruginosa*,[34] *Rhodopseudomonas palustris*,[35] and *Rhodospirillum centenum*[36]). Their chromophore can be bound either to a cysteine or to a histidine (via a Schiff's base linkage). An interesting new member of the phytochrome family was recently found in *Agrobacterium tumefaciens*.[37] Here, the chromophore is bound through pyrrole ring A to an N-terminal cysteine,

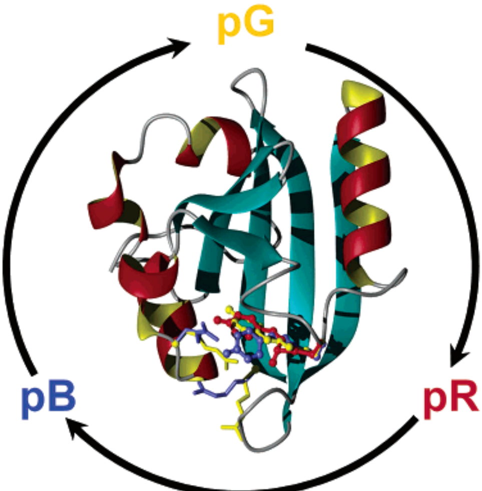

FIGURE 3. Structure and photocycle of PYP. The photocycle shows the key intermediates that are found at room temperature. The three-dimensional structure shows the protein in the three corresponding states, with the changes in the chromophore configuration and the protein conformation highlighted.

outside the GAF domain (Figure 2) that usually binds the chromophore.

Most bacteriophytochromes contain a regular histidine kinase domain at their C-terminus, and a cognate response regulator has been identified in many systems. Accordingly, they can function in light-regulated gene expression,[35] light-induced tactic responses,[38] or resetting of the circadian clock.[39]

Whereas a lot of information has been gained on the output part of the signal transduction cascade initiated by phytochromes, relatively little is known about actual phytochrome signaling states. This may be due in part to the slow Pfr−Pr state conversion and their overlapping absorption spectra, which considerably complicates spectroscopic studies. Especially, though, the lack of detailed structural information on any form of phytochrome slows further progress.

4. Xanthopsins

The family of the xanthopsins is the photoreceptor family that carries *trans-p*-coumaric acid, through a thiol-ester linkage, as its light-sensitive chromophore. Photoactive Yellow Protein (PYP) from *Ectothiorhodospira halophila* is its archetype. PYP is a water-soluble protein of which the structure has been solved at 0.82-Å resolution.[40] PYP displays a typical α/β fold, with a central five-stranded β-sheet and helical segments on either side, which has become the prototype for the PAS domain, a key element in biological signal transfer (Figure 3).

PYP is the photoreceptor for a blue-light-induced avoidance response in *Ec. halophila*.[41] Blue light absorption leads to chromophore isomerization and formation of the signaling state pB.[42,43] Initially the cis configuration of the chromophore is strained, but it relaxes through several picosecond and nanosecond intermediates, until at the time scale of a few hundred microseconds a proton is transferred from E46 (a hydrogen-bonding partner of the chromophore in the receptor state) to the chromophore.[44]

Formation of the pB state shows characteristics typical for a (partial) protein unfolding reaction;[45] its rate (i.e., 10^4 s^{-1}) is compatible with this interpretation. Surprisingly, the degree of transient unfolding in the pB state is dependent on the mesoscopic context of the sensor protein: whereas the crystal structure of the pB state shows that most structural changes take place in the chromophore binding pocket, multidimensional NMR shows structural changes throughout much larger parts of the protein.[46]

PYP from *Ec. halophila* is by far the best-studied xanthopsin. The PYP−phytochrome fusion protein Ppr from *Rs. centenum* is of particular interest because it is the first xanthopsin of which the biological function has been proven genetically: the protein was demonstrated to regulate chalcone synthase gene expression in response to blue light.[36]

Considering the detailed knowledge available for primary photochemistry and signal generation in PYP from *Ec. halophila*, plus our understanding of the biological function of the Ppr system from *Rs. centenum* as a light-regulated two-component system that modulates gene expression, the xanthopsin family also is a candidate to facilitate complete (i.e., based on first principles) understanding of biological signal transfer.

5. Cryptochromes

The cryptochrome family, named after the long-hidden nature of its crucial chromophore, is the oldest family of flavin-containing photoreceptors. These blue/green-light photosensors occur in lower and higher eukaryotes [including mammals (*Homo sapiens*), insects (*Drosophila*), plants (*Arabidopsis*), and algae (*Chlamydomonas*)] and a prokaryote.[47] They are involved in processes ranging from synchronization of the circadian clock in animals to hypocotyl elongation, seed germination, and pigment accumulation in plants. In many higher organisms they are redundant, with Cry2 being proteolytically sensitive under prolonged illumination.

This family of photosensors was identified 10 years ago[6] via their similarity with (bacterial) photolyases. The shared characteristic feature is the joint involvement of two chromophores in photosensing, i.e., a flavin and a pterin, which are incorporated in their N-terminal homologous domain. Some of the amino acids involved in DNA binding are conserved between cryptochromes and photolyases.

Most cryptochromes have a considerable part of the polypeptide chain extending beyond the N-terminal homology domain, in which specific sequence features can be recognized, like subcellular localization signals, a tropomyosin motif, and/or target sites for protein phosphorylation. In agreement with this, it has been recently reported that Cry2 from *Arabidopsis* shows a light-dependent phosphorylation.[48] Together with the observation

that the C-terminal domain of Cry1 of *Arabidopsis* appears to be constitutively activated, this then leads to a model in which light absorption in the N-terminal domain liberates target sites for phosphorylation, and in which the phosphorylated Cry protein is the stably activated form.

The cryptochrome family adds an exciting dimension to the primary photochemistry of photosensing, considering that *E*/*Z* isomerization of neither the pterin nor the flavin is feasible. This has led to many speculations on its primary photochemistry: light-induced transfer of either an exciton or an electron has been proposed, either intra- or intermolecularly (see, e.g., ref 49). Indeed, in photolyase an electron is transiently transferred from the flavin to the DNA-derived, covalently coupled thymidine dimer. The pterin acts merely as an antenna for the flavin, by transferring its excitation energy to the latter via Förster resonance energy transfer.

Crucial in answering the question of whether light-induced electron transfer can take place in cryptochromes is the redox state of the flavin in vivo. The flavin in photolyase is present as $FADH_2$ or as the $FADH^-$ semiquinone radical (which can be photoactivated to the fully reduced form[50]). The flavin chromophore in cryptochrome, however, must be present in the oxidized form, considering the wavelength dependence of the inhibition of hypocotyl elongation.[51]

Lin[52] reported that the photochemistry of cryptochrome is based on (reversible) electron transfer, on the basis of the observed inhibition of cryptochrome function by diphenylene-iodonium in intact cells. Recently, independent evidence was provided that activation of cryptochrome is based on reversible electron transfer.[53]

6. The LOV Domains of Phototropins

The phototropin family, named after its primary representatives that mediate several light responses in plants, like phototropism, chloroplast movement, stomatal opening, and the rapid inhibition of hypocotyl growth,[54] also uses a flavin derivative (i.e., FMN) as its light-sensitive chromophore. The light-sensitive domain that generates signaling in this photoreceptor family is referred to as the LOV domain, also—like the xanthopsins—a subfamily of the PAS domain family. Actually, all known phototropins contain two of these LOV domains, of which the second (i.e., LOV2) is the most important for their light-regulated serine/threonine kinase activity.[54,55] These LOV domains occur in many signal transduction proteins from plants, green algae, and bacteria (like WC-I from *Neurospora crassa*[56] and YtvA from *Bacillus subtilis*[57]).

Upon blue-light excitation, the LOV domains of phototropins are activated through (i) intersystem crossing from an excited singlet to a triplet state, (ii) excited-state proton transfer (note, however, that ab initio theory predicts hydrogen rather than proton transfer[58]), and (iii) covalent adduct formation between the C4 atom of the isoalloxazine ring and the sulfur atom of a nearby conserved cysteine. This sequence of events has firmly been demonstrated with a range of spectroscopic and structural techniques. Signaling state formation is accompanied by disruption of the planar configuration of the flavin, which in turn is transmitted all the way to the surface of the LOV domain, where it leads to disruption of a strongly conserved salt bridge.[55] The latter process may be a unifying feature of signal generation in all PAS domains, including PYP.

The covalent adduct state—presumably the signaling state of the LOV domain—is formed on the microsecond time scale and recovers to the ground state extremely slowly (with rates varying from 10^{-1} to 10^{-4} s^{-1}). Nevertheless, these rates are in the relevant physiological range, and may also be modulated by their mesoscopic context. Formation of the signaling state presumably is also accompanied by a significant conformational transition in the apo-protein part of the LOV domains, as is indicated by ^{31}P and ^{1}H NMR signals originating from this state.[59]

7. BLUF-Domain-Containing Proteins

Even a third family of photoreceptor proteins makes use of a flavin chromophore. This is the so-called BLUF (for "sensors for *bl*ue-light *u*sing *F*AD")-domain-containing family of photosensors that bind FAD as their chromophore.[8] Members of this family are involved in photophobic responses of *Euglena gracilis*[60] and in transcriptional regulation in *Rhodobacter sphaeroides*.[8,61] Secondary structure predictions suggest that the fold of the BLUF domain is distinct from all known flavin-binding folds. Experiments to confirm this with multidimensional NMR spectroscopy are in progress (M. A. van der Horst et al., unpublished experiments). Phylogenetically, this domain is widely distributed among the proteobacteria and the cyanobacteria.

Many BLUF domains are part of multidomain proteins involved in catalytic conversion of regulatory cyclic nucleotides, either cAMP or bis-(3′,5′)-cyclic diguanylate, a regulatory "alarmone" in the bacterial domain, presumably as regulatory domains modulating the catalytic activity of these enzymes in response to absorption of blue photons. However, just like with many other multidomain proteins (like, e.g., the sensory kinases of two-component systems), the BLUF domains can also be present as a small, single-domain protein [e.g., ORF(729−1178) from *Klebsiella pneumoniae*]. In agreement with this, the BLUF-domain-containing proteins from *Eu. gracilis* have been demonstrated to be blue-light-activated adenylyl cyclases (PACs[60]).

The BLUF domains bind FAD noncovalently, as do the LOV domains with FMN. Surprisingly, the fluorescence excitation spectrum of the PAC proteins does not show vibrational fine structure. This contrasts the fluorescence properties of all LOV domains and the absorption characteristics of the AppA protein from *Rb. sphaeroides*.[61] This latter protein is a regulatory protein that integrates redox and light signals in the control of expression of photosynthesis gene clusters,[61,62] as a transcriptional anti-repressor, interacting with PpsR. A detailed model has

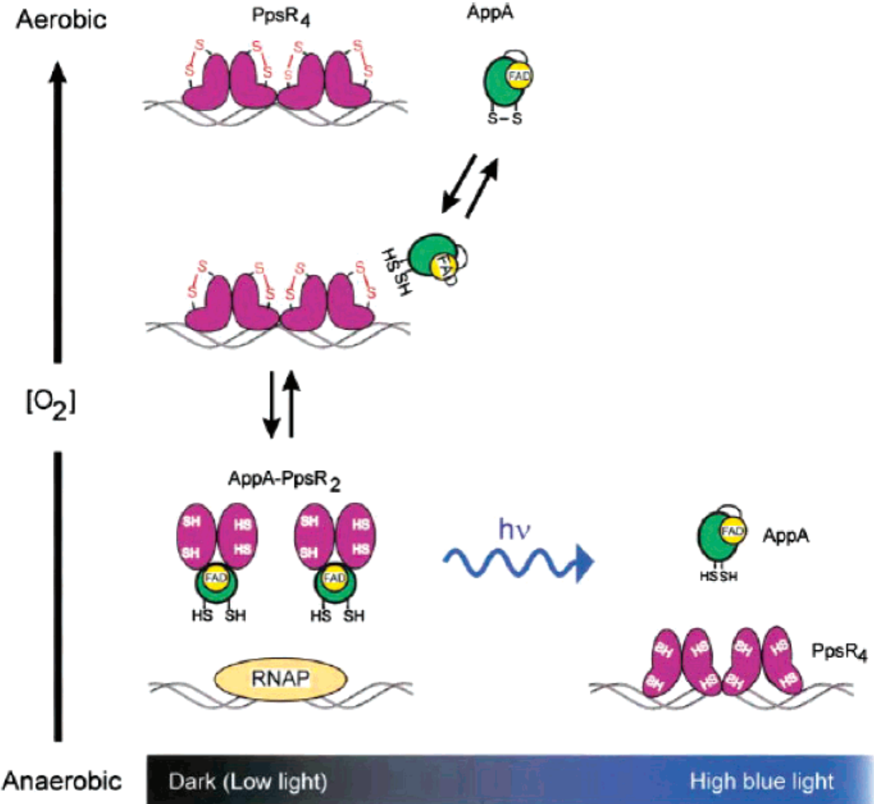

FIGURE 4. Model depicting the action of the transcriptional antirepressor AppA in the regulation of expression of photosynthesis genes in *Rb. sphaeroides* through interaction with the transcriptional regulator PpsR (for further details, see text). Reprinted with permission from *Cell* (http://www.cell.com), ref 61. Copyright 2002 Cell Press.

been proposed (see Figure 4) to explain its function at the molecular level, implying that light absorption would disrupt the interaction between AppA and PpsR dimers, thereby freeing PpsR for repressive interactions of tetrameric PpsR with the relevant promoter region.[61]

The initial characterization of the primary photochemistry of AppA has revealed a number of surprising features: Blue light generates a long-living intermediate—that presumably is the signaling state—with a lifetime of hundreds of seconds and a small (~10 nm) red shift of its absorption spectrum. Site-directed mutagenesis revealed that a conserved tyrosine in the N-terminus of AppA is critically required for photochemistry, and preliminary evidence for the involvement of reversible proton transfer from the flavin (to a tyrosinate?) has been obtained.[63]

Thus, although AppA-mediated signal transduction is well understood in its downstream parts, its initial photochemistry and structural transitions leading to signaling state formation still remain to be resolved.

8. Perspectives

Among the photoreceptors abundant in plants, a dichotomy is discernible concerning the intensity of their mutual interactions. Whereas the phototropins function in more or less linear response pathways, the cryptochromes and phytochromes jointly regulate—through a very complex network—a variety of responses, at levels varying from the transcriptional to the post-translational. It is a challenge to rationalize the underlying mutual interactions from knowledge about the dynamical changes in the structure of the photoreceptor proteins that are initiated by light absorption.

A striking feature for many photoreceptors is that, after photoexcitation of the chromophore, an intramolecular proton transfer takes place. This may be an important (electrostatic) feature required for the subsequent conformational change that drives a photoreceptor protein into its signaling state. Many photoreceptor proteins furthermore display light-induced branching reactions in their photocycle, particularly originating from long-lived blue-shifted intermediates, bringing the protein back in its receptor state. This has been shown for many (archaeal) rhodopsins (e.g., ref 64) and xanthopsins (e.g., ref 65), and now also for a LOV domain (J. Kennis et al., Free University Amsterdam, unpublished observations). Particularly for photoreceptor families with extremely low recovery rates ($<10^{-3}$ s^{-1}, like some phototropins and BLUF proteins), this has important functional consequences: under many conditions they may actually operate as “two-photon switches”, such that short-wavelength irradiation negates the effect of visible light.

Signal transduction research is dominated by so-called “two-state models”; i.e., a particular sensor either is, or is not, in its signaling state, and as a consequence only two structural states are relevant for consideration. Only rarely are observations made that require more complex models,

like the "three-state model" for VirA from *Agrobacterium tumefaciens*.[66] Nevertheless, photosensor proteins go through a cycle with much more complex transitions than the simple "two-state model". Therefore, another challenge in photosensor research is to translate our understanding of the spatial and temporal characteristics of the transitions in the photosensor structure into simple mathematical models with high predictive capacity.

The intrinsic dynamics of a protein can be analyzed by molecular dynamics modeling (up to the nanosecond time scale for a single protein molecule in a box of water molecules). However, currently available algorithms and/or accessible computational capacity does not allow one to extend these simulations for regular photoreceptor proteins to the millisecond or second time scale relevant for enzyme catalytic turnover and signal transfer. In one particular class of "proteins" this may be different, however. Recently, an analysis was reported of ultrafast light-induced conformational transitions in a small peptide, designed as a light-sensitive switch.[67] The results obtained were in good accordance with MD predictions; for this system, predictions and measurements can overlap in the time domain because of the small size of the system. It can be anticipated that design and analysis of increasingly complex systems of this type will bring us closer to detailed understanding of the intramolecular basis of light sensing, just like the detailed "films" that are being recorded with time-resolved Laue diffraction at ambient temperature.[68]

Ultimately, such "design photosensors" may compete with those originating from biology in light-mediated steering of relevant genetic circuits.[69]

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