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Photoreceptors Take Charge: Emerging Principles for Light Sensing

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Abstract

The first stage in biological signaling is based on changes in the functional state of a receptor protein triggered by interaction of the receptor with its ligand(s). The light-triggered nature of photoreceptors allows studies on the mechanism of such changes in receptor proteins using a wide range of biophysical methods and with superb time resolution. Here, we critically evaluate current understanding of proton and electron transfer in photosensory proteins and their involvement both in primary photochemistry and subsequent processes that lead to the formation of the signaling state. An insight emerging from multiple families of photoreceptors is that ultrafast primary photochemistry is followed by slower proton transfer steps that contribute to triggering large protein conformational changes during signaling state formation. We discuss themes and principles for light sensing shared by the six photoreceptor families: rhodopsins, phytochromes, photoactive yellow proteins, light-oxygen-voltage proteins, blue-light sensors using flavin, and cryptochromes.

Contents

1. INTRODUCTION

We review time-resolved studies on a diverse set of photoreceptors and aim to derive general principles for the molecular mechanism that causes their activation.

1.1. Deriving Molecular Mechanisms of Photoreceptor Activation

Research on photosensory proteins has provided rich data on the molecular mechanisms of six distinct types of photosensory modules: rhodopsins; phytochromes; photoactive yellow proteins (PYPs, also referred to as xanthopsins); light-oxygen-voltage (LOV) proteins; cryptochromes; and blue-light using flavin (BLUF) proteins (16, 28, 75, 90, 134). In this review, we aim to derive emerging themes and generally applicable principles governing the molecular mechanism underlying the function of these proteins. We do not discuss the more recently discovered photoreceptors UVR8 (ultraviolet resistance locus 8) from plants and algae (110), B_{12}-dependent CarH from myxobacteria (99) discovered in studies on carotenoid biosynthesis, the orange carotenoid protein from cyanobacteria (139), and the UV receptor LITE-1 from *Caenorhabditis elegans* (36) because limited experimental data are available for these proteins.

While the set of six well-studied types of photosensory proteins exhibit extensive diversity in their spectral, structural, and biophysical properties, they utilize just four different chromophores (**Figure 1**). Therefore, they represent a valuable data set for deriving generally applicable mechanisms in functional protein dynamics and the molecular mechanism of receptor activation. Photoreceptors provide a unique opportunity for understanding these processes because their

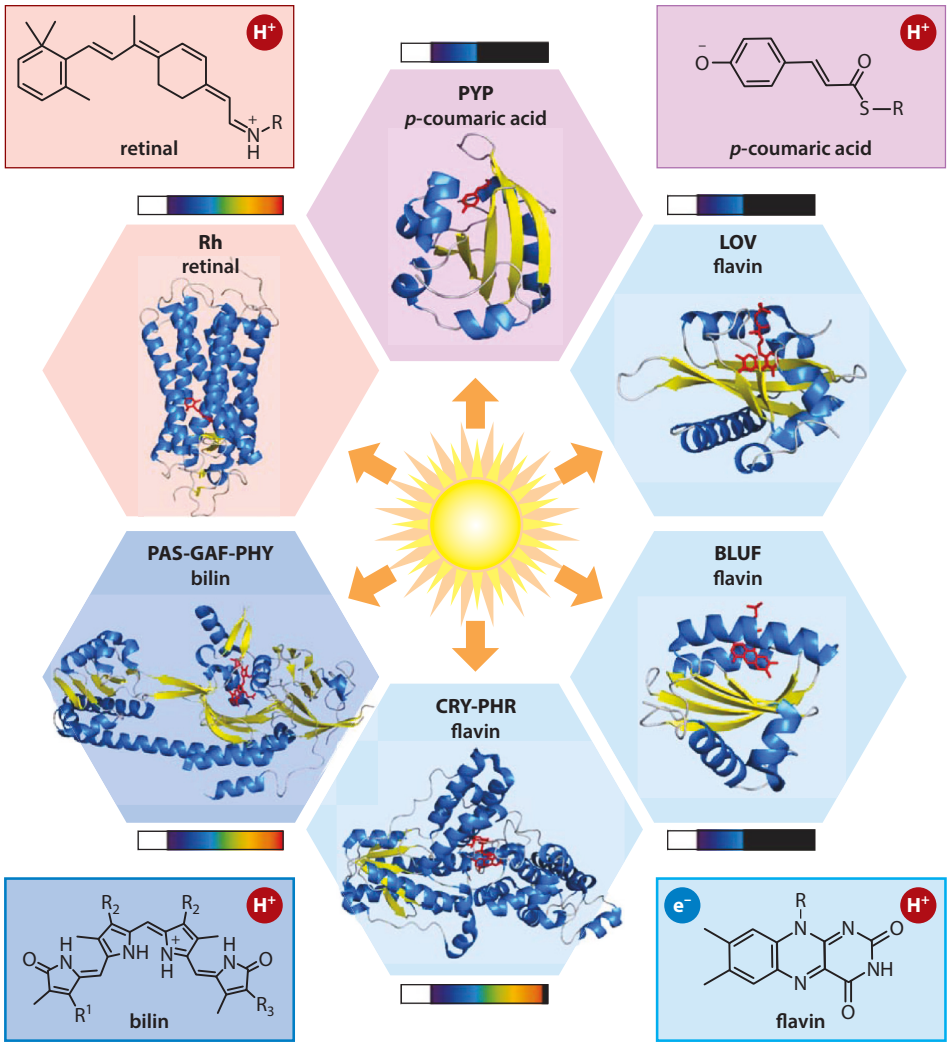

Figure 1

Overview of six types of photosensory proteins. For rhodopsin, PYP, LOV proteins, BLUF proteins, cryptochrome, and phytochrome, a representative crystal structure of the sensory domains is indicated, together with the spectral region they cover. The chemical structures of the four chromophores bound to the photoreceptors are shown. PDB IDs for the depicted rhodopsin, PYP, LOV, BLUF, cryptochrome, and phytochrome are 1F88, 1NWZ, 2V0U, 2BYC, 1U3D, and 2VEA, respectively. The spectral region absorbed by members of each photoreceptor type are indicated in the colored bars. Abbreviations: BLUF, blue-light using flavin; CRY-PHR, cryptochrome-photolyase; e^-, electron; H^+, hydrogen; LOV, light-oxygen-voltage; PAS-GAF-PHY, Per-ARNT-Sim–cGMP phosphodiesterase/adenylyl cyclase/FhlA–phytochrome-specific domain; PYP, photoactive yellow protein; Rh, rhodopsin.

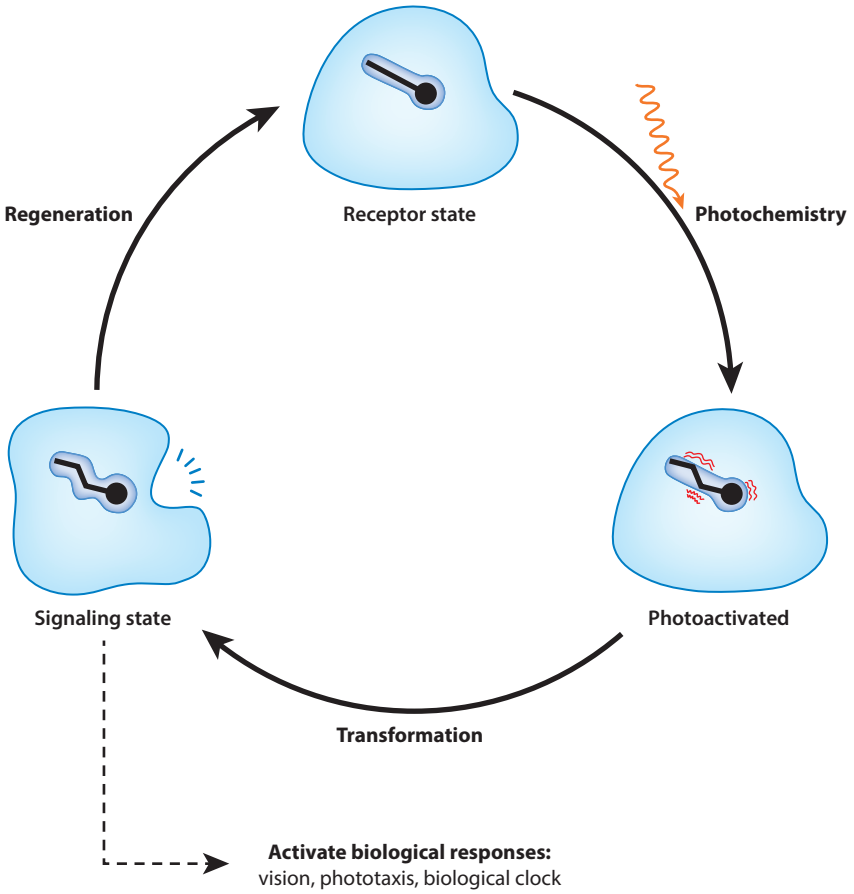

Figure 2

Shared properties of most photoreceptors. Light excitation of the chromophore leads to an electronically excited state that undergoes photochemistry. In picoseconds, the chromophore returns to the electronic ground state with different geometry and/or oxidation state, which triggers subsequent structural evolution, leading to the formation of the signaling state. In the signaling state, the photoreceptor protein relays a signal to a subsequent component in a signal transduction chain, triggering a cellular response.

light-triggered nature allows for experimental studies across the entire relevant time range, from femtoseconds to the formation and decay of signaling states of the receptor on a much longer timescale. Here, we focus on selected photoreceptor proteins for which time-resolved biophysical data are available.

The application of time-resolved techniques can reveal the sequence of molecular events leading to receptor activation (**Figure 2**). This temporal sequence of events provides direct, powerful clues regarding the causal chain of events that converts the absorbance of a photon into protein conformational changes for biological signaling. A question of general relevance for protein science is how the ultrafast, local structural changes at the active site during the primary photochemical event are converted into long-lived global conformational changes in the receptor protein. Here, we present recent progress on this question, with emphasis on the occurrence of proton transfer, changes in hydrogen bonding, and strain induced by the primary photochemistry.

1.2. Diversity in Chromophore and Fold of Photoreceptors

The six types of photoreceptor proteins contain four distinct chromophores: retinal in the rhodopsins; bilin in the phytochromes; flavin in LOV proteins, cryptochrome, and BLUF proteins; and *p*-coumaric acid (*p*CA) in PYP. While the rhodopsins form transmembrane seven-α-helical bundles (101), the other five photoreceptors are water soluble (**Figure 1**). PYP (104) and LOV proteins (18) adopt a Per-ARNT-Sim (PAS) domain structure, cryptochromes share the same fold composed of Rossmann-like α/β and α subdomains as photolyases (12), and BLUF proteins form a unique flavin-binding fold (1, 55, 61). In the case of phytochromes, multiple protein domains, particularly PAS, GAF (cGMP phosphodiesterase/adenylyl cyclase/FhlA), and PHY (phytochrome-specific) domains, are involved in embedding the larger bilin chromophore (29), although considerable variability in domain composition occurs (112). Linked to these sensory domains, a variety of effectors have been identified, mostly on the C-terminal side but sometimes also found N-terminally.

2. SEQUENCE OF MOLECULAR EVENTS DURING RECEPTOR ACTIVATION

To identify general principles in the molecular mechanism of photoreceptor activation, we first review knowledge on the series of light-induced molecular events in the six types of well-studied photoreceptors.

2.1. Rhodopsins

Extensive data are available for three distinct types of rhodopsins (28): the animal rhodopsins and the microbial rhodopsins, which consist of the microbial sensory rhodopsins (SR) and the channelrhodopsins (ChR) from algae (37). The chromophore in all rhodopsins is a retinal chromophore linked to a lysine side chain through a Schiff base, and the primary photochemical event is the photoisomerization of a C=C bond in the retinal. In animal rhodopsins, the retinal initially is present in the 11-*cis* conformation, which is converted to all-*trans* retinal in the primary photoproduct (145). In the microbial rhodopsins (14, 45) the initial all-*trans* retinal is converted to the 13-*cis* conformation. These isomerization events occur within one picosecond with a quantum yield of ≥0.6, while storing approximately 50% of the energy of the absorbed photon (28).

The structural changes that occur in both the retinal and the protein upon the formation of this first (red-shifted) photoproduct for rhodopsin (92) are quite small owing to concurrent rotation over the isomerizing C=C double bond and C–C single bonds in the retinal chromophore (see 138). The substantial changes in backbone conformation upon the formation of the primary photoproduct of ChR (109) are unusual in this respect.

In most rhodopsins, the Schiff base is protonated in the initial dark state of the protein, and ultrafast retinal photoisomerization is followed by slower Schiff base deprotonation. For bovine rhodopsin, this deprotonation event occurs on a timescale of approximately 2 ms (23), with Glu113 as the proton acceptor. This step results in the formation of the Meta II intermediate and proceeds with the occurrence of substantial protein conformational changes. Importantly, Schiff base deprotonation precedes the conformational changes that result in the activation of rhodopsin (3). In sensory rhodopsin I (SRI), the Schiff base deprotonation event occurs with a time constant of 270 μs, with His166 as the proton acceptor. For SRII from *Halobacterium salinarum*, the proton acceptor is Asp73, which is protonated within 1 ms. In channelrhodopsin-2, either Glu123 or Asp253 accepts a proton from the Schiff base with a time constant of 10 μs (67, 74, 119). In the light-driven proton pump bacteriorhodopsin, the protonated Schiff base (PSB) and the proton

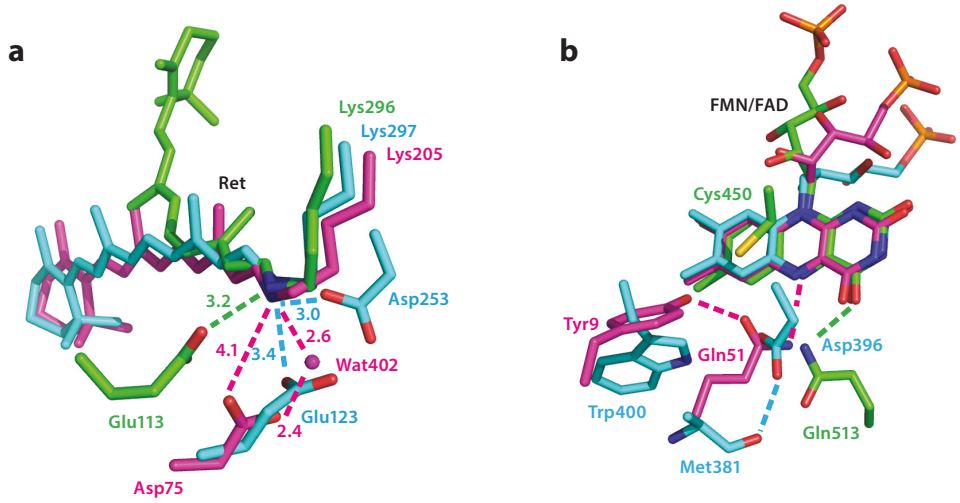

Figure 3

Chromophore binding pockets of photosensors containing retinal and flavin and their proton/electron acceptors/donors. (*a*) Overlay of the retinal chromophore of animal rhodopsin (*green*; PDB ID 1GZM), sensory rhodopsin II (*pink*; PDB ID 1JGJ), and channelrhodopsin (*cyan*; PDB ID 3UG9). For ChR2, two possible proton acceptors are included. (*b*) Overlay of the flavin chromophore in LOV proteins (*green*; PDB ID 1N9L), BLUF proteins (*pink*; PDB ID 2BYC), and plant cryptochrome (*cyan*; PDB ID 1U3D). The electron donors are included for LOV proteins (Cys450), BLUF proteins (Tyr9), and cryptochrome (W400). The flavin N(5) is hydrogen-bonded in BLUF proteins by Gln51, allowing for a proton-coupled electron transfer from Tyr9 but not to the proton donors in LOV proteins (C450) or in cryptochrome (Asp396). The adenosine moiety of FAD in cryptochrome has been omitted for clarity. Abbreviations: BLUF, blue-light using flavin; ChR2, channelrhodopsin-2; FAD, flavin adenine dinucleotide; FMN, flavin mononucleotide FMN; LOV, light-oxygen-voltage.

acceptor Asp85 are connected through a water molecule (56, 78), and a corresponding water molecule (but with lower electron density) was observed in SRII from *Natronobacterium pharaonis* (77), where Asp75 is the proton acceptor (**Figure 3*a***). However, in animal rhodopsin, the PSB and Glu113 form a direct salt bridge (72, 101), as is the case for the PSB in ChR2 (57). This issue is likely to be of great importance for understanding the mechanism of light-triggered proton transfer in the rhodopsins (31, 56).

Schiff base deprotonation is generally accompanied by functionally important conformational changes. For rhodopsin, Schiff base deprotonation results in the formation of the Meta II state in ~2 ms, which involves protein conformational changes that activate the receptor (23, 146). For SRI, the S373 state is formed in 270 μs and involves substantial conformational changes (8, 11). In ChR2 from *Chlamydomonas*, retinal *trans* to *cis* isomerization causes substantial conformational changes in protein backbone during the formation of the early red-shifted P1 (or P500) state (96). Subsequent Schiff base deprotonation upon formation of the blue-shifted P2 (or P390) state without further conformational changes occurs with a time constant of ~25 μs (109). The decay of this state into the late red-shifted P3 (or P520) state in ~0.15 ms involves Schiff base reprotonation and small conformational changes that convert ChR2 into its conductive state.

2.2. Phytochromes

Phytochrome photoreceptors utilize linear tetrapyrrole chromophores (bilins) as primary light absorbers that, upon photoexcitation, initiate a complex set of dynamics that activate an output

domain (e.g., histidine kinases) (113). While phytochromes and their bacterial bacteriophytochromes (BphP) homologs are qualitatively similar to the cyanobacteriochrome photoreceptor family, they differ significantly in spectral responses, photodynamics, and protein architectures. Here, we focus on the forward dynamics in plant phytochromes and bacteriophytochromes.

Plant phytochromes and BphP exhibit the greatest range of activity of the known photoreceptor families (114). This is a consequence of the intrinsic variability in the bilin chromophores and the differing protein architectures. Variation in the bilin chromophore can involve changes in multiple dihedral angles, protonation state of the nitrogen atom in each of the four pyrrole rings, and hydrogen bonding of these groups. Furthermore, several different bilin chromophores have been identified. BphPs use the biliverdin-IXR chromophore generated from heme, while plant and cyanobacterial phytochromes utilize the more reduced phytochromobilin and phycocyanobilin chromophores, respectively.

Canonical phytochromes reversibly switch between red-absorbing P_r and far-red-absorbing states P_{fr}. While this light-adapted protein can eventually change the chromophore conformation back to the dark-adapted form through the thermal relaxation, this reverse process can also be accelerated by photoexcitation.

Our understanding of phytochrome photoactivity has advanced greatly in the past decade owing to the characterization of multiple crystal and nuclear magnetic resonance (NMR) structures (29, 124) complemented with spectroscopic studies. In most phytochromes, the D-ring of the bilin in the dark-adapted P_r state occupies a *Z* conformation with respect to other rings. Photoexcitation of P_r initiates an excited-state isomerization reaction with the D-ring twisting to generate a primary lumi-R photoproduct with the D-ring in a putative *E* configuration (20, 43). Most ultrafast studies on phytochromes resolve multi-exponential excited-state decay kinetics, which have been interpreted in varying ways, including an excited-state equilibrium (47) and partial *Z*-to-*E* isomerization in the excited state (20). The reaction occurs with a low quantum yield (<15%) (58, 69) and on a picosecond timescale (59). Photothermal beam deflection measurements indicated that lumi-R in oat phytochrome is a highly strained intermediate (86).

The lumi-R intermediate evolves through a series of Meta intermediates to generate the light-active P_{fr} state, including Meta-R_a and Meta-R_c. While the dynamics involved are still unclear, temperature-dependent X-ray crystallography indicated a so-called flip-and-rotate mechanism responsible for BphP activity whereby after isomerization, the whole bilin rotates within the binding pocket (143). The recent light-adapted structure of a different BphP homolog identified an opening (with a time constant of 4 ms) of the BphP dimer probably induced by a secondary structural change at a critical so-called tongue region connecting two domains together (129). Solid-state NMR spectroscopy has revealed that the P_r to P_{fr} transition involves extensive changes in the internal hydrogen-bonding network and switching of salt bridges within the protein (124).

Raman studies indicate that Meta-Rc in BphP has a deprotonated bilin, which occurs on a millisecond timescale and requires the reprotonation of the bilin to generate P_{fr} (9, 135) [this is unclear for plant phytochromes (62, 88)]. In addition, evidence has been reported for the transient release of a proton to the solvent during population of the Meta-R_c intermediate (9, 135).

2.3. Photoactive Yellow Proteins

Most biophysical studies of PYP have used the homolog from *Halorhodospira halophila* (41). Ultraviolet–visible spectroscopy (UV/vis) absorbance studies from the femtosecond to the second time range, coupled with global analysis approaches, have identified a number of intermediates: the primary photoproduct I_0, which decays to the early red-shifted pR state in ∼5 ns, followed

by the formation of the late blue-shifted pB photocycle intermediate in ~250 μs and the final recovery of the initial state in ~250 ms (46, 71, 85).

The PYP photocycle is initiated by the *trans* to *cis* photoisomerization of the central C=C moiety of the *p*CA chromophore (65, 131). The structural changes upon photoisomerization have been studied intensively. Early Fourier transform infrared (FTIR) spectroscopy studies revealed that *p*CA isomerization during the entry of PYP into its photocycle involves both double- and single-bond rotations that flip the C=O group of the chromophore (140). This process leaves the hydrogen bond between the phenolic oxygen of the *p*CA and the protonated side chain of active-site residue Glu46 intact (140, 141). Subsequent vibrational studies with progressively better time resolution have largely confirmed this view (17, 38, 44, 68, 132). In addition, both cryotrapping and time-resolved X-ray crystallography have yielded rich structural information on the initial events in the PYP photocycle (34, 48, 103, 120).

An important emerging insight is that the structural changes in the initial event of the PYP photocycle are surprisingly small. This is possible because of the combined rotation over both the isomerizing C=C double bond and single bonds. As a result, the ring of the *p*CA chromophore does not flip in the primary event, which would involve substantial structural changes (140, 141).

X-ray crystallographic work has resulted in the identification of the highly strained I_0 primary photoproduct and the implications of its structure on the mechanism of *p*CA photoisomerization. However, recent ultrafast UV/vis absorbance studies on the PYP homolog from *Rhodospirillum centenum* (53) revealed that this protein enters its photocycle with a similar quantum yield but without the formation of an I_0 intermediate. Instead, the excited state evolves immediately to the early red-shifted pR photoproduct (87). This result implies that the I_0 intermediate is not required for photocycle entry. An important take-home lesson is that the occurrence of a molecular event or the population of an intermediate does not provide conclusive proof that this intermediate or process is required for function.

Key insights into the conversion of the early pR intermediate into the pB state were obtained through time-resolved FTIR difference spectroscopy (141). This approach allowed the real-time detection of the deprotonation of Glu46 and the protonation of the *p*CA chromophore with the same time constant of 250 μs, providing direct evidence for proton transfer from Glu46 to the *p*CA. Following this proton transfer event, large amide I difference signals develop with a time constant of 2 ms. These data clearly kinetically separate a faster proton transfer event followed by slower global protein conformational changes.

2.4. Light-Oxygen-Voltage Proteins

LOV proteins bind an oxidized flavin as their chromophore. The photoproduct is a covalent adduct between flavin and an adjacent cysteine residue (19, 117) (**Figure 3*b***). LOV proteins are the only photoreceptor in which photochemistry takes place from the triplet excited state (128). Therefore, the primary change proceeds very slowly within a few microseconds. The reason might be the low reactivity of cysteine for electron transfer, but the precise reaction mechanism of adduct formation has been under debate for many years. It was proposed early on that an ionic intermediate is formed within nanoseconds by protonation of the triplet excited state of flavin, but this proposal has been refuted by electron paramagnetic resonance spectroscopy (118) and time-resolved FTIR spectroscopy (106). Clear evidence for an intermediate state at room temperature is missing, but a concerted reaction has been ruled out as well by introducing a competing reaction channel by a tyrosine (80). Most likely, electron transfer from cysteine to flavin is the first step (118) and rate limiting, followed by intersystem crossing, proton transfer, and radical recombination in an unresolved sequence.

Therefore, the most intuitive primary process to initiate signaling would be a strain or steric clash resulting from the covalent bond formation to the flavin and the resulting sp^3 hybridization of a carbon in the planar aromatic isoalloxazine. Instead, a rearrangement of the hydrogen-bond network of the flavin by rotation of a glutamine side chain has been implicated from X-ray crystallography and resolved by FTIR spectroscopy (19, 97). This rearrangement takes place concomitant with adduct formation (106) and channels the signal to the β-sheet surface as visualized by molecular dynamics simulations (105), causing the release of the C-terminal Jα helix as observed by NMR spectroscopy (40). The dissociation of the latter and subsequent unfolding have been interpreted by transient grating spectroscopy to proceed with $\tau = 300$ μs and $\tau = 1$ ms, respectively (93), but recent time-resolved infrared spectroscopic experiments point to an unfolding already in the first process with $\tau = 240$ μs (64). The release of the Jα is modulated by the unfolding of an extension at the N-terminus, the A'α helix (147). In bacterial LOV proteins, a different role of the Jα helix undergoing a rotation of coiled coils has been revealed (89), but time-resolved information on this process is missing. These conformational changes finally lead to a release of effector inhibition or the formation or dissociation of receptor dimers. Strikingly, the light-induced formation of a hydrogen bond between the protonated N(5) of flavin and the glutamine has been shown to be sufficient for conformational changes in a cysteine-less mutant of a fungal LOV proteins, which induce dimerization and signaling in the absence of any adduct formation (144).

2.5. Blue-Light Using Flavin Proteins

In BLUF proteins, the flavin remains oxidized after illumination with only a small red shift by about 10 nm in the absorption spectrum (81), which is observed already within hundreds of picoseconds in transient absorption spectra (33). The change was assigned by FTIR spectroscopy to hydrogen-bond rearrangements that weaken the bond strength of the two carbonyl moieties of flavin (82). The primary changes leading to this rearrangement include the formation of a flavin radical by electron transfer and proton transfer from tyrosine (**Figure 3*b***), which recombines on the ultrafast timescale as indicated by infrared spectroscopy (32); however, this intermediate seems not to be necessary in all BLUF proteins (79). The proposed product (24, 115, 126) of this rearrangement has been identified by a combination of FTIR double difference spectroscopy and quantum chemical calculations as a tautomerized glutamine close to flavin (25). The final rotamer of the tautomerized glutamine in the light state is still under strong debate, as is the role of other residues in the flavin binding pocket such as a methionine and a tryptophan (1, 54, 61).

A register shift of the β5 strand has been suggested from infrared spectroscopy to stabilize the reorganized hydrogen-bonding network (83). This shift has not been time resolved, but some conformational changes in BLUF proteins have been shown to occur with $\tau = 13$ μs by transient grating spectroscopy (94). The structure of a BLUF domain with its native effector provided information on the interdomain communication (5). The signal was suggested to proceed via the β5 strand and C-terminal helices to the effector. From this and following studies, the impression emerges that the signaling pathway from the BLUF domain to the effector might vary depending on the type of effector.

2.6. Cryptochromes

In sensory cryptochromes, directly after excitation of the oxidized flavin, electron transfer of tryptophan to flavin occurs with a time constant of $\tau = 400$ fs, as demonstrated by transient absorption spectroscopy (13, 50) (**Figure 3*b***). On the picosecond timescale, the hole of the tryptophan cation radical is transported toward the surface of the protein by a series of tryptophan residues, the

tryptophan triad (12, 35). The identity of the tryptophans involved is under debate, because it has been demonstrated on a DASH cryptochrome that both the distance and the orientation of the tryptophans need to be taken into account for the identification (7). Moreover, the promiscuous nature of electron transfer leads to many possible pathways via tryptophans and tyrosines, rendering the study of point mutations arbitrary. A long debate on the lacking requirement of tryptophans for the generation of a signal in plant cryptochrome in vivo (73) has been settled by the finding that alternative electron transfer pathways may be active in the presence of cellular nucleotides (27). The role of the tryptophan triad can clearly be assigned to increase the distance of charge separation by which the unproductive recombination reactions are considerably slowed down.

In plant cryptochromes, proton transfer to flavin is observed with a time constant of $\tau = 2$ μs (70), decoupled from the ultrafast electron transfer. The proton donor has been identified as Asp396 close to flavin (42, 130). It has been suggested from theoretical work that an ultrafast proton transfer takes place (123). According to flash photolysis experiments, this pathway becomes relevant to some extent only in the absence of ATP (91). Step-scan FTIR spectroscopy revealed that the β-sheet of the α/β subdomain within the sensory domain at a distance of ∼25 Å to flavin becomes destabilized with a time constant of $\tau = 500$ μs (130). Finally, the C-terminal extension outside the sensory domain undergoes a conformational change as observed by transient grating spectroscopy with a time constant of $\tau = 400$ ms (63). The neutral flavin radical state has been identified as the signaling state of plant cryptochromes in steady-state experiments (4, 10).

For insect cryptochromes, time-resolved data are not available, and it is under debate whether the dark form of the flavin is the oxidized flavin or the anion radical in vivo (100, 133). Illumination of the oxidized flavin in vitro leads to formation of the anion radical without subsequent proton transfer to flavin (6). This difference to plant cryptochrome can be attributed to the presence of a cysteine close to flavin instead of the aspartic acid (148).

3. EMERGING PRINCIPLES

A number of recurring themes emerge from the studies of the mechanism of photoactivation of different photoreceptors. We now identify these themes and discuss the general mechanisms for photoreceptor activation that they imply.

3.1. Minimal Atomic Motions During the Formation of the Primary Photoproduct

In the case of the three flavin-containing photoreceptor types (LOV proteins, BLUF proteins, and cryptochrome), the aromatic system of the flavin isoalloxazine cannot undergo isomerization and other changes in geometry are also highly restricted. The observation that the conformational changes upon the formation of the flavin-cysteine adduct at low temperature are small (51) is in line with the notion that formation of the primary photoproduct involves only small atomic motions.

In the case of the three photoreceptor types (rhodopsins, phytochromes, and PYP) that involve C=C photoisomerization as the primary photochemical step, the atomic motions in the chromophore have the potential to be much larger in amplitude if rotations would cause a large part of the chromophore to "flip" to a new position. In striking contrast with this notion, chromophore photoisomerization in PYP, Rh, and SRII results in very small atomic motions. In each of these cases (except for ChR; see Section 2.1 above), this is achieved by concomitant rotation not only over the isomerizing C=C double bond but also of C–C single bonds.

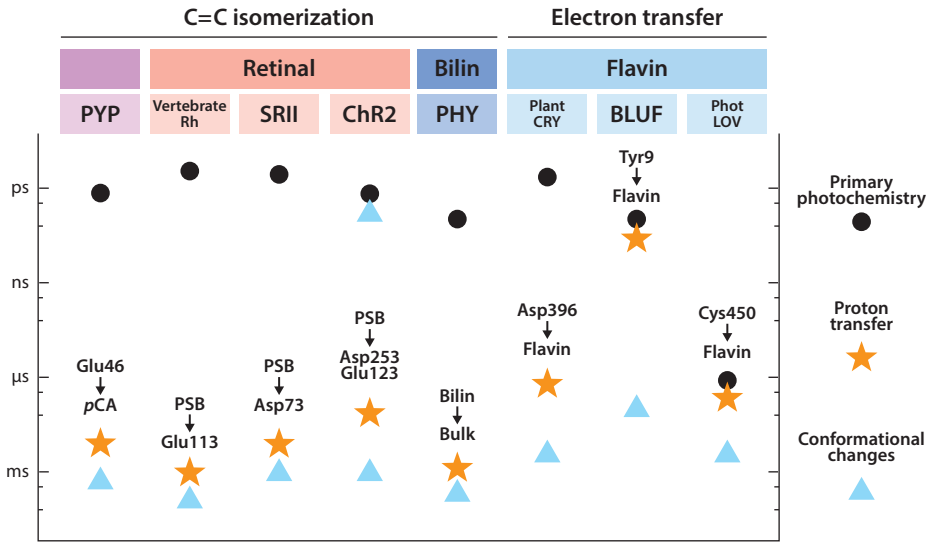

Figure 4

Dissecting the kinetics of key events in photoreceptor activation. The rates for the primary photochemical events (*black circles*), proton transfers involving the chromophore (*orange stars*), and large protein conformational changes for formation of the signaling state (*blue triangles*) in the sensory domains are indicated on a logarithmic time scale from picoseconds to milliseconds. Abbreviations: BLUF, blue-light using flavin; ChR2, channelrhodopsin-2; CRY, cryptochrome; LOV, light-oxygen-voltage; *p*CA, *p*-coumaric acid; Phot, phototropin, an LOV-containing plant photoreceptor; PHY, phytochrome-specific domain; PSB, protonated Schiff base; PYP, photoactive yellow protein; Rh, rhodopsin; SRII, sensory rhodopsin II.

These considerations imply that the occurrence of concomitant double- and single-bond isomerization in the chromophore to minimize atomic motions during primary photochemistry is an important and widespread strategy that allows photoreceptors to efficiently use the electronically excited state on a (sub)picosecond timescale to generate a photoproduct with a high quantum yield within the tightly packed interior of a protein.

3.2. From Ultrafast Photochemistry to Microsecond Proton Transfer to Subsequent Conformational Changes

Figure 4 reveals an important recurrent theme in different photoreceptors: The initial ultrafast photochemical event is followed by proton transfer to/from the chromophore on a microsecond timescale and subsequent protein conformational changes on a millisecond timescale. This observation leads to the possibility that proton transfer is a major contributor to the mechanism that converts the initial local photochemical event into global protein conformational changes for signaling. In this model, a number of important questions arise. How does the primary photochemical event cause subsequent proton transfer? Why is the proton transfer step (typically microseconds) so much slower than the initial photochemical event (typically picoseconds)? And finally, does proton transfer drive subsequent protein conformational changes; and if it does (see next section), how?

In these considerations, it should be noted that the proton transfer events in LOV proteins and BLUF proteins are part of a proton-coupled electron transfer (PCET) process and thus are mechanistically distinct from proton transfer in the other photoreceptor families. PCET is a

general observation in many reactions involving electron transfer. Of the three flavin-binding receptor families, both BLUF (32) and LOV (80) proteins show a PCET, but such process has not been finally proven for LOV proteins. In contrast, the cryptochromes undergo either a decoupled proton transfer as in the case of plant cryptochrome (70) or no proton transfer to flavin at all as found for insect (type I) cryptochrome (6).

We suggest two possible explanations for this difference between, on one hand, LOV and BLUF proteins and, on the other hand, cryptochrome. In the first hypothesis, PCET relies on the presence of a hydrogen bond to flavin N(5), which is indeed present in BLUF proteins and provides a linking hydrogen-bond structure to the electron-donating tyrosine via a glutamine (1, 54, 61) (**Figure 3*b***). In plant cryptochromes, such a hydrogen bond is absent because the aspartic acid 396 is hydrogen bonded to the opposite side to a backbone carbonyl as indicated by X-ray crystallography (12). In contradiction to this hypothesis, such a hydrogen bond is not present in LOV proteins in the dark (19); although there, the flavin most likely undergoes PCET from the cysteine.

The second hypothesis claims that PCET is dominant for the tyrosine in BLUF proteins and the cysteine in LOV proteins because the cation radicals resulting from the electron transfer are of high acidity with a $pK_a = -2$ for $TyrOH^{\bullet+}$. In agreement with this hypothesis, the tryptophan cation radical formed in cryptochromes is even stabilized into the nano- to microsecond time range (70, 91) by the transport of the hole via the electron wire and, in particular, by its significantly lower acidity with a $pK_a = 4$ for $TrpNH^{\bullet+}$.

We propose that the widespread occurrence of proton transfer upon primary photochemistry in a range of photoreceptors reflects the important role that this process can play in driving protein conformational changes for signaling state formation, as reviewed in the next section.

3.3. Electrostatic Epicenter for Photoreceptor Activation

For multiple photoreceptor systems, evidence has been reported that the proton/electron transfer processes that occur upon photoactivation are critical to driving the protein conformational changes needed for the formation of the signaling state of the photoreceptor. In the case of PYP, proton transfer from the protonated side chain of Glu46 to the deprotonated phenolic group of the *p*CA (49, 140) results in the movement of a buried negative charge to a hydrophobic pocket (140, 141). The energetically unfavorable effect of placing a charged side chain in a hydrophobic pocket in the early blue-shifted photointermediate pB′ causes the subsequent large conformational changes [a so-called protein quake (2)] upon the formation of the late blue-shifted pB photointermediate. The transition from pB′ to the pB state is spectroscopically silent in UV/vis absorbance spectroscopy but was first predicted (140) and subsequently observed (141) using FTIR spectroscopy, resulting in the formulation of the electrostatic epicenter model. Further evidence for the role of the negative charge of the side chain of Glu46 as the electrostatic epicenter for driving pB formation is that light-induced conformational changes in the E46Q mutant are greatly reduced (22, 141).

Electrostatic epicenters may play a role in receptor activation of other receptor proteins. The light-induced generation of a charge occurs in plant cryptochromes as a consequence of ultrafast electron transfer: A negative charge resides on the flavin anion radical formed, and a positively charged counterpart is produced at the electron donor. Even after protonation of the flavin anion radical in plant cryptochrome by the adjacent aspartic acid, a negative, buried charge remains on an aspartate within the flavin binding pocket until the reaction is fully reverted (130). However, a crucial role of proton transfer as a charge relocalization that may initiate a sequence of folding changes in the protein has been challenged. The D396C mutant completely blocks the proton

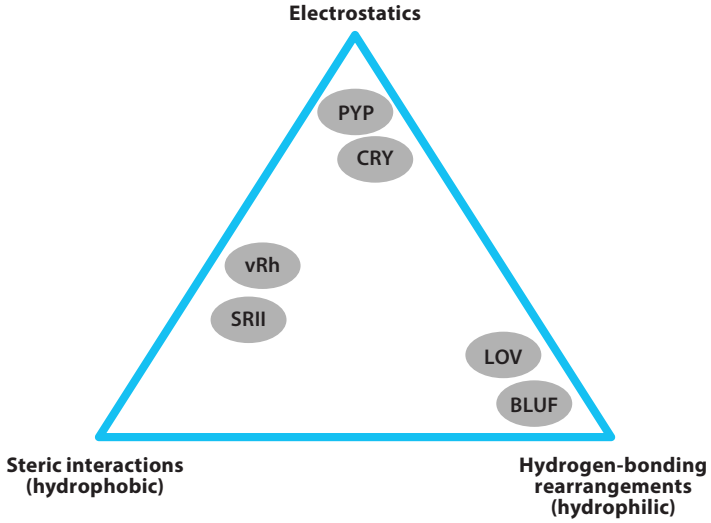

Figure 5

Dissecting contributions to the driving force that converts the initial local photochemical event into large protein conformational changes for biological signaling. The limiting cases are given by the edges of the triangle. The position of the receptor families reflects the contribution of primary effects that most likely are key for driving the signaling. Abbreviations: BLUF, blue-light using flavin; CRY, cryptochrome; LOV, light-oxygen-voltage; PYP, photoactive yellow protein; SRII, sensory rhodopsin II; vRh, vertebrate rhodopsin.

transfer, generating the flavin anion radical as a photoproduct similar to insect cryptochromes. In the D396C mutant lacking the proton transfer, changes in secondary structure of turn elements were observed by infrared difference spectroscopy similar to those of the wild type (42). In this mutant, the lifetime of the signaling state, the flavin radical, is reduced drastically by six orders of magnitude (42). The proton donation accordingly has been suggested to act as an intrinsic stabilizer of the signaling state in addition to external additives such as ATP. Currently, most evidence points to a model in which the charge of the anion radical of flavin drives conformational changes in plant cryptochrome, but more time-resolved studies are necessary to support this statement.

4. DISSECTING DRIVING FORCES FOR THE FORMATION OF THE SIGNALING STATE

In addition to buried charges that serve as an electrostatic epicenter, electrostatic interactions can also make other contributions to receptor activation, as discussed for the rhodopsins below. More generally, we propose that formation of the signaling state can be productively analyzed by considering three major contributors as driving forces for the conformational changes involved: (*a*) proton and/or electron transfer, or more generally electrostatics; (*b*) changes in hydrogen bonding; and (*c*) distortion of the chromophore and strain/steric clashes between the chromophore and groups in its binding pocket (**Figure 5**).

4.1. Release of an Electrostatic Constraint in Driving Rhodopsin Receptor Activation

A distinct form of electrostatic interactions that drive receptor activation was observed in both rhodopsin (125) and SRII (126). Instead of using an electrostatic epicenter, the neutralization of

a salt bridge at the active site through light-activated proton transfer drives rhodopsin receptor activation. In the initial state of Rh, the interaction between the PSB and the deprotonated side chain of Glu113 is energetically favorable and constrains the protein in a conformation that is inactive for signal relay. This constraint is removed by proton transfer from the Schiff base to Glu113 upon light activation, and the resulting protein conformational changes are essential for the formation of the signaling state of rhodopsin. Direct support for this model is the constitutive activation of rhodopsin mutants in which mutation of Glu113 permanently disrupts the constraining interaction between the PSB and residue 113 (108). In striking parallel, mutation of Asp73 results in constitutive signaling by SRII (125). Initial evidence points to a similar situation in BphP involving transient deprotonation of the bilin, with a concomitant relay of a proton to the solvent (9, 135). Intriguingly, disruption of this process by a point mutation of a conserved Asp residue blocks BphP photoswitching (137).

4.2. Rearrangement in Hydrogen-Bonding Network

The hydrogen-bonding network around the flavin plays a key role in the signaling mechanism of LOV and BLUF proteins. In both cases, a key event is the light-induced rearrangement at around the flavin N(5) involving a conserved glutamine residue despite the different folds. For LOV proteins, glutamine rotation is essential for signaling (95), whereas deletion of the reactive cysteine does not necessarily abrogate signaling (144). For BLUF, the tautomerization (with or without rotation) of glutamine leads to strong changes in the network around flavin (25). In line with a key role for this residue in signaling, substitutions of glutamine in BLUF proteins lead to pseudolit forms that constitutively drive signaling (26).

4.3. Buildup of Strains and Steric Clashes

A widely discussed factor for driving conformational changes is strain in a distorted chromophore in early photointermediates, perturbation of the stabilizing interactions between the chromophore and its binding pocket, and steric clashes between the chromophore and groups in the binding pocket. The issue has been investigated most extensively for the rhodopsins (see 28). Such strain is often viewed as a key contributor to storage of energy of the photon in the primary photoproduct. In addition, release of this strain in the form of conformational changes can in principle contribute to causing proton transfer or protein conformational changes for signaling, and experimental evidence for functionally important strain has been reported for rhodopsin (102, 116) and phytochrome (121, 143) as well as the flavin-cysteine adduct of LOV proteins (76). However, direct evidence demonstrating the functional necessity of light-induced distortions at the photoactive site of photoreceptors in driving the formation of the signaling state is often lacking. In addition, reliably quantifying the energetic contribution has proven challenging. The novel approach of Raman optical activity is promising in this respect (39, 66).

Steric interactions between the retinal (particularly the β-ionone ring and 9-methyl group) and its binding pocket contribute to the conformational changes that occur during formation of the Meta II signaling state (52, 60, 84, 122, 136). However, proton transfer from the Schiff base to the proton acceptor is also required for full activation of rhodopsin (111), leading to the view that both steric interactions and proton transfer from the PSB are needed for full activation of rhodopsin (15, 60, 98). A similar situation appears to occur in the archaeal sensory rhodopsins (125, 142).

4.4. Three Mechanisms for Photoreceptor Activation

The analysis provided here implies the existence of three mechanistically distinct groups of photoreceptors (**Figure 5**). The first group consists of LOV and BLUF proteins. After an initial PCET process, rotation/tautomerization of an active-site glutamine side chain results in changes in hydrogen bonding, which then trigger conformational changes for signaling. In this case, it is not proton transfer but changes in hydrogen bonding that drive conformational changes for signaling. The second group contains the rhodopsins where C=C isomerization leads to proton transfer, which removes an attractive charge–charge interaction that constrains the conformation of the protein. Both strain in the chromophore and proton transfer contribute to driving conformational changes for signaling. The final group is composed of PYP and cryptochrome: The primary photochemistry event (C=C isomerization and flavin photoreduction) creates an electrostatic epicenter consisting of a new buried charge that drives conformational changes for receptor activation.

Where phytochromes fit in this grouping remains to be established. In addition, one should note that it is likely that not all homologs of the above photoreceptors adhere to the above scheme; the classification is intended to help analyze mechanisms that convert the initial event into conformational changes upon signaling state formation.

5. IMPLICATIONS FOR NONPHOTOSENSORY PROTEINS

To what extent can the insights gained here be used to better understand proteins that are not triggered by light? Proteins with a light-triggered function have contributed greatly to our understanding of electron transfer (particularly based on chlorophyll-containing photosynthetic reaction centers) and proton transfer (particularly bacteriorhodopsin). Here, we propose that the role of creating, removing, or relocating buried charges in triggering conformational changes can provide a framework to analyze a wide range of proteins in signal transduction. An example is provided by the BRAF gene in melanoma cell lines, which often contains the V599E or V559D mutation (21). This mutation introduces a new charge and causes the constitutive activation of the receptor. It has been proposed that introduction of a negative charge to the position of Val599 mimics phosphorylation of the nearby residues Thr598 and Ser601 that occur during the normal activation process (21, 107). A second example is that signaling by methyl-accepting chemotaxis proteins in *Escherichia coli* involves both protein phosphorylation and the methylation of carboxylic acids (30, 127). These processes alter the presence of charged groups in the protein. For both these examples, whether the model for receptor activation reviewed here indeed operates remains to be established.

6. KEY OPEN QUESTIONS

1. For most photoreceptors, the driving forces for signaling state formation remain only partially elucidated. In particular, it remains challenging to experimentally quantify the contribution of strain at the photoactive site to receptor activation. More generally, it remains to be seen whether the classification of driving forces for generating conformational changes proposed here is of value for guiding further research on photoreceptors, and whether it remains valid.
2. Structural changes upon formation of the receptor state have been characterized in multiple photosensory proteins. However, in many cases, whether (all) these conformational changes are required for biological signaling has not been experimentally determined. An important

example is that the light-induced conformational changes in PYP have been studied quite extensively, but experimental studies determining which of these structural motions are indeed needed for in vivo signaling is largely lacking.

3. Much progress has been made on identifying the groups that form the proton transfer pathway in multiple photoreceptors and revealing the temporal order in which these proton donor and acceptor groups change their protonation state upon photoexcitation. However, key questions remain regarding the structural and mechanistic basis that drives these directional proton transfer processes in the interior of a protein. Even for the well-studied proton transfer events in rhodopsins and in PYP, the mechanistic drivers for proton transfer have not been fully identified.
4. While a rich body of information exists on the molecular mechanism of photoreceptor activation, much less information is available on the mechanisms by which the signal is transferred from the signaling state of the receptor to its downstream signaling partner. Recent technological advances in crystallography using X-ray lasers and cryo-electron microscopy may help address this gap in knowledge. Here, we focused on how photo absorbance can drive photoreceptor activation. The next frontier is to understand how the signal is relayed from the activated photoreceptor to the next component in the signal transduction chain.

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